MANILA, PHILIPPINES

GARA

GAP ANALYSIS WORKSHOP

DECEMBER 5-7, 2023

Global African Swine Fever Research Alliance

GARA Gap Analysis Workshop
Manila, Philippines | December 5-7, 2023

Program Agenda

Day 1: Tuesday, December 5, 2023

8:30 - 9:15 AM	Check In **CRDF Global**
9:15 - 9:30 AM	Welcome Message **Paul C. Limson,** *Chief Veterinary Officer*, Department of Agriculture, Bureau of Animal Industry
9:30 - 9:45 AM	GARA Introductory Remarks **Cyril Gay**, *Senior National Program Leader*, Animal Production and Protection, USDA-ARS
9:45 - 10:15 AM	Update on ASF in Asia **Yooni Oh**, *Animal Production and Health Officer,* ASF programme for Asia and the Pacific, Food and Agriculture Organization (FAO)
10:15 - 10:50 AM	**Coffee**
10:50 - 11:50 PM	Country Reports – Philippines, Vietnam, Thailand, South Korea **Samuel Joseph M. Castro**, Philippines Country Report **Dao Duy Tung**, Vietnam Country Report **To be announced**, South Korea Country Report **Dachrit Nilubol**, Thailand Country Report
11:50 - 1:20 PM	**Lunch**
1:20 - 1:50 PM	State-of-the-art ASF Epidemiology **Erika Chenais**, *Associate Professor*, National Veterinary Institute
1:50 - 2:05 PM	An Overview of CAHFS International Research and Activities Supporting ASF Prevention and Control and Veterinary Capacity Building **Rachel A. Schambow,** *Postdoctoral Associate,* Center for Animal Health and Food Safety, College of Veterinary Medicine, University of Minnesota
2:05 - 2:20 PM	Mapping Key Players in the Vietnamese Pig Value Chain to Inform Strategies for Improved African Swine Fever Biosecurity Regulation Adoption **Le Thi Thu Ha,** *Researcher,* Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)

2:20 - 2:35 PM	Enhanced Accessibility to Modeling of ASFV transmission Dynamics for Policymakers **Dirk Pfeiffer,** *Chow Tak Fung Chair Professor of One Health,* City University of Hong Kong
2:35 - 2:50 PM	Case of ASF in the Critically Endangered Visayan Warty Pig (Sus cebrifrons) **Matthew Ward,** *Executive Director,* Talarak Foundation
2:50 - 3:20 PM	State-of-the-art Diagnostics **Aruna Ambagala,** *Mammalian Diseases Unit Head,* National Centre for Foreign Animal Diseases, Canadian Food Inspection Agency
3:20 - 4:00 PM	**Coffee**
4:00 - 4:15 PM	ASF Nanogold Biosensor Test Kit: An Alternative Decentralized ASF Detection Assay **Clarissa Yvonne J. Domingo,** *Professor,* Department of Pathobiology, Central Luzon State University
4:15 - 4:30 PM	Effect of High Temperature Exposure and Laboratory Processing Techniques on the Diagnostic Performance of Dry Swabs for the Detection of ASFV **David T. Williams,** *Diagnostics and Mammalian Infectious Diseases Research Group Leader,* CSIRO Australian Centre for Disease Preparedness
4:30 - 4:45 PM	Detection of ASFV Infection in Pigs using Clinical, Environmental, and Non-invasive Samples Through the Application of a Minimal Equipment Colorimetric LAMP PCR **Adriana Muñoz,** Institute of Agrifood Reseach and Technology, Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain
4:45 - 5:00 PM	**Wrap Up**

Day 2: Wednesday, December 6, 2023

9:00 - 9:05 AM	Introduction to Day 2 **GARA Scientific Committee**
9:05 - 9:20 AM	ASF Nanogold Biosensor Test Kit: An Alternative Decentralized ASF Detection Assay **Clarissa Yvonne J. Domingo,** *Professor,* Department of Pathobiology, Central Luzon State University
9:20 - 9:35 AM	Effect of High Temperature Exposure and Laboratory Processing Techniques on the Diagnostic Performance of Dry Swabs for the Detection of ASFV **David T. Williams,** *Diagnostics and Mammalian Infectious Diseases Research Group Leader,* CSIRO Australian Centre for Disease Preparedness
9:35 - 9:50 AM	Detection of ASFV Infection in Pigs using Clinical, Environmental, and Non-invasive Samples Through the Application of a Minimal Equipment Colorimetric LAMP PCR **Adriana Muñoz,** Institute of Agrifood Reseach and Technology, Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain
9:50 - 10:00 AM	**Coffee**
10:00 - 10:30 AM	Introduction to the Gap Analysis Model **Erika Chenais**, *Associate Professor,* National Veterinary Institute **Karl Stahl**, *State Epizootiologist and Department Head*, National Veterinary Institute, SVA
10:30 - 11:30 AM	**Gap Analysis – Epidemiology and Diagnostics – Part 1**
11:30 - 11:40 AM	**Break**
11:40 AM - 1:30 PM	**Gap Analysis – Epidemiology and Diagnostics – Part 2**
1:30 - 2:30 PM	**Lunch**
2:30 - 3:20 PM	Updates on the Center of Excellence for African Swine Fever: Fixing the Genotyping Problem in ASFV **Douglas Gladue**, *Senior Scientist*, Plum Island Animal Disease Center (PIADC), USDA-ARS
3:20 - 4:00 PM	**Coffee**

4:00 - 4:15 PM	Assessment of Virological Response, Survival Rates and Persistent Infection of Pigs following Natural Field African Swine Fever Virus Exposure **Dachrit Nilubol,** *Assistant Professor,* Chulalongkorn University
4:15 - 4:30 PM	Whole Genome Sequences of African Swine Fever Virus from Outbreaks in Several Provinces in Luzon and Mindanao, Philippines, from 2020-2022 **Andrew D. Montecillo,** *Assistant Professor/PhD Researcher*, University of the Philippines Los Baños, and BioAssets Corporation, Philippines
4:30 - 4:45 PM	New Insights into Transcriptional Dysregulation Following Infection of Domestic Pigs with Moderately Virulent African Swine Fever Virus **Samantha Davis,** *Postdoctoral Researcher,* Commonwealth Scientific and Industrial Research Organization (CSIRO)
4:45 - 5:00 PM	**Wrap Up** *Please stay at the end of this session for a group photo.*
5:00 - 6:30 PM	**Break**
6:30 - 7:15 PM	**Dinner Keynote: African Swine Fever – From Humble Beginnings to Pandemic** **Mary Louise Penrith,** *Extraordinary Professor,* Department of Veterinary Tropical Diseases, University of Pretoria
7:15 - 7:25 PM	**Cultural Performance**
7:25 - 9:15 PM	**Dinner** *Craft Beer provided by BioAssets Corporation.*

