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Animal Health Task Force

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ON

PRIORITY ANIMAL DISEASES IN 2024: EMERGING AND ZOONOTIC INFLUENZA VIRUSES, AFRICAN SWINE FEVER, AND BOVINE TUBERCULOSIS

June 11-13, 2024

ABSTRACTS

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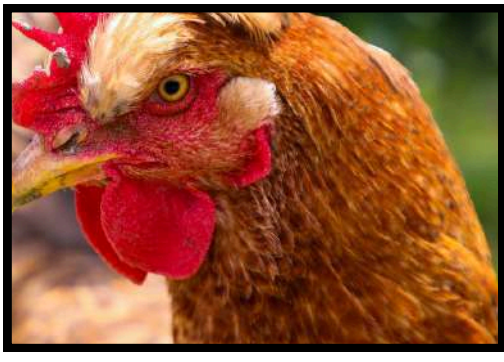

June 11th

Emerging and Zoonotic Influenza Viruses

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MMC Carlos Javier Alcázar Ramiro
SENASICA, Mexico

Current Situation of Avian Influenza in Mexico

Avian influenza is caused by viruses of the genus Influenzavirus A, of the Orthomyxoviridae family, which are widely distributed among wild birds without causing significant problems; however, under certain conditions they can infect poultry, which may not get sick, present a mild clinical picture, or become severely ill.

Since its appearance, the avian influenza virus has caused serious repercussions to the poultry industry in almost all the countries of the world, in addition to the havoc it has caused in other susceptible animal species, as well as the effects on public health considering its zoonotic potential.

For this reason, Senasica carries out epidemiological surveillance in Mexico to timely detect the presence of this disease in domestic poultry and wildlife. During the period from January 1, 2023 to May 25, 2024, 86 positive cases of avian influenza subtypes H5N1, H5N2 and H7N3 were detected in different states of the Mexican Republic.

H5N1
Thirty-three positive cases were detected in Aguascalientes (4), Baja California (3), Chihuahua (2), State of Mexico (1), Guanajuato (2), Jalisco (8), Oaxaca (1), Puebla (2), Sonora (2), Veracruz (1) and Yucatan (7), of which 11 were identified in commercial poultry, 7 in backyard poultry, 1 in poultry from a collection center and 14 in wild birds, with an approximate population of 1,147,737 affected birds.

H5N2
Twenty-five positive cases were confirmed in the State of Mexico (3), Guanajuato (6), Jalisco (12), Michoacán (1), Puebla (2) and Tabasco (1), of which 13 were identified in commercial birds, 11 in backyard poultry and 1 in wild birds, with an approximate population of 1,536,084 affected birds.

MMC Carlos Javier Alcázar Ramiro
SENASICA, Mexico

7N3
Twenty-eight positive cases were detected in Aguascalientes (2), Guanajuato (8), Jalisco (3), Michoacán (1), Puebla (12), San Luis Potosí (1) and Zacatecas (1), of which 11 were identified in commercial poultry and 17 in backyard poultry, with an approximate population of 2,505,719 affected birds.

In the production units and backyard premises, anti-epidemic measures were applied consisting of the slaughter of the birds and their sanitary disposal, cleaning, washing and disinfection of the facilities, material and equipment, as well as a period of sanitary vacuum, which has contributed to exhaustively controlling the circulation of the virus in commercial flocks.

As a result of the timely actions implemented by Senasica, all cases of avian influenza have been controlled, so that national poultry farming has not been put at risk; however, producers continue to be encouraged to strengthen biosecurity measures in poultry production units, with a total of 2,712 units registered with a biosecurity certificate in force as of April 30, 2024, with the collaboration of more than 400 Authorized Responsible Veterinarians in poultry.

MVZ Néstor Avendaño
OIRSA, Dominican Republic

Regional Actions and Current Situation on Zoonotic Influenza in OIRSA Member Countries

Introduction:
Avian influenza (AI) is a highly contagious viral disease that affects domestic and wild birds, and occasionally mammals, including humans. There are two main types of AI: low pathogenic (LPAI) and highly pathogenic (HPAI), the latter being the most worrisome due to its severity and rapid spread. The H5 and H7 subtypes are particularly associated with high pathogenicity.

Current Situation and Transmission:
Since 2020, a variant of avian influenza A(H5N1) virus, belonging to the H5 clade 2.3.4.4b, has caused significant deaths in wild birds and poultry in Africa, Asia, and Europe. In 2021, the virus spread to North America, and in 2022 to Central and South America, with several outbreaks reported in the Americas in 2023. Avian influenza is transmitted primarily by the fecal-oral route, through contaminated food and water, as well as through fomites such as equipment, clothing and vehicles. The ability of the virus to vary antigenically facilitates the emergence of new outbreaks.

Impact on Birds and Mammals:
HPAI affects multiple organs in birds, leading to severe and often fatal disease. Clinical signs include ocular and nasal discharge, coughing, swelling, cyanosis, incoordination and diarrhea. In mammals, the detection of the virus in species such as foxes, seals and minks, and the recent identification in dairy cows in the United States, raises concerns about its possible adaptation to infect humans more easily.

Human Cases and Surveillance:
Since 2022, sporadic cases of human infections with HPAI A(H5N1) virus A(H5N1) have been identified, generally associated with poultry exposures. Although the risk of human-to-human transmission is low, the rapid evolution of the virus and the overall

MVZ Néstor Avendaño
OIRSA, Dominican Republic

prevalence of outbreaks require ongoing surveillance. In 2024, the first probable cases of mammal-to-human transmission were reported in Texas and Michigan, associated with infected dairy cows.

Regional Situation:
In Costa Rica, Guatemala, Honduras, Mexico, and Panama, multiple outbreaks of HPAI have been detected in wild and backyard birds since late 2022. To date, no human cases have been identified in these countries, but surveillance and biosecurity measures remain crucial. OMSA recommends maintaining and strengthening surveillance systems and on-farm biosecurity measures, in addition to timely reporting of outbreaks.