Day 3: Thursday, December 7, 2023

9:00 - 9:10 AM	Introduction to Day 3 **GARA Scientific Committee**
9:10 - 9:40 AM	China Country Report Updates on ASFV Virology from China **Dongming Zhao**, *Professor,* Chinese Academy of Agricultural Sciences
9:40 - 10:10 AM	State-of-the-art ASF Vaccines **Douglas Gladue**, *Senior Scientist*, Plum Island Animal Disease Center (PIADC), USDA-ARS
10:10 -10:40 AM	ASF Vaccine International Standards **Cyril Gay**, *Senior National Program Leader*, Animal Production and Protection, USDA-ARS
10:40 - 10:55 AM	AVAC ASF Live Vaccine the Key Solution to African Swine Fever **Nguyen Van Diep**, *CEO,* AVAC Vietnam Joint Stock Company
10:55 - 11:05 AM	**Coffee**
11:05 - 11:30 AM	NAVET-ASFVAC: A Safe and Highly Effective Vaccine for Preventing African Swine Fever **Trần Xuân Hạnh,** Navetco
11:30 - 11:45 AM	African Swine Fever Live Attenuated Virus Vaccine Safety, Efficacy and Evaluation in 3-Week and 6-week-old Pigs **Rachel Madera,** *Senior Research Scientist/Lab Manager,* Kansas State University
11:45 AM - 12:00 PM	Characterization of far-distant African Swine Fever Challenge Models. The Case of Ghana2014, Benin97/1, Georgia10, and Kenya1033 **Anna Lacasta-Marin,** *Animal and Human Health Scientist,* International Livestock Research Institute
12:05 - 12:15 PM	**Poster Viewing**
12:15 - 1:15 PM	**Lunch**
1:15 - 3:15 PM	**Gap Analysis – Vaccines and Virology**
3:15 - 3:25 PM	**Coffee**
3:25 - 4:45 PM	**Discussion and Wrap Up**

Speaker Information

Aruna Ambagala, *Mammalian Diseases Unit Head,* National Centre for Foreign Animal Diseases, Canadian Food Inspection Agency

State-of-the-art Diagnostics - Oral Fluid as an Aggregate Sample Type Early of African Swine Fever: Results from Field Studies in Vietnam

Samuel Joseph M. Castro, *Veterinarian III,* Bureau of Animal Industry – ASF Task Force

Philippines Country Report

Erika Chenais, *Associate Professor*, National Veterinary Institute

State-of-the-art ASF Epidemiology

Introduction to the Gap Analysis Model

Samantha Davis, *Postdoctoral Researcher,* Commonwealth Scientific and Industrial Research Organization (CSIRO)

New Insights into Transcriptional Dysregulation Following Infection of Domestic Pigs with Moderately Virulent African Swine Fever Virus

Clarissa Yvonne J. Domingo, *Professor,* Department of Pathobiology, Central Luzon State University

ASF Nanogold Biosensor Test Kit: An Alternative Decentralized ASF Detection Assay

Dao Duy Tung, Vietnam National Institute of Veterinary Research

Vietnam Country Report

Cyril Gay, *Senior National Program Leader*, Animal Production and Protection, USDA-ARS

GARA Introductory Remarks

ASF Vaccine International Standards

Douglas Gladue, *Director*, Center of Excellence for African Swine Fever Genomics, and *Senior Scientist*, Plum Island Animal Disease Center (PIADC), USDA-ARS

Centers of Excellence

Updates on the Center of Excellence for African Swine Fever: Fixing the Genotyping Problem in ASFV

State-of-the-art ASF Vaccines

Trần Xuân Hạnh, Navetco

NAVET-ASFVAC: A Safe and Highly Effective Vaccine for Preventing African Swine Fever

Anna Lacasta-Marin, *Animal and Human Health Scientist,* International Livestock Research Institute

Characterization of far-distant African Swine Fever Challenge Models. The Case of Ghana2014, Benin97/1, Georgia10, and Kenya1033

Paul Limson, *Chief Veterinary Officer*, Department of Agriculture, Bureau of Animal Industry

Welcome Message

Rachel Madera, *Senior Research Scientist /Lab Manager,* Kansas State University

African Swine Fever Live Attenuated Virus Vaccine Safety, Efficacy and Evaluation in 3-Week and 6-week-old Pigs

Andrew D. Montecillo, *Assistant Professor/PhD Researcher,* University of the Philippines Los Baños and BioAssets Corporation

Whole Genome Sequences of African Swine Fever Virus from Outbreaks in Several Provinces in Luzon and Mindanao, Philippines, from 2020-2022

Adriana Muñoz, Institute of Agrifood Reseach and Technology, Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain

Detection of ASFV Infection in Pigs using Clinical, Environmental, and Non-invasive Samples Through the Application of a Minimal Equipment Colorimetric LAMP PCR

Dachrit Nilubol, *Assistant Professor,* Chulalongkorn University

Thailand Country Report

Assessment of Virological Response, Survival Rates and Persistent Infection of Pigs following Natural Field African Swine Fever Virus Exposure

Yooni Oh, *Animal Production and Health Officer,* ASF programme for Asia and the Pacific, Food and Agriculture Organization (FAO)

Update on ASF in Asia

Mary Louise Penrith, *Extraordinary Professor,* Department of Veterinary Tropical Diseases, University of Pretoria

Keynote: African Swine Fever – From Humble Beginnings to Pandemic

Dirk Pfeiffer, *Chow Tak Fung Chair Professor of One Health,* City University of Hong Kong

Enhanced Accessibility to Modeling of ASFV transmission Dynamics for Policymakers

Rachel A. Schambow, *Postdoctoral Associate,* Center for Animal Health and Food Safety, College of Veterinary Medicine, University of Minnesota

An Overview of CAHFS International Research and Activities Supporting ASF Prevention and Control and Veterinary Capacity Building

Karl Stahl, *State Epizootiologist and Department Head*, National Veterinary Institute, SVA

Introduction to the Gap Analysis Model

Le Thi Thu Ha, *Researcher,* Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)

Mapping Key Players in the Vietnamese Pig Value Chain to Inform Strategies for Improved African Swine Fever Biosecurity Regulation Adoption

Nguyen Van Diep, *CEO,* AVAC Vietnam Joint Stock Company

AVAC ASF Live Vaccine the Key Solution to African Swine Fever

Matthew Ward, *Executive Director,* Talarak Foundation

Case of ASF in the Critically Endangered Visayan Warty Pig (Sus cebrifrons)

David T. Williams, *Diagnostics and Mammalian Infectious Diseases Research Group Leader,* CSIRO Australian Centre for Disease Preparedness

Effect of High Temperature Exposure and Laboratory Processing Techniques on the Diagnostic Performance of Dry Swabs for the Detection of ASFV

Dongming Zhao, *Professor,* Chinese Academy of Agricultural Sciences

China Country Report

Updates on ASFV Virology from China

Poster Presenter Information

Zyne K. Baybay

Building a Mobile Biocontainment Laboratory to Bridge the Gaps in Animal Health Point of Need Diagnostics

Ann Catherine Y. Cabrera

Comparison of MinION Rapid Kits for Sequencing Multiple Displacement Amplification Products of African Swine Fever Virus

Cherry P. Fernandez-Colorado, Chair and Assistant Professor, University of the Philippines Los Baños

Molecular Surveillance of ASFv in Raw Meat and Processed Pork Products in the Philippines

Jean Nepomuscene Hakizimana

Updates on the African swine fever situation in Tanzania and the description of the African swine fever virus genotypes II and IX in Rwanda

Allan Michael C. Mamites

Whole Genome Sequencing of African Swine Fever Virus Whole Blood Samples from Philippines, 2019-2022 Using the Illumina Platform

James O'Dwyer

Emergence of microvariants of ASFV Genotype II in the Asia-Pacific

Mary Lyn Pelingon

Development of Optimized Protocols using Commercial Cell Lines for the Isolation of African Swine Fever Virus Field Samples in the Philippines

Muhammad Salman

The full-length genome analysis of African Swine Fever virus Genotype II from Thailand compared to other Asian countries

Le Thi Thu Ha

African Swine Fever Impacts and Control Measures on Pig Production in Vietnam since 2019: A Scoping Review

Le Van Phan

African Swine Fever Outbreaks in Vietnam during the 2019 – 2022 Period

David Williams

Comparative evaluation of qPCR diagnostic tests for the detection of African swine fever virus DNA in oral swabs, swine oral fluids and whole blood

Oral Presentation Abstracts

ASF Nanogold Biosensor Test Kit: An Alternative Decentralized ASF Detection Assay

Clarissa Yvonne J. Domingo, Central Luzon State University

The ASF Nanogold Biosensor test kit amplifies the P72 target gene using three pairs of primers designed from the Philippine ASF isolates. It includes its own in-house DNA extraction component and amplifies using a heat block set at 58oC for 30 minutes. Results are visualized using synthesized gold nanoparticles while interpretation is done with the use of a downloadable program application exclusively made for the test kit. Upon taking a photo with an android phone of the tubes after the reaction, the APP will interpret the test results to the user.

The study determined the positivity rate of ASF using the test kit on blood, oral and fecal swabs from twenty pigs and five surface swabbing from ten hauling vehicles per abattoir from seven abattoirs in 5 provinces of Region III; from raw and processed meat taken from wet markets and grocery stores; from water samples and environmental surface swabs of 532 pig farms in 11 provinces in the country; and from 26 commercial feeds bought from agricultural stores for testing. Representatives from each sample type were subjected to parallel testing with conventional PCR. Likewise, diagnostic validity of the test kit against Real-Time PCR was established.

Blood, oral and fecal swabs, raw and processed meat, water samples, environmental surface swabs and feeds had higher positivity rate using the ASF test kit as compared to conventional PCR. Using similar DNA extraction method and the forward and reverse primers of the ASF Nanogold Biosensor test kit, the diagnostic sensitivity and specificity when pitted against Real-Time PCR were 96% to 100% and 77% to 85.7%, respectively, regardless of sample type. The overall proportion agreement or accuracy of the ASF test kit, regardless of sample type, when pitted against real-time PCR, had a Kappa coefficient of 0.75 (substantial) to 0.91 (almost perfect).

Effect of high temperature exposure and laboratory processing techniques on the diagnostic performance of dry swabs for the detection of African swine fever virus

David T. Williams, CSIRO, Australian Centre for Disease Preparedness

One of the key surveillance strategies for the early detection of African swine fever (ASF) incursion into a country is sampling wild pig populations. In Australia, the remote northern regions are considered a high-risk pathway for ASF incursion due to the combination of high numbers of feral pigs and close proximity to countries where ASF is present. These regions primarily consist of isolated arid rangelands with high average environmental temperatures. We undertook a series of experiments to assess a sampling system based on dry swabs that might be distributed to land managers owners, stockpersons and recreational hunters. A specific objective of these experiments was to assess whether the high temperatures encountered in outback Australia for storage of swabs over an extended period might reduce the diagnostic sensitivity (DSe) of realtime PCR (qPCR) to detect ASF virus (ASFV). We show that storage of FLOKSwabs or Genotube swabs in incubators at either 35°C or 45°C for over 9 months did not reduce DSe, as compared to storage at room temperatures of ~22°C. A follow-on experiment to assess if high temperatures following collection of blood containing ASFV using these swabs – to simulate transport delays in between sampling and processing in a laboratory – demonstrated that DSe was not reduced. A third experiment was undertaken to assess an improved DNA extraction method for samples collected using Genotube swabs to obtain viral load quantifications more comparable to those obtained with FLOKSwabs. Taken together, this study demonstrates that dry swabs can provide the basis for an effective low-cost surveillance system for ASF in situations where extended exposure to high environmental temperatures is unavoidable.