International Support:
Organizations such as OIRSA support countries in the prevention and control of HPAI by providing outreach materials, promoting training workshops and strengthening regional laboratories. Specific projects are carried out for the detection and control of respiratory zoonoses in Central America and the Dominican Republic.

Conclusion:
The presentation provides a comprehensive update on the current status of highly pathogenic avian influenza, its public health and economic implications, and effective prevention and control strategies. It is essential for animal health professionals, poultry producers and animal health policy makers to stay informed and prepared for this global threat.

Dr Oliver Lung
CFIA, Canada

High-Throughput Sequencing, Bioinformatics Analysis and Reporting of High Consequence Viruses during the COVID-19 Pandemic and H5N1 Avian Influenza Outbreak - A Canadian Food Inspection Agency National Centre for Foreign Animal Disease Perspective

Sequencing enables unequivocal identification and comprehensive genomic characterization of microbial pathogens from known, unknown, unexpected and mixed infections, making it invaluable for epidemiological investigations of infectious disease outbreaks. The Canadian Food Inspection Agency (CFIA) National Centre for Foreign Animal Disease (NCFAD) Genomics Unit provides genomics and bioinformatics support for outbreaks, diagnostics, surveillance, and research on known, novel, and unexpected infectious diseases in animals. In addition to containment level (CL) 2 sequencing capabilities, NCFAD's Genomics Unit operates a unique CL3 sequencing facility, allowing the convenient sequencing of samples from CL3 and CL4 laboratories. The NCFAD's Genomics Unit use of both short-read Illumina and long-read Oxford Nanopore sequencing technologies, along with automation in both the wet- and dry-lab, has allowed it to build the capability and capacity to sequence, analyze, and accurately characterize large volumes of diverse samples. The NCFAD's Genomics Unit supports and participates in collaborative projects with a wide range of government and NGO partners. The presentation will cover how we have structured our operations to maximize efficiency and reproducibility through automation and deployment of systems for genomics data and information management, analysis and reporting to better support scientific research and response to animal health emergencies such as the ongoing H5N1 avian influenza outbreak and SARS-CoV-2 surveillance.

Dr Anthony Signore et al
CFIA, Canada

Phylodynamics of the H5N1 Avian Influenza Outbreak in North America Reveals the Emergence of Reassortants with Increased Fitness

In November 2021, an A/Goose/Guangdong/1/96 lineage highly pathogenic avian influenza virus (HPAIV) bearing the hemagglutinin (HA) gene of clade 2.3.4.4b (subtype H5N1) was detected for the first time in North America. This initial incursion led to an outbreak that spread across the continent, was detected in >80 wildlife species and affected >90 million domestic birds in Canada and the USA. From samples collected in Canada during this outbreak, the National Centre for Foreign Animal Disease has sequenced 2,124 complete H5N1 genomes. These data, in conjunction with previously published data, were used to perform multifaceted phylodynamic analyses. By jointly analyzing whole-genome sequences, collection dates, locations and host information, we provide substantial insights into the emergence, evolution, spatiotemporal spread and host dynamics of H5N1 in North America. Our analyses reveal significant viral diversification, as 37 distinct H5N1 genotypes emerged in North America in the 18 months following its introduction. Despite sharing a recent common ancestor, these genotypes display clear differences in host dynamics, geographic distribution and relative fitness. While Anseriformes and Charadriiformes are well known reservoirs for avian influenza viruses, our analyses find Charadriformes played a reduced role in the spread of the H5N1 viruses in North America. Phylogeographic reconstructions revealed geographic regions that are key to transcontinental viral spread and foster the emergence of novel reassortants with increased fitness. These insights into the diversification, host dynamics and spatiotemporal diffusion of H5N1 HPAI virus will inform ongoing surveillance efforts to minimize damages to ecological, animal and human health as these viruses continue to circulate throughout North America.

Dr José Iván Sánchez Betancourt
UNAM, Mexico

Swine Influenza in Mexico

Swine influenza is a worldwide disease that causes damage to the respiratory system of pigs (Bouvier & Palese, 2008). Influenza viruses belong to the Orthomixoviridae family and have a genome composed of eight segments of ssRNA (-), where each one encodes for one or two proteins (Flint, J; Racaniello, 2001; King, Adams, Carstens, & Lefkowitz, 2012). The evolution of these viruses occurs through antigenic drift (Drift), characterized by the selection of new strains that contain amino acid changes in hemagglutinin (HA) and neuraminidase (NA) proteins; these changes are responsible for seasonal influenza infections (Carrat & Flahault, 2007; Treanor, 2004). In addition, these viruses can also have genetic rearrangements (Shift) associated with the emergence of pandemic viruses; these occur when a host cell is infected by more than one different virus subtype and the viral genomic segments are rearranged, generating a new combination (Boni, 2008; Webster, Laver, Air, & Schild, 1982; Zambon, 1999). The research was carried out using 486 pig lung tissue samples from different production units located in twelve states of Mexico (Jalisco, State of Mexico, Guanajuato, Sonora, Michoacan, Puebla, Morelos, Yucatan, Queretaro, Hidalgo, Veracruz and Nuevo Leon). The criterion for obtaining the samples was to use pigs that presented suggestive signology to infection with influenza virus. The samples were remitted and processed in the Laboratory of the Medicine and Zootechnics Swine Department (DMZC) from the Veterinary Medicine and Zootechnics Faculty (FMVZ), National Autonomous University of Mexico (UNAM).

We processed the tissues samples by qRT-PCR and then by simple RT-PCR to obtained the sequences. By sequencing and analyzing phylogenetically the eight segments that form the virus genome, the following subtypes were identified: H1N1, H3N2, H1N2 and H5N2; of which, a H1N1 subtype had a high genetic relationship with the human influenza virus. In addition, a H1N2 subtype related to the porcine H1N2 virus reported in the United States was identified, as well as, two other viruses of avian origin from the H5N2 subtype. Particularly for the H5N2 subtype, this is the first time that its presence has been reported in Mexican pigs. The analysis of these sequences demonstrates that in the swine population of Mexico, circulate viruses that have suffered punctual-specific mutations and rearrangements of their proteins with different subtypes, which have successfully adapted to the Mexican swine population.