Detection of ASFV infection in pigs using clinical, environmental, and non-invasive samples through the application of a minimal equipment colorimetric LAMP PCR

Adriana Muñoz, Institute of Agrifood Reseach and Technology, Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB)

African swine fever virus (ASFV) currently represents the biggest threat to the porcine industry worldwide, with high economic impact and severe animal health and welfare concerns. Outbreaks have occurred in Europe and Asia, since 2007, and, in 2021, ASFV was detected on Hispaniola, Caribbean. Given the lack of a global vaccination strategy against ASFV, control of the virus relies on molecular surveillance. In this work we used the recently minimal equipment colorimetric ASFV LAMP PCR test developed, to determine the kinetics of detection of ASFV Georgia strain in 20 infected pigs. A large panel of tissues, clinical and environmental samples were collected. Samples that were collected using a non-invasive protocol for the infected animals were also included. All samples were previously evaluated using the WOAH recommended qPCR. From day 3 post infection (dpi), almost in the absence of clinical signs, the presence of the ASFV DNA was detected in blood, serum, oral swabs, as well as in different tissues, including bone marrow (of great relevance for the diagnosis of the virus in wild boars). On day 5, a strong ASFV DNA load was detected in feces, as well as in the air and the walls of the pen. The detection of the ASFV DNA remained with a high load on the walls and in the air until 7 dpi. Also, at 7 dpi, a very high viral load was detected in all the samples, except in the feces. During the presentation we will discuss about the application of this diagnostic tool in a pen-side format and the detection levels per sample will be presented. Special emphasis will be placed on the type of sample that can be collected non-invasively in the infected animals, as well as on the environmental sample collection protocol and its relevance for the ASFV diagnosis.

Oral fluid as an aggregate sample type early of African Swine fever: Results from field studies in Vietnam

Aruna Ambagala, CFIA-National Centre For Foreign Animal Disease

Active surveillance based on individual sampling is labor-intensive and costly and therefore impractical for early detection of African swine fever. Pen-based aggregate oral (rope) fluid testing is a non-invasive alternative that requires significantly lower financial and human resources. Oral fluid can be collected by non-veterinary staff, require minimal resources, impose little disruption to animals, and is widely being used in North America as a routine sample type for the detection of endemic swine pathogens. Using four independent experimental studies, we previously demonstrated that the ASFV genome can be detected in oral fluids as early as 3-5 days in commercial size pig pens at ~4%–5% prevalence.

The objective of this study was to conduct a field evaluation of oral fluid for the early detection of ASFV genome in an ASF endemic country. Three independent studies (#1-3) were conducted between 2021 and 2023 in Vietnam. In all three studies, apparently-healthy fattening pigs sourced from ASF infected farms were used. Upon purchase, the pigs were moved to a clean study farm, randomly assigned to pens, and pen-based oral fluid and individual whole blood samples were collected once daily. After completing each study, the oral fluid and whole blood samples were tested by ASF specific real–time PCR. Beta actin was tested as the endogenous control in all the samples. In Study #1 (94 pigs assigned to 4 pens, A-D), the first viremic pig was detected Pen B at day 3 and the infection spread to other pens rather slowly with time. In Pen B, ASFV oral fluid samples tested positive on day 6 at 4% prevalence. In Study # 2 (177 pigs assigned to 8 pens; A-H), many pigs (20-74% prevalence) were viremic on the first day of sampling. As a result, ASFV genomic material was detected in oral fluids from all the pens starting day 1. In Study # 3 (104 pigs assigned to 4 pens, A-D), the first ASFV genome detection in oral fluid was on day 1 in Pen B at 4%, and in Pen D at 9% within-pen prevalence. The three studies provided evidence that oral fluids can be a valuable screening tool in detecting an ASF incursion within 0-3 days of initial detection of viremia in a pen. At the time of the earliest detection in oral fluids, the pigs were clinically normal and there were no mortalities in the pens.

The data from the three independent large scale field studies confirmed the findings from our previous experimental study and further supports the use of oral fluid as a reliable aggregate sample that can be used for screening commercial swine herds for early detection of ASF.

An overview of CAHFS international research and activities supporting ASF prevention and control and veterinary capacity building

Rachel A. Schambow, Center for Animal Healthy and Food Safety, College of Veterinary Medicine, University of Minnesota

This presentation provides an overview of ongoing research projects and activities conducted by the University of Minnesota-Center for Animal Health and Food Safety (CAHFS) and its international partners to support ASF prevention and control globally. Point-of-care (POC) diagnostic tests for ASF are a relatively emerging technology whose accuracy and field usefulness are not well-characterized. To address this, we are investigating the utility of the IndiField ASF POC-PCR-based platform in the Dominican Republic using blood samples from swine farms. In the Philippines, spatiotemporal analysis of ASF spread was conducted using 19,697 outbreaks from 2019-2022. Outbreaks were clustered temporally and spatially with a distinct season pattern. Following this work, risk factor analysis was performed with a workshop of Philippine veterinary experts using qualitative and quantitative methods. Findings suggested that swill or contaminated feed, insufficient biosecurity protocols, and personnel movements were top risk factors for ASF spread. Spatiotemporal and descriptive analysis has also been performed on outbreak data from the Dominican Republic collected from 2022-2023. Preliminary results suggest inadequate biosecurity and husbandry practices may contribute to clustering of the disease amongst backyard producers. From 2020-2022, CAHFS implemented ASF-specific training to the Department of Animal Health in Vietnam, training 40 participants. In 2023, ProgRESSVet-SE Asia was launched to continue training with Vietnam and 25 Philippine participants in alignment with WOAH Advanced Competencies for veterinary service professionals related to ASF data analysis, surveillance, diagnostics, and more. In the US, early detection of a theoretical ASF introduction may be difficult due to highly prevalent, similarly presenting diseases. Using conjoint analysis, responses from workshop participants were evaluated to estimate and compare the relative likelihood of seeing clinical signs/necropsy findings with ASF and Porcine Reproductive and Respiratory Syndrome outbreaks. These results will help in the development of surveillance protocols. Together, these projects support international ASF prevention and control.

Mapping key players in the Vietnamese pig value chain to inform strategies for improved African Swine Fever biosecurity regulation adoption

Le Thi Thu Ha, Centre de coopération Internationale en Recherche Agronomique pour le Développement - CIRAD

Since the outbreak of African Swine Fever (ASF) in February 2019, the Vietnamese government has implemented proactive strategies such as diagnosis, culling, disinfection, controlled pig movement, and biosecurity measures. Understanding the perspectives of stakeholders on these regulations is critical for developing recommendations to improve their implementation.

A qualitative study was conducted in Vietnam using stakeholder mapping and analysis. Based on a focal point meeting with the Department of Animal Health, two regulations, the National Plan for ASF Prevention and Control and Circular No. 24/2022/TT-BNNPTNT, outlining animal disease-free zones and establishments, were selected for study.

Between March and May 2023, thirty semi-structured interviews and two focus group discussions were conducted with representatives from governmental authorities, international organizations, cooperatives, and private sector representing the pig supply chain. The findings revealed that illegal slaughterhouses and traders were critical players in the pig supply chain, significantly contributing to the spread of ASF. Farmers typically receive advice from two

sources: local authorities, who inform them about ASF control strategies, and the private sector, which provides technical assistance on livestock production.

Farmers are motivated to follow biosecurity regulations and obtain VietGAHP certification for cost savings and improved farm sustainability (lower disease risks, increased productivity). VietGAHP elevates farms' reputation and branding, while a free-disease certificate allows pig sales during outbreaks, enhancing trade and reducing price manipulation vulnerability. Farmers face constraints during free-disease establishment construction due to limited knowledge, high costs (inspection, expert hiring), and trust issues with official vets regarding sampling and biosecurity. Due to a lack of financial and land resources, establishing a minimum safety distance between farms and residential areas is difficult. Furthermore, the registration processes for these certificates are complex but their benefits in terms of production and output are unsatisfactory, preventing farmers from obtaining them.

Enhanced accessibility to modelling of ASFV transmission dynamics for policymakers

Dirk Pfeiffer, City University of Hong Kong and Royal Veterinary College

The Epidemix modelling application was developed as a user-friendly online interface for infectious disease simulation models. It allows those involved in disease control decision-making to examine complex disease transmission dynamics. Recently, the tool, developed with reproducible open-source technology, was extended to include a dedicated model of within-farm spread of African swine fever virus (ASFV). Epidemix allows examining the pattern of spread over time within a pig farm with respect to the number of pigs in the different states of the ASFV pathogenesis process, i.e. susceptible, latently infected, infectious, recovered, or dead. It is then possible to vary assumptions about all parameters, for example, to examine the impact of new strains of ASFV on the time it takes for farmers to detect the presence of the virus in their herd since introduction based on increased morbidity or mortality. Users can assess and compare the impact of different control strategies, such as culling, or potential vaccination, once effective vaccines become available. During outbreak investigations, the model can be used to identify plausible time windows for virus introduction providing valuable information to inform backward and forward tracing activities. Moreover, Epidemix assists in determining trigger values for excess morbidity and mortality, which, in turn, inform on-farm ASFV surveillance efforts involving molecular diagnostic testing as distinct from clinical observation. To capture parameter uncertainty or variability, the model utilises a Monte Carlo simulation approach that presents a range of possible numbers of pigs in different pathogenesis states over time. This means that users will have access to both worst-case and best-case scenarios. The Epidemix interface presents outputs through tables and interactive graphs, which can be tailored to user needs. Furthermore, a case study specifically for ASFV has also been developed to guide users through potential decision-making scenarios.

Case of ASF in the critically endangered Visayan warty pig (Sus cebifrons)

Matthew Ward, Talarak Foundation Inc.

ASF hit the West Visayas region of the Philippines (Cebu, Negros and Panay islands) in 2021, bringing national concern about the impact on the regions pork industry and the endemic Visayan warty pig. The virus spread through Cebu island then Iloilo city (Panay island), bordering Negros to the East and West respectively. Although national attention was being paid to the virus, and port authorities and cities were provided with educational materials, the virus quickly spread to Negros in early 2023.

The Talarak Foundation have been active in biodiversity conservation on Negros since 2010, with captive breeding centers in two cities and a nature reserve for captive bred releases in another. The Visayan warty pig is a focal species for conservation with Talarak, being regionally endemic and critically endangered, the spread of ASF within its range is very concerning. In order to protect our captive stocks we implemented biosecurity measures, imposing strict disinfection procedures for warty pig keepers, disinfection sprays and foot-baths at site entrances, and limiting

access to pig areas. Unfortunately this was not enough, as animals from one of our breeding centers contracted the virus. After one mortality of a healthy animal, we isolated pigs from their usual breeding groups and had the rest of the stock sampled. 8 of 12 sampled pigs returned positive for ASF, with all possible measures being taken to keep negative pigs away and clean. Ultimately all 16 individuals at the site died within 12 days, with symptoms of reddening skin, lethargy, loss of appetite, apparent soreness, and occasionally respiratory difficulties, showing 24hrs prior to death. Since this incident our other locations have increased biosecurity measures drastically, limiting entry to the sites to essential personnel only, and aiming to implement stronger measures to avoid possible contamination from external food sources or wild animals.

New insights into transcriptional dysregulation following infection of domestic pigs with moderately virulent African Swine Fever virus

Samantha Davis, Commonwealth Scientific and Industrial Research Organisation

Pork industries in China and neighbouring countries have been devastated by African swine fever (ASF). Lower virulence forms of ASF have emerged in China and Europe that have caused subacute and chronic forms of disease. Knowing the systemic disease state of such strains can contribute to the development of next-generation vaccines and treatments. Our study examined the systemic transcriptome of pigs (n = 3) infected with a naturally occurring moderately virulent ASFV isolate Malta/78, using bulk RNAseq. Whole blood specimens collected on days 0, 3, 6, and 13 post-inoculation (dpi), and clinical endpoints (ED) were compared to identify significant differently expressed genes (DEGs) and GO pathways and KEGG pathways were analysed. After infection, pigs produced clinical signs and pathology consistent with ASF, with clinical endpoints at day 18 or 21. At 3 dpi, the number of significant DEGs was limited; notably, macrophage activation pathways were highly downregulated. At 6 dpi, when peak levels of viremia were found, immunomodulatory viral proteins dominated (e.g. E184L IFN signalling antagonist). Upregulation of DEGs peaked at 6 dpi, while downregulated DEGs peaked at 13 dpi, suggesting regulation of the immune responses occurred from day 13. Notably, the IL-18 and IL-1a genes were significantly downregulated from 6 dpi until ED, likely due to viral proteins which inhibit the MyD88-dependent toll-like-receptor signalling pathway. Inflammatory genes were significantly upregulated at 6 dpi onwards (e.g., CCL2, leading to upregulation of inflammatory innate immune pathways). At ED, upregulation of repair/ homeostatic pathways was observed. Many inflammatory DEGs were shared with those observed in companion studies with ASFV Georgia/07 (high virulence), but these were not upregulated in OURT88/3 (low virulence) infected pigs. Forty-six DEGs were shared with all three strains. Overall, this data further highlights the systemic dysregulation of host immune responses, driven by viral proteins and inhibition of antiviral pathways.