Dr José Iván Sánchez Betancourt
UNAM, Mexico

Origin of the genetic segments of swine influenza viruses identified in Mexico

SEGMENT	1	2	3	4	5	6	7	8
PROTEIN	PB2	PB1	PA	HA	NP	NA	M	NS
A/swine/Mexico/GtoDMZC01/2014(H1N2)	S	S	S	S	S	S	S	S
A/swine/Mexico/GtoDMZC02/2014(H5N2)	C	C	C	C	C	C	C	C
A/swine/Mexico/EdoMexDMZC03/2015(H5N2)	C	C	C	C	C	C	C	C
A/swine/Mexico/GtoDMZC04/2015(H1N1)	H	H	H	H	H	H	H	H
A/swine/Mexico/JalDMZC05/2015(H1N1)	S	S	S	S	S	S	S	S
A/swine/Mexico/GtoDMZC09/2015(H1N1)	S	S	S	S	S	S	S	S
A/swine/Mexico/HgoDMZC11/2015(H3N2)	S	S	S	S	S	S	S	S
A/swine/Mexico/JalDMZC12/2015(H3N2)	S	S	S	S	S	S	S	S

The Mexican isolates are located in the left side column and the segments of each protein are represented by a color chart depending on the subtype to which they belong: H1N1 (blue), H3N2 (pink), H1N2 (gray) and H5N2 (orange). The letters that are inside the coloring boxes indicate the species to which they correspond: swine (S), chicken (C) and human (H).

There is evidence that new subtypes of influenza virus have evolved genetically and have been rearranged with human viruses and from other species; therefore, the 3 aim of our study was to identify and characterize the genetic changes that have been generated in the different subtypes of the swine influenza virus in Mexican pigs.

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Dr José Iván Sánchez Betancourt
UNAM, Mexico

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Dr Alberto Jorge Galindo Barboza et al
UNAM, Mexico

Swine Influenza, Serological and Molecular Situation in the State of Jalisco

A convenience-directed study was conducted in swine farms. Nasal swab samples and oral fluids were collected from pigs in 13 farms with a clinical history of respiratory illness associated with swine influenza virus. Five individual serum samples were collected from the production line (3-21 weeks of age). 4 positive farms were identified in nasal swab samples (5/33.15%), two farms in oral fluid samples (2/7.28%) by real-time RT-PCR. Sequencing allowed the identification of two main subtypes, H3N1 and H3N2. In all cases, the sequences obtained from HA correspond to subtypes of porcine origin, identified in North America or the USA; for NA, four sequences obtained are of porcine origin and two from viruses identified as human influenza; the samples correspond to Mexican strains or from USA. In relation to the detection of antibodies, positivity was identified in all the analyzed farms, however, there were negative individuals or individuals with low titers. The highest average titers for the H1N1 subtype were identified between weeks 12 and 18. In the case of the H3N2 subtype, the highest mean titer was at week 6 and 9.

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African Swine Fever

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Dr Silvia Kreindel
Dr Yussaira Castillo et al
USDA, USA & Dominican Republic

African Swine Fever in the Dominican Republic - USDA/APHIS Update at three years of its Detection

In 2021, ASF was detected in the Dominican Republic (DR) and Haiti. Together, these countries make up the island of Hispaniola. Both countries were previously affected by ASF in 1978, which they dealt with by eradicating all susceptible swine on the island. In response to the 2021 reintroduction, the Government of the Dominican Republic, and the veterinary authority, with international support, employed a series of emergency strategies focused on containing and eradicating the disease.

Since African swine fever (ASF) was detected in the Dominican Republic in July 2021, it has negatively affected the country's pork sector. Assessing the epidemiological situation is crucial to help local authorities and industry stakeholders control the disease. Here, data on reported outbreaks in the Dominican Republic were evaluated. Data on clinical presentation, biosecurity measures and suspected reasons for introduction were classified and summarized. The majority (78%) of outbreaks occurred on backyard farms with generally poor biosecurity. In all farm types, most pigs were still alive at the time of depopulation.

These results provide critical information on the status of the ASF epidemic in the Dominican Republic and will help government officials and regional swine industry leaders develop effective ASF control strategies.

Dr Aruna Ambagala
Dr Kalhari Goonewardene
CFIA, Canada

Novel Diagnostic Tools for Early Detection of ASF

African swine fever virus (ASFV) is continuing to spread across the globe and has reached closer to North America. As a result, heightened surveillance measures are in place to protect North American swineherds. Scientists have developed and explored novel diagnostic tools; focused on alternative less invasive sample types and field deployable assays to improve the early detection capacity.

Oral fluid is a non-invasive aggregate sample type that is widely used in the industry to detect several endemic swine pathogens. Using four independent animal experiments we demonstrated that ASFV genomic material can be detected in pen-based oral fluid samples as early as 3-5 days post infection (dpi) at the pen prevalence at its lowest (4-5%). A large-scale field validation conducted during 2021 – 2023 in Vietnam enduring highly uncontrollable field conditions indicated ASFV genomic detection within 0-3 days of identifying the first viremia in a given pen.

Processing fluids (PF), is another aggregate sample type generated from the industry practice of piglet processing. Using two moderately virulent strains of ASFV, we demonstrated that ASFV genome could be detected in PF as early as 3-4 dpi. Passive surveillance of dead pigs based on WOAH-recommended individual tissue samples requires opening up the carcasses. It is time-consuming, requires skilled labor and often leads to contamination of the premises. We demonstrated that superficial inguinal lymph nodes (SILNs) which can be collected in minutes with no to minimum environmental contamination from dead pigs, can be used to detect pigs succumbed to highly and moderately virulent ASFV strains with 100% sensitivity.

ASFV can survive in meat and meat products for several months, leading to long-distance transmission of ASF. Using highly, moderately and low virulent ASFV strains, we demonstrated that meat exudate can be used to detect both ASFV genomic material and antibodies to ASFV.