African swine fever live attenuated virus vaccine safety, efficacy and evaluation in 3-week and 6-week-old pigs

Rachel Madera, Kansas State University

African swine fever (ASF) virus is a high consequence swine pathogen that is currently affecting domestic pigs and feral boars in several countries. It originated in Africa in the 1920s, first reached Asia in 2018 and in 2021 reported in Dominican Republic and Haiti. ASF has never been reported in the United States and is classified as USDA Select Agent that requires research in high level biocontainment and biosecurity. ASF clinical symptoms include high fever, decrease in appetite, weakness, skin lesions, severe hemorrhage that usually leads to death with up to 100% fatality rate. Without commercially ASF vaccine currently available, it is of outmost importance to develop a safe and efficacious vaccine that is readily available in the event of ASF outbreaks. We are working on a live attenuated virus (LAV) ASF vaccine that is attenuated by serial passaging in cell culture. Preliminary studies in 3-week and 6-week-old pigs show that pigs vaccinated with ASF LAV vaccine display protective immunity upon virulent ASF Vietnam virus challenge at 4 weeks post vaccination. During the 3-week challenge period, the 3-week and 6-week-old

vaccinated pigs appear normal with no observed fever and continuous weight gain. Further studies are needed to further evaluate the safety and efficacy of this ASF LAV vaccine.

AVAC ASF Live Vaccine The Key Solution to African Swine Fever

Nguyen Van Diep, AVAC Vietnam Joint Stock Company

African swine fever (ASF) poses a significant threat, causing substantial economic losses in the global pig industry. To address this challenge, AVAC Viet Nam Joint Stock Company (AVAC) has developed the AVAC ASF LIVE vaccine from the attenuated ASF-G-ΔMGF vaccine strain propagated in a macrophage-derived cell line (DMAC). Our series of experiments and field trials demonstrated the safety and efficacy of the AVAC ASF LIVE vaccine for piglets from 4 weeks of age and older. The vaccinated piglets developed protective immunity within 2 weeks post-vaccination, and were alive and healthy when being administered a 10-fold overdose of the protective dose or after a challenge with a highly virulent virus strain 4 weeks post-vaccination. Also, the vaccinated pigs did not shed the vaccine virus. To date, more than 600,000 piglets have been vaccinated with AVAC ASF LIVE and the ELISA test revealed around 93% positivity for ASF antibodies at 4 weeks post-vaccination. In addition, the vaccine neither impacts the growth performance of the vaccinated pigs nor interferes with other vaccines. Given the safety and high efficacy as well as other advantages, the AVAC ASF LIVE is considered a key solution for controlling the African Swine Fever Virus.

Characterisation of far-distant African swine fever challenge models. The case of Ghana2014, Benin97/1, Gerogia10 and Kenya1033

Anna Lacasta-Marin, ILRI

African swine fever virus (ASFV) is the causative agent of African swine fever (ASF), a haemorrhagic pig disease which ranges in severity to a highly lethal, nearly 100% mortality rate in infected pigs. The disease is endemic in Africa but is rapidly becoming a global threat, with continuous outbreaks in Eastern Europe, Asia and the Caribbean. In Asia and Europe, only one ASFV genotype is circulating in a specific area (Genotype I in Sardinia and Genotype II in Asia and Eastern Europe). In contrast, several genotypes circulate simultaneously in sub-Saharan Africa, where the disease is endemic. Between 2005-2019, 15 genotypes were found in Tanzania and neighbouring countries. Reports from Uganda and Kenya indicated that genotype IX and X are the most found there, but Genotype II is also present. In addition, Genotype II and XV were present in Tanzania. The simultaneous presence of different genotypes poses a challenge to the control of ASFV and the development of vaccines.

Live attenuated vaccines (LAV) are very effective in protecting pigs against homologous viruses (genetically closely related); however, it is yet to be evaluated their cross-protection against other genetically far-distant ASFV strains. In line with this, one of the major gaps in ASFV research is the variability in challenge stocks, doses and routes of administration used by the different institutions. At the International Livestock Research Institute (ILRI, Kenya), we are systematically developing challenge models with a panel of far-distant ASFV isolates to be used in in vivo cross-protection experiments. Up to date, the panel includes lethal dose 100 (LD100) challenge models for Ghana2014 (genotype-I), Benin97/1 (genotype-I), Georgia10 (genotype-II) and Kenya1033 (genotype-IX). The challenges are well characterised, including the progression of clinical signs, haematology parameters, virus in blood, viral nasal shedding, and gross pathology. ILRI would like to offer well-characterised challenge models for the use of the ASFV community to harmonise the research, offering reliable and repeatable challenge experiment outcomes.

Assessment of virological response, survival rates and persistent infection of pigs following natural field African swine fever virus exposure

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African Swine fever (ASF) is a haemorrhagic disease characterized with high mortality. ASF has spread throughout the Asian continent. Following an outbreak, mortality is varied. Convalescent sows have ASF detected in weaned pigs. The objectives of the study were to determine virological response and the survival rate of pigs following natural field ASF exposure. Shedding period and the presence of ASF in organs of pigs survived from natural field ASF exposure were additionally evaluated. In the study, a population of 200 female pigs, at approximately 40 kilograms BW, procured from an ASFV-free source, were introduced into a herd currently experiencing an ASF outbreak. Upon arrival, all pigs were housed in one building, tunnel ventilated, and randomly allocated into 10 pens of 20 pigs each. Following a week of introduction, pigs were subsequently exposed to ASFV under natural field conditions. Blood samples were collected weekly for 19 weeks post exposure (WPE) and assayed for the presence of ASF DNA by realtime PCR against P72 gene. Survival analysis was performed on weekly basis. Sentinel pigs were introduced at 4, 8, 12 and 16 (WPE). At 19 WPE, one pig per pen was randomly selected and necropsy was performed. Organs including tonsil, spleen, lymph node and ovary were collected and assayed for the presence of ASF DNA by realtime PCR against P72 gene. Following 2 WPE, all pigs have detectable viremia, and mortality was observed. Mortality stopped at 3 WPE, and the average survival rate at 3 WPE was 35% (highest: 70%; lowest:0%). None of pigs died after 3 WPE. Sentinel pigs introduced developed clinical disease. ASFV was detected in 100%, 40%, 40% and 20% of tonsil, spleen, lymph node and ovaries, respectively. The results of the study demonstrated that pigs following infection can develop into a carrier state.