Dr Aruna Ambagala
Dr Kalhari Goonewardene
CFIA, Canada

Field-deployable portable assays can be used in field when laboratory access is limited. Battery powered portable thermocyclers provide handy molecular testing capacity in the field. Field validation testing done using portable real time PCR instrument demonstrated successful transfer of ASFV diagnostic real time PCR assay for field level testing with high sensitivity and specificity.

Antibody based lateral flow assays (LFA) are cheap, quick and easy to perform in the field. We evaluated a few commercial LFA assays for ASFV antigen detection. The assays were less sensitive compared to molecular assays but they can be used at herd level when access to portable molecular assays or central laboratories is not possible.

In conclusion, these novel alternative sample types permitting efficient collection for surveillance and outbreak control and testing platforms with portability and field utility are definitely benefiting countries currently battling with ASF and the North American region to keep ASF out of their soil.

Dr Sandra Julieta Cuevas Romero et al
INIFAP, Mexico

Capacity Building for ASF Diagnostics in Mexico: Generation of Reagents Required for Serological Assays

African swine fever (ASF) is a disease that affects both domestic and wild swine and has spread across Central Europe, East and Southeast Asia, and Africa. ASFV was recently found in the Dominican Republic (DR) and Haiti, marking the first ASFV diagnosis in the Western Hemisphere in almost 40 years. This fact poses a significant risk to the rest of the Americas and places a significant burden on health authorities to implement appropriate measures to avoid the spread of ASF to any other nations in the Americas. The goal of this Research Project titled "Capacity building for ASF diagnostics in México: Generation of reagents required for serological assays" is to lay the groundwork for the development of diagnostic tools for early and large-scale detection of ASF that can be used in biosafety level 2 laboratories in Mexico. The overall goal is to produce two recombinant proteins (p30 and p72 of ASFV) in our low-cost E. coli vector system. These antigens can be utilized to create serological techniques like indirect ELISA (I-ELISA), which detects antibodies in a sensitive and specific assay. The reference strain for this experiment was African swine fever, which was identified in Georgia in 2007. Five ORFs from five different ASF proteins (p, 30, 54, 72, 49, and 205) were used to convert the Escherichia coli (E. coli) strain TOP10, which served as a cloning host, with the PASK 33 BSA plasmid. These two proteins (p30 and 72) were chosen as potential antigens for this study. Bioinformatics investigation of the p72 ASF proteins using the Maximum Likelihood technique was done, and in this analysis involved 34 amino acid sequences, with a total of 647 locations in the final dataset indicate a 99.54% of identity related to Genotype I sequences, 100% identity with Genotype II (20/30), and 97.83% with other genotypes. The same study was performed with the p30 ASF protein, and the findings show 97.85% identity with Genotype I sequences, 100% identity with Genotype II (9/42) and 83.94% with other genotypes. This investigation shows that p72 and p30 are conserved proteins and are suitable representative proteins of the ASF virus to create in a recombinant expression system and obtain the proteins for adaption to the indirect ELISA.

Dr José Luis Cerriteño Sánchez et al
INIFAP, Mexico

Constructing a System for Expressing Recombinant ASF Proteins

Producing pork and pork products is a constant challenge for the industry due to the epidemic diseases that affect this sector. Serological assays, specially ELISA, are critical for surveillance in order to regain freedom after a potential African swine fever (ASF) outbreak. Currently all ASFV ELISA kits are produced outside of Mexico and are expensive. The aim of this work was to produce two recombinant proteins (p30 and p72 of ASFV) that can be used to develop serological tools such as indirect ELISA. The antigenicity of the African swine fever protein p30 and p72 was characterized in silico analysis to predict the antigenic sites and select the appropriate protein region for expression, which contains the most representative epitopes and antigenic site. In silico characterization of p30 protein antigenicity was made up of 194 amino acid residues and a weight of 22.39 kDa. The amplified protein weighed 19,983 kDa and contained 174 amino acid residues, beginning with glutamic acid (Glu, E) at residue 12 and ending with leucine (Leu, L) at residue 186. The antigenicity index was determined using surface probability, and the hydrophilic regions revealed the presence of 7 antigenic determinants (epitopes) and one glycosylated site. . On the other hand, the complete sequence of the p72 protein was made up of 646 amino acid residues and a weight of 73,155 kDa. The protein fragment has a weight of 28,651 kDa with 254 amino acid residues, begins with glutamic acid (Glu, E) at residue 73 and ends with proline (Pro, P) at residue 326, selected from the N-region terminal with the highest antigenicity index, surface probability, with hydrophilic regions, with the presence of ten antigenic determinants (epitopes) and one glycosylated site. Transformation of two proteins was carried out in E. coli Top10 bacteria to obtain the insert, which were then used to obtain transformed producer strains. In this approach, the BL21 strain served as recombinant expression vector. Once the vector has been generated in E. coli TOP 10, it can be transformed into E. coli BL21, a strain specifically designed for the production of recombinant proteins. This strategy is utilized to ensure the stability and correct replication of the vector before its introduction into the BL21 strain, thus optimizing the production of the recombinant protein. The antigenicity, specificity and the expression of recombinant proteins (p30 and p72) were assessed by Western Blot (WB) analysis.

Dr José Luis Cerriteño Sánchez et al
INIFAP, Mexico

Finally, a recombinant expression system was successfully developed in E.coli as an expression vector for the p72 and p30 proteins of the African swine fever virus, allowing for the stage of adaption to the indirect ELISA technique. This will be accomplished using ASF reference sera available at the CFIA-National Centre for Foreign Animal Disease, Winnipeg, Canada. NCFAD.

MVZ Alonso Steffani
OIRSA, Dominican Republic

African Swine Fever. Current Situation and Actions in Free Countries of the Region (OIRSA)

On July 28, 2021, ASF diagnosis was confirmed in swine samples from the DR. The samples were tested at Foreing Animal Disease Diagnostic Laboratory (FADDL) in the United States. The virus was isolated and sequenced. Officially, the World Organization for Animal Health (OIE) was notified, and a sanitary emergency was declared in the country.

OIRSA SUPPORT IN DR

OIRSA, at the request of the Ministry of Agriculture and the General Directorate of Livestock of the DR (MARD- DIGEGA), assists and accompanies the country's response to the ASFV emergency from the creation of an action plan and implementation of the first High Level Commission to develop the actions to be taken immediately to the current actions.

In this regard, actions have been carried out from the containment of the outbreak, development of continuous training sessions for the official veterinary service of the DR, strengthening of diagnostic capacity in the official animal health laboratory (LAVECEN), including international accreditation of ASF and CSF tests under ISO 17025: 2017; outreach campaigns to the general public, the systematization of processes through the official MARD application, SIDIAGRO agricultural information system, which provides information on pig farms nationwide.

The capacity of the national quarantine services has also been strengthened through the training and implementation of agricultural detection canine units with emphasis on pork products and by-products, thanks to the support provided by the SENASICA Canine School, MEXICO, and the Canine School of Panama. These dogs also provided support for the ASF outreach campaign for travelers at airports.

MVZ Alonso Steffani
OIRSA, Dominican Republic

Additionally, through the strategic alliance between MARD, the USDA Animal and Plant Inspection Service (APHIS) and OIRSA, actions were implemented that have allowed the country to strengthen field response, maintaining and increasing control and eradication actions, as well as increasing biosecurity and digitalization of processes in LAVECEN, and strengthening national quarantine services, both outbound and inbound to the country.

OIRSA SUPPORT IN FREE COUNTRIES

In the free countries of the OIRSA region, countries are accompanied in the development of work plans that include actions such as:

- Strengthening diagnostic capacity in the region.
- Strengthening of the technical capacity and field response of the Official Veterinary Services of the countries.
- Creation, updating and dissemination of information material on ASFV for the general public.
- Implementation and operation of non-intrusive inspections at the countries' entry quarantines using agricultural detection canine binomials, thanks to the collaboration of the canine schools of Mexico and Panama.
- Creation of a Regional Technical Commission on Swine Health.
- Continued management of resources to strengthen ASFV prevention and exclusion plans.

CONCLUSION

Thanks to the measures taken by the countries and the willingness of major cooperating partners to provide strategic support, ASFV has been contained within the Dominican territory, thus keeping the countries of central and northern America free of the disease, as a result of the implementation of non-intrusive inspection using agricultural detection canine binomials at the exit of international airports, and the implementation of national regulations that strengthen this activity.

MVZ Alejandro Zaldívar Gómez
MVZ Andrés Josafat Iniesta Valencia
SENASICA, Mexico

Update in the Development of a Quantitative Tool to Evaluate the Risk of Introduction of ASF to Mexico

Mexico faces a constant threat from African Swine Fever (ASF), a highly contagious viral disease affecting pigs that has caused significant losses in the global swine industry. The implementation of tools for ASF risk analysis and management is crucial to protect animal health and the country's economy. Currently, available risk analysis platforms are expensive or require specialized training to operate, which limits their accessibility to Veterinary Services and other industry professionals. In response to this need, the objective is to develop an interactive, accessible and scalable platform that facilitates both qualitative and quantitative risk analyses to assess the risk of introduction and spread of ASF in Mexico.

In the initial phase of the project, the databases needed to assess the risk of ASF introduction and spread in Mexico have been identified. In this strategy, the importance of focusing the analysis on the spread of the disease has been recognized, due to the absence of previous studies on this aspect, since the national Veterinary Services have mainly evaluated the risk of introduction of the disease.

The platform has been developed using Shiny, an RStudio library that allows the creation of interactive web applications. Meanwhile, the programming has been done in R, a freely available programming language designed for statistical analysis and data visualization. In addition, it offers competitive advantages, including scalability and an active community that continuously contributes to its development and improvement.

Similarly, the initial diagnosis revealed the need to focus efforts on optimizing data input and building scenario trees to operationalize the platform's functions. This systematic approach ensures that data are consistently integrated and analyzed to guarantee the consistency and reproducibility of risk analyses.

MVZ Alejandro Zaldívar Gómez
MVZ Andrés Josafat Iniesta Valencia
SENASICA, Mexico

The platform seeks to develop an intuitive environment that simplifies data entry and analysis, as well as to build a work routine that allows users to perform qualitative and quantitative risk analysis and to generate reports and visualizations that facilitate informed decision making.

Preliminary results indicate that the platform has the potential to become a tool for PPP risk management, representing a significant step forward in Mexico's ability to address PPP risk. In the next stages of the project, it is planned to carry out regional risk assessments and deepen quantitative analyses.

During the process, the tool has been successfully developed so that it can be of use in various health aspects. Likewise, through the information provided for the development of the project, related to risk analysis, it is expected to make a transition from qualitative to quantitative analysis at the internal level.

Several meetings have been held between the developers and the technical area, where it has been identified that the project can be transversal, and if some variables are changed, the tool could be used to analyze other diseases.

To consolidate the above, it is necessary to have knowledge of the R program, so the developers have trained the area to be able to handle this language, since it is considered of utmost importance that it transcends and is not static for a single disease.

Dr Aruna Ambagala
Dr Kalhari Goonewardene et al
CFIA, Canada

Characterization of a Field Isolate of ASFV with Deletions in the MGF Region from Vietnam

Since the introduction of ASFV into Asia, the emergence of naturally attenuated or illegally introduced attenuated ASFV strains has been a major concern. These concerns later became a reality when China reported the identification of live-attenuated ASFV strains circulating in pig farms. Here we are reporting the characterization of a genetically modified live-attenuated ASFV field strain isolated from a family farm that experienced ASFV outbreaks in Northern Vietnam. The isolate, ASFV-GUS-Vietnam, belongs to p72 genotype II, has six multi-gene family (MGF) genes deleted, and an Escherichia coli GusA gene (GUS) inserted. When six 6–8-week-old pigs were inoculated with the virus oro-nasally (2 × 105 TCID50/pig), they developed viremia, mild fever, lethargy, and inappetence, and shed the virus in oral and nasal secretions and feces. One of the pigs developed severe clinical signs and was euthanized 12 days post-infection, while the remaining five pigs recovered. When ASFV-GUS-Vietnam was inoculated intramuscularly (2 × 103 TCID50/pig) into four 6-8 weeks old pigs, they also developed viremia, mild fever, lethargy, inappetence, and shed the virus in their oral and nasal secretions and feces. Two contact pigs housed together with the four intramuscularly inoculated pigs, started to develop fever, viremia, loss of appetite, and lethargy 12 days post-contact, confirming horizontal transmission of ASFV-GUS-Vietnam. One of the contact pigs died of ASF on day 23 post-contact, while the other one recovered. The pigs that survived the exposure to ASFV-GUS-Vietnam via the mucosal or parenteral route were fully- protected against the highly virulent ASFV Georgia 2007/1 challenge.