Whole genome sequences of African Swine Fever virus from outbreaks in several provinces in Luzon and in Mindanao, Philippines from 2020 to 2022

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African Swine Fever (ASF) outbreak was first recorded in the Philippines in July 2019, and it has been spreading throughout provinces in Luzon, Visayas, and in Mindanao resulting to severe economic consequences for the swine industry. Here we present our collaborative work on whole-genome sequencing of ASF virus field strains from outbreaks in several provinces in Luzon and in Mindanao from 2020 to 2022. The coding-complete genomes were sequenced using the rapid PCR barcoding kit (RPB004) on a minION mk1b R9.4.1 flowcell or through the tiled amplicon sequencing protocol using 32 primer pairs on nanopore sequencing machine. Scaffolds were generated using the LILO pipeline and gaps due to primer dropouts were identified and were filled in by targeting the drop out regions with individual primer pairs. The first de novo assembled ASFV whole genome is 192,377 bp long and belongs to the clade with ASFV genotype II strains from Timor-Leste, China, Ukraine, Belgium, and Poland. Locally circulating strains can be classified as CD2v serogroup 8, CVR subtype I, and IGR variant II. Conserved and variable genome sites may be used in tracing the spread and evolution of the circulating strains. Genomic epidemiology of ASFV plays a crucial role in disease surveillance, control, and local prevention efforts. It provides valuable insights into the virus's evolution, transmission patterns, and potential sources, ultimately aiding in the protection of swine populations and the preservation of the swine industry in the country.

Poster Presentation Abstracts

Building a Mobile Biocontainment Laboratory to Bridge the Gaps in Animal Health Point of Need Diagnostics

Zyne K. Baybay, BioAssets Corporation

Rapid and efficient diagnostic testing and surveillance are necessary to prevent or minimize the effects of infectious diseases among livestock animals that continue to threaten the global food security. To aid our country's lack of point-of-need diagnostics for animal diseases, BioAssets has built the first mobile biocontainment laboratory (MBL) unit in the country, through the BRIDGES: Brisk Response through In-location Diagnostics and Gene Sequencing Project. The MBL is a viable laboratory on wheels and a resource-appropriate solution capable of deployment throughout the Philippines for surveillance and rapid diagnostic response to disease outbreaks such as African Swine Fever and Avian Influenza. The MBL's interior was retrofitted into a functional and compact high containment laboratory divided into three compartments- office, analytical and biocontainment areas. Through a risk and evidence-based approach, the MBL's workflow covers sample drop-off and receipt, sample inactivation, on-site testing, on-site analysis, and release of results. Moreover, initial testing activities conducted using the MBL showed its potential to be a stand-alone, self-sufficient laboratory. Its features will support continuity of operation, capacity building and research activities while maintaining biosafety and biosecurity. By reaching remote and resource-limited areas, the MBL will enable and empower veterinarians, farmers, researchers, and diagnosticians to rapidly respond to disease outbreaks.

Comparison of MinION Rapid Kits for Sequencing Multiple Displacement Amplification Products of African Swine Fever Virus

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African swine fever virus (ASFV) causes African swine fever (ASF), a highly contagious and lethal disease affecting both wild and domestic pigs. With no effective treatments or vaccines available, early detection is crucial for successful control measures. To support this effort, identifying a cost-effective, rapid, and reliable method for whole genome sequencing of ASFV is needed. For this study, five whole blood samples from Leyte, Philippines were pre-processed through host methylated-DNA depletion, multiple displacement amplification, and T7 endonuclease digestion to enrich viral content. Three samples were multiplexed and prepared using Rapid Barcoding Kit (SQK-RBK004 or RBK), while the remaining two were prepared using Rapid PCR Barcoding Kit (SQK-RPB004 or RPB). They were then sequenced using the R9.4.1 flow cell and MinION Mk1B device for 6 hours each. RBK resulted in 14.2 GB of data, 476.88k reads, 1.34 Gb, and N50 of 4.52 kb, while RPB produced 18 GB, 582.02k, 1.7 Gb, and 3.2 kb, respectively. De novo assembly of raw reads from the RBK run resulted in largest contig lengths of 55-199 kbp with 28-76X coverage, while RPB samples resulted in lengths of 190-193 kbp with 238-378X coverage. Notably, the RBK samples exhibited a genome coverage of 3-81% at 20X depth, whereas RPB samples achieved higher coverage of 97-98% at the same depth when aligned to the ASFV Georgia 2007/1 (Genbank FR682468.2) reference. Although RBK produced a substantial amount of data with longer read lengths and reasonable coverage, RPB generated more data with higher genome coverage in the same amount of time, albeit with shorter read lengths. Additionally, better flow cell health was observed when RPB was used, potentially extending its reusability. Further research and optimization are needed to enhance these methodologies for comprehensive ASFV whole genome sequencing.

Molecular Surveillance of ASFv in Raw Meat and Processed Pork Products in the Philippines

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African swine fever (ASF) is a hemorrhagic viral disease of domestic and wild pigs that causes almost 100% mortality. Although, the Philippines being an archipelago has a geographical advantage in terms of the risk of transmission of ASF, the disease has continuously spread throughout the country. The source of the ASF outbreak in the Philippines has been attributed to the feeding of leftover food scraps or "swill" to pigs and from frozen raw meat and other pork products. Detection of the virus in raw meat and processed pork products and the possibility of disease transmission have not been extensively studied in the Philippines. Therefore, we conducted a surveillance study and detected the presence of ASFv in raw pork meat and processed pork products from randomly selected wet markets in the Philippines. The study collected a total of 384 raw meat and 384 processed pork products from randomly selected municipalities of 21 provinces based on the current ASF zoning status of each province. DNA was extracted from the samples and qPCR assay was performed to amplify the presence of the ASFv VP72 gene, which encodes the major structural protein of ASFv. Out of 384 total raw meat samples collected, 39/384 (10.16%) samples were tested ASFv-positive using qPCR assay. On the other hand, out of 384 total processed pork products, 41/384 (10.68%) were ASF-positive. The results of this study revealed that ASFv is still present throughout the country and that there is a need to craft contingency plans on pre-slaughter inspection and testing before raw meats are used to make processed products and distributed into the markets.