This study show that ASFV-GUS-Vietnam field isolate is able to induce complete protection against highly virulent homologous ASFV challenge in the majority of pigs, but has the potential for horizontal transmission, and can be fatal in some animals. The study highlights the need for proper monitoring and surveillance when ASFV live-attenuated virus-based vaccines are used in the field for ASF control in endemic countries.

Dr Douglas Gladue
Veterinary Pharmaceuticals Seek Labs, USA

Understanding African Swine Fever Virus Genomics and Implications for Therapeutic or Preventative Approaches

African swine fever virus (ASFV) contains a complex DNA Genome typically containing approximately 180-190 open reading frames. Although ASFV has been causing outbreaks since 1921, ASFV genome analysis was mostly limited to sequencing of a single gene for disease tracking, with only a few select genomes fully sequenced. However since the recent relative low cost of next-generation sequencing, many researchers fully sequenced isolates of ASFV. Recently comparative analysis of full genomes of ASFV has resulted in characterization by full-genome analysis or biotypes. This information provides additional insights into potential targets for therapeutic use, and potential for determining grouping for vaccine matching prediction. This new information has been critical for determine potential universal treatments for ASFV. Here we will discuss the potential for CRISPR treatment of ASFV, with a first-generation CRISPR treatment resulting in 57% protection against intra-muscular challenge of virulent ASFV from the current outbreak strain in Asia.

June 13th

Bovine Tuberculosis

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Dr Olga Andrievskaia
CFIA, Canada

Genetic Diversity of *Mycobacterium bovis* Strains in Canada: an Updated

Bovine tuberculosis (bTb) is an important infectious disease that poses a risk to public health, livestock, and wildlife. In Canada, the century-long mandatory National Bovine Tuberculosis Eradication Program has virtually eliminated the disease in livestock, with only rare sporadic outbreaks. Over the last ten years, three localized bTb outbreaks have been reported in Canadian cattle: in Alberta in 2016 (AB2016), British Columbia in 2018 (BC2018), and Saskatchewan in 2023 (SK2023). Tuberculosis has not been detected in wild cervids in Riding Mountain National Park (RMNP), Manitoba, since 2014. However, one wildlife bTb reservoir still persists within a population of free-ranging wood bison in and around Wood Buffalo National Park (WBNP), located in the northern part of the country.

Historically, bTb outbreaks in Canadian livestock were associated with Mycobacterium bovis spoligotypes SB0130, SB0140, SB0145, SB0265, SB0673, SB1069, SB1070, and SB1071. Tuberculosis in wildlife was caused by M. bovis spoligotypes SB130 in WBNP, and SB1071 in RMNP. Since 2016, whole-genome single nucleotide polymorphism (SNP)-based genotyping was routinely applied in epidemiological investigations to assess the genetic relatedness between new bTb cases and historical M. bovis isolates. Whole genome sequencing (WGS) was performed using Illumina Nextera XT DNA libraries and the Illumina MiSeq platform, and the sequencing data were analysed using the bioinformatics tool vSNP (https://github.com/USDA-VS/vSNP). Single nucleotide polymorphism (SNP) differences were used to determine the phylogenetic relationships between the isolates. WGS SNP genotyping clearly demonstrated that each of the three bTb outbreaks (AB2016, associated with M. bovis spologotype SB0673; BC2018, spoligotype SB0971; SK2023, spoligotype SB0971) was caused by new genetically distinct strains that exhibited significant SNP differences when compared with historical Canadian isolates of animal origin. Our analysis of available genome sequences of Canadian M. bovis strains of human origin revealed a larger diversity of M. bovis genotypes; yet, there was no evidence of M. bovis zoonotic and reverse zoonotic transmission events in Canada. The pathways through which the new M. bovis strains were sporadically introduced into livestock during the last decade were not conclusively determined.

Dr José Ángel Gutiérrez Pabello
UNAM, Mexico

Mycobacterium bovis Naturally Infected Calves Present a Higher Bacterial Load and Proinflammatory Response than Adult Cattle

Granulomas are characteristic bovine tuberculosis lesions; studying this structure has improved our understanding of tuberculosis pathogenesis. However, the immune response that develops in granulomas of young cattle naturally infected with Mycobacterium bovis (M. bovis) has not been fully studied. Our previous work described an atypical pattern in granulomatous lesions of cattle younger than 4 months (calves) naturally infected previously M. bovis that did not correspond to the histological classification previously proposed. Histologically, granulomas from calves lack a connective tissue capsule and have fewer multinucleated giant cells (MGCs) and more acid-fast bacilli (AFB) than the classic tuberculosis lesions found in cattle older than 1 year (adults); this suggests a deficient immune response against M. bovis infection in young animals. Therefore, we used IHC and digital pathology analysis to characterize the in situ immune response of granulomas from young and adult cattle. The immunolabeling quantification showed that granulomas from calves had more mycobacteria, CD3+ cells, IFN-γ, TNF-α, and inducible nitric oxide synthase (iNOS) than those of adult cattle. Furthermore, calf granulomas showed lower immunolabeling of MAC387+, CD79+, and WC1+ cells without connective tissue surrounding the lesion and were associated with less vimentin, Alpha Smooth Muscle Actin (α-SMA), and TGF-β compared with granulomas from adult cattle. Our results suggest that the immune responses in granulomas of cattle naturally infected with M. bovis may be age dependent. This implies that an exacerbated proinflammatory response may be associated with active tuberculosis, producing more necrosis and a lower microbicidal capacity in the granulomas of calves naturally infected with M. bovis.

Dr Om Surujballi et al
CFIA, Canada

Use of the IDEXX *Mycobacterium bovis* Antibody Detection Test in Investigation of Three Bovine Tuberculosis Outbreaks in Canadian Cattle

Canada experienced three outbreaks of bovine tuberculosis (bTB) in cattle caused by Mycobacterium bovis, within the past ten years; in 2016, 2018 and most recently in 2023. In Canada, bTB is a reportable disease that is regulated by the Canadian Food Inspection Agency (CFIA). The CFIA's policy objective is to eradicate bTB in livestock by finding all cases of M. bovis, preventing further spread of the disease and determining the extent and origin of the disease.

The outbreak response following each detection included ante-mortem testing with the caudal fold skin test (CFT), the ELISA and in a limited number of herds, the interferon gamma test (Bovigam®).

Infected/index herds, as well as epidemiologically linked herds (contact, trace-in, trace-out, and proximity herds) were subject to ante-mortem testing protocols structured for sensitivity commensurate with the risk level of the herd category, e.g. in parallel testing with CFT, ELISA and, where feasible, Bovigam, in herds with a high risk of being exposed/infected. ELISA was used in 2016 and 2018 in different herd categories while in 2023, ELISA was only used in the index herd. The ELISA was always applied in parallel with CFT.

A stamping out policy applied where all cattle on the index premises were destroyed with post mortem (PM) examination of animals >6 months old. All CFT reactors and/or ELISA positive animals received an enhanced PM examination while test-negative animals were subject to a standard PM. In epidemiologically linked herds, all CFT reactors and/or ELISA positive animals were also destroyed with an enhanced PM. Regardless of live animal test results, all trace-out animals were destroyed with a PM examination.

Dr Om Surujballi et al
CFIA, Canada

During each PM examination, a standard set of tissues and all bTB-compatible lesions were collected and submitted for confirmatory testing. Confirmatory testing for bTB includes histopathological examination of tissues and PCR testing of paraffin-embedded formalin-fixed (PEFF) tissues in which acid-fast organisms are observed and bacteriological culture of tissues for isolation of Mycobacteria spp.

Cattle from which PEFF samples yield PCR-positive results for Mycobacterium Tuberculosis Complex (MTC) organisms, and/or from which M. bovis is cultured are classified as infected with bTB (gold standard).

The ELISA used in the three investigations was the Idexx® Mycobacterium bovis Antibody Test kit which is listed in the World Organization for Animal Health register of diagnostic kits. For all three investigations, in the majority of herds, serum for ELISA was collected on the day of injection of tuberculin for the CFT. In some herds, serum was collected on the day of reading of the CFT. However, only for the 2023 investigation, a second set of serum samples was additionally obtained post-CFT (at slaughter) from 89/108 cattle in the index herd.

This presentation will focus on the performance of the serological test (Idexx ELISA) that was deployed in the three investigations and discuss the potential for this or a similar test to be used as a diagnostic in bTB investigations to either complement the skin test, or, as a stand-alone test for identification of bTB-exposed animals. The potential for the ELISA for use as a screening test in a bTB surveillance program will also be discussed.

Dr Paola Boggiatto et al
USDA, USA

Oral Vaccination of White-Tailed Deer against Bovine Tuberculosis Using Alginate Sphere Encapsulation of BCG

In the United States, white-tailed deer (Odocoileus virginianus) are a wildlife reservoir for Mycobacterium bovis, the causative agent of bovine tuberculosis. Initially identified in Michigan in 1975 and again in 1994, the apparent prevalence of M. bovis in free-ranging white-tailed deer in the northeaster lower peninsula of this state has remained at 1-2% for over a decade. Despite extensive efforts, M. bovis remains endemic in deer and is a continuous threat to cattle producers in northern Michigan. We have previously shown that oral vaccination of deer with the human tuberculosis vaccine, bacille Calmette-Guérin (BCG), can provide protection to white-tailed deer against experimental challenge with virulent M. bovis. This protection is characterized by reduced lesion severity, however it requires direct administration of the liquid vaccine into the posterior oral cavity. While successful, this approach would not work for delivery of vaccine in free-ranging wildlife populations. In the work presented here, we developed a vaccine delivery platform using sodium alginate. Through a process of reverse spherification, sodium alginate was used to generate spheres with a soft-membranous exterior and a liquid interior containing BCG. We utilized these spheres to load BCG and vaccinate deer orally by allowing the deer to consume these spheres. Following vaccination, we tracked peripheral T cell immune responses to determine if BCG contained within the sodium alginate spheres would induce measurable immune responses. Our data demonstrated that consumption of the spheres resulted in exposure to BCG and the development of immune responses, similar to those observed when deer are vaccinated parenterally with BCG. The work presented here demonstrates that sodium alginate spheres can be used to effectively deliver oral BCG to white-tailed deer.

Dr Feliciano Milián Suazo et al
UQA, Mexico

Vaccination Strategies against Bovine Tuberculosis

Bovine tuberculosis (bTb) remains one of the diseases that causes the greatest damage to livestock in many countries of the world, in addition to being a public health risk (Phillips et al., 2003). Developed countries have managed to reduce bTb prevalence to minimum rates with the “test and slaughter” strategy, contrary to what has happened in developing countries, where this strategy is not feasible because it is costly. Given this limitation, producers request alternative solutions that do not imply the slaughter of animals, this is where the possibility of BCG vaccination comes in; a vaccine that, in spite of being used for approximately 100 years in children, is not used in cattle.

Experimental work with BCG with different strains, routes of application and vaccine doses and challenge doses, in domestic and wild animals, have shown that the vaccine significantly reduces pathological damage in vaccinated versus unvaccinated animals. It has been observed that low doses are better than high doses, that the age of application is not an important factor, and that the use of a protein booster improves protection.