Updates on the African swine fever situation in Tanzania and the description of the African swine fever virus genotypes II and IX in Rwanda

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African swine fever (ASF) is a devastating viral hemorrhagic disease caused by the ASF virus (ASFV) that can kill up to 100% of domestic pigs and wild boars, for which there is still no effective vaccine or treatment at this moment. The domestic pig industry in Tanzania and Rwanda is highly threatened by ASF, with several outbreaks reported yearly to the World Organization for Animal Health by respective veterinary authorities. Despite the endemic status, no ASFV isolate from Rwanda has been genetically characterized, and the ASF situation in Tanzania needs to be regularly updated. This study reports, for the first time, the ASFV genotypes causing outbreaks in Rwanda and the updated situation of ASFV in Tanzania. The ASF confirmation was performed by polymerase chain reaction (PCR) followed by molecular characterization of the causative ASFV by genome sequencing and phylogenetic reconstruction. After genetic analysis, the ASFV strains responsible for the 2021 outbreak in eastern Rwanda clustered within genotype II, while the strain from the 2023 outbreak in northern Rwanda clustered within genotype IX. On the other hand, the ASFV strains involved in reported ASF outbreaks in domestic pigs between January 2021 and August 2023 in Tanzania clustered within genotypes II, IX, and X. Of concern is the extension of the geographical distribution of genotype II in eastern Africa. This genotype was reported for the first time in Tanzania at the Tanzania-Malawi border in 2011, followed by a relentless spread of the virus northwards along major highways before it reached Rwanda in 2021. This highly virulent ASFV genotype will most likely reach other eastern African countries threatening the regional domestic pig industry. The results of this study call for science-driven and regional approaches to enable the timely identification of ASF outbreaks for effective containment and prevention.

Whole Genome Sequencing of African Swine Fever Virus Whole Blood Samples from Philippines, 2019-2022 Using the Illumina Platform

Allan Michael C. Mamites, National Institute of Molecular Biology and Biotechnology, National Institutes of Health, University of the Philippines

African Swine Fever (ASF) is a lethal transboundary disease of swine caused by African Swine Fever virus (ASFV). It was first reported in the Philippines in July 2019 and has since spread to all regions except Metro Manila. The absence of effective treatments and vaccines highlights the importance of biosurveillance and epidemiological investigations to craft informed disease management programs. Whole genome sequencing is an effective surveillance tool since it provides crucial information on viral evolution and transmission, but such data on ASFV is currently lacking in the country. To address this, we sequenced 50 ASFV whole blood samples biobanked in the Bureau of Animal Industry - Animal Disease Diagnostic Reference Laboratory using the Illumina platform. The samples were collected from 23 provinces across 10 regions from 2019 to 2022. In brief, methylated host DNA was depleted from total nucleic acid extract then the remaining microbial DNA was subjected to multiple displacement amplification prior to sequencing. Out of 50 samples, 49 have acceptable genome coverage of >10X depth and >90% breadth when reads were mapped against ASFV Georgia 2007/1 reference genome. Draft de novo assembly yielded an average of three contigs with an average 99% genome fraction. The average length of the assembled largest contig is 184 kbp with 159 mean annotated genes. Moving forward, we intend to perform hybrid assembly with Oxford Nanopore sequencing and in-depth characterization such as analysis of molecular targets, phylogenomic analysis, and variant calling. To our knowledge, this is the largest repository of Philippine ASFV sequences and is expected to be a valuable resource for further studies of ASFV in the country.

Emergence of microvariants of ASFV Genotype II in the Asia-Pacific

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African Swine Fever Virus (ASFV) is a highly stable DNA virus showing little genetic variation among genomes, particularly within each defined genotype of the species. This genetic stability has often posed challenges in tracking variants and identifying potential transmission chains as ASFV spreads into new regions. This limitation arises from the reduced ability of targeted gene amplification techniques in detecting microevolution. While mutations within individual sequenced genes are infrequent, the application of whole genome sequencing enables the identification of neutral and functional mutations across the entire genome, contributing to a deeper understanding of ASFV evolution and epidemiology. In this study, we sequenced 25 ASFV genomes collected from Viet Nam, Timor-Leste, and Papua New Guinea, classifying mutations across ASFV genes and non-coding regions while identifying mutations which may act as candidates for geographic based population genetic structuring. Overall, ASFV samples showed >99.8% genetic similarity to the Georgia 2007/8 outbreak strain. Nonetheless, emergent genetic clusters rooted in geographic location were apparent. Each cluster appeared to be driven by a relatively small number of mutations exclusively detected within samples from a single country, suggesting ongoing microevolution within ASFV as it spread throughout the Asia-Pacific region. Analysis of coding regions within the ASFV sequences revealed several point mutations resulting in variations to transcribed proteins, however, only one mutation for a single sample was detected in any gene commonly used in ASFV strain classifications. This work highlights the benefits of incorporating whole genome sequencing into ongoing ASFV surveillance to better reflect the natural population genetic structuring occurring within the species as it has spread across the Asia-Pacific region.

Development of Optimized Protocols using Commercial Cell Lines for the Isolation of African Swine Fever Virus Field Samples in the Philippines

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African Swine Fever (ASF) is a highly lethal viral disease causing devastating mortality in wild and domestic pigs. With no treatment or vaccine available, outbreaks have since spread worldwide, affecting several regions in Europe and Asia, including the Philippines. It is considered a major socio-economic concern in the pig industry, attributing to significant detrimental impacts on animal welfare and the livelihood of farmers. Due to this, it is critical to strengthen our surveillance efforts, epidemiological investigations, and pathogen detection. In the Philippines, research studies on African Swine Fever Virus (ASFV) are limited to the detection of viral genomes. Diagnosis from field samples is conducted using real-time PCR rather than handling the live infectious virus as recommended by the World Organization for Animal Health. Virus isolation is an essential laboratory tool for confirming active infection, obtaining virus stocks, viral pathogenesis, vaccine development and downstream analysis such as whole genome characterization. Currently, the isolation of ASFV from field isolates requires the use of primary cells. It is known to predominantly infect macrophages and monocytes without significant genetic modifications and loss of immunogenicity of the adapted strain. However, replication of ASFV to this culture is labor-intensive and time-consuming. Consequently, numerous efforts have been made to replicate these virulent isolates in competent ASFV-susceptible cell lines. The Institute of Molecular Biology and Biotechnology of the National Institutes of Health - UP Manila aims to establish sustainable and standardized protocols for the adaptation of ASFV field samples using MA-104 and 293T cell lines.

The full-length genome analysis of African Swine Fever virus Genotype II from Thailand compared to other Asian