In field studies it was observed that the vaccine induces an immune response similar to that observed experimentally, and that it is totally safe in pregnant animals. In these studies, an efficacy between 22 and 86% has been observed, a difference that may be due to the parameters used as a response, from positivity to the tuberculin test, to the counting and classification of lesions, and the isolation and counting of bacteria; it is the latter that report efficacies of approximately 86%. Recent work has shown that, in combination, prevention of lesion development with prevention of dissemination, the vaccine can achieve an efficacy as high as 89%. It has also been shown that vaccination with BCG and/or Protein Filtrate (CFP) does not induce IFN-gamma response with CFP-10 and ESAT-6 antigens, which allows to make differential diagnosis, vaccinated vs. infected. The overall conclusion is that the vaccine, alone or combined with protein as a booster, is a tool that can significantly help the current control programs to eliminate tuberculosis from cattle herds.

Dr Feliciano Milián Suazo et al
UQA, Mexico

How and when to vaccinate. Depends on a diagnosis of risk of infection of calves in the herd, studies have shown that the age of the animals to be vaccinated is not critical in vaccine efficacy. **BCG strain to be used.** It has been shown that there is no significant difference between the most commonly used strains, Danish 1331; Pasteur 1173; Glaxo 107; Tokyo 172-1; Russia-Ir; Brazil and Phipps. **Time vaccinating**. Depends on goals and activities committed to achieve them, approximately 7 to 10 years. **Route of application**. Subcutaneous route. **Dose.** doses of 1x104 to 1x106 colony forming units. **Vaccination strategies**. It can be started by vaccinating the whole herd, vaccinating heifers or vaccinating calves, depending on goals and time to achieve them.

Dr Carly Kanipe
USDA, USA

Histopathologic Differences in Granulomas of BCG Vaccinated & non-Vaccinated Cattle with Bovine Tuberculosis

Mycobacterium bovis (M. bovis) is the zoonotic bacterium responsible for bovine tuberculosis. An attenuated form of M. bovis, Bacillus Calmette-Guerin (BCG), is a modified live vaccine known to provide variable protection in cattle and other species. Protection for this vaccine is defined as a reduction in disease severity rather than prevention of infection and is determined by evaluation of the characteristic lesion of tuberculosis: the granuloma. Despite its recognized ability to decrease disease severity, the mechanism by which BCG imparts protection remains poorly understood. Understanding the histopathologic differences between granulomas which form in BCG vaccinates compared to non-vaccinates may help identify how BCG imparts protection and lead to an improved vaccine. Utilizing special stains and image analysis software, we examined 88 lymph nodes obtained from BGC-vaccinated and non-vaccinated animals experimentally infected with M. bovis. We evaluated the number of granulomas, their size, severity (grade), density of multinucleated giant cells (MNGC), and the amounts of necrosis, mineralization, and fibrosis. BCG vaccinates had fewer granulomas overall and smaller high-grade granulomas with less necrosis than non-vaccinates. The relative numbers of high- and low- grade lesions were similar as were the amounts of mineralization and the density of MNGC. The amount of fibrosis was higher in low-grade granulomas from vaccinates compared to non-vaccinates. Collectively, these findings suggest that BCG vaccination reduces bacterial establishment, resulting in the formation of fewer granulomas. In granulomas that form, BCG has a protective effect by containing their size, reducing the relative amount of necrosis, and increasing fibrosis in low-grade lesions. Vaccination did not affect the amount of mineralization or density of MNGC.

Dr Olga Andrievskaia
CFIA, Canada

Evaluation of Oxford Nanopore Long-Read Sequencing as a Versatile Tool for *Mycobacterium bovis* Genotyping and Bovine Tuberculosis Outbreak Investigations

Bovine tuberculosis (bTb) is a reportable zoonotic bacterial disease primarily caused by Mycobacterium bovis, a member of the Mycobacterium tuberculosis complex (MTC). Genotyping of M. bovis isolates is essential for epidemiological investigation of bTb outbreaks and justifying regulatory actions. Spoligotyping, variable number tandem repeat (VNTR) typing, and whole genome sequencing (WGS)-based single nucleotide polymorphism (SNP) typing are genotyping approaches that rely on different technological platforms and vary in resolution power. Worldwide, these methods are unevenly applied by veterinary and human health laboratories, which complicates information exchange between different jurisdictions and comparison with international MTC genotype databases. Our study aimed to evaluate whether Oxford Nanopore long-read sequencing can provide a single technical approach to generate accurate in silico-derived spoligo-, VNTR-, and WGS SNP-typing information.

We analysed 90 Canadian M. bovis isolates collected between 1985 and 2023, representing distinct genetic clusters. The isolates were sequenced using native barcoding kits, various flowcell formats (R9.4, R10.3, R10.4), and a Nanopore MinION MK1B device. Nanopore sequencing reads were basecalled in super-accuracy mode, and analysed by bioinformatics tools:
Spoligotyper (https://github.com/duceppemo/Spoligotyper),
MIRUReader (https://github.com/phglab/MIRUReader),
and vSNP3 (https://github.com/USDA-VS/vSNP).

The Nanopore sequencing-based in silico genotyping results were compared with spoligo- and VNTR types generated by traditional wet lab protocols, and with Illumina WGS SNP-typing. VNTR types were identified with 100% accuracy from R9.4 Nanopore sequencing reads at coverage >50X and from R10 reads at coverage >22X. The R9.4

Dr Olga Andrievskaia
CFIA, Canada

sequencing did not generate data for accurate in silico spoligotyping or SNP-based cluster assignment. At the Nanopore R10 sequencing coverage >22 X, in silico spoligotypes were identified with an accuracy of 98%, and the M. bovis strain assignation to SNP-based phylogenetic clusters was concordant with Illumina-based clustering and epidemiological data. The distances between genomes based on Nanopore SNP differences were greater than those based on Illumina SNPs.

Overall, Oxford Nanopore long-read sequencing can be considered as a versatile cost-efficient alternative to conventional M. bovis genotyping wet lab protocols. It can provide comprehensive in silico genotyping data for high-resolution bTb epidemiological investigations and large-scale inter-laboratory information exchange.

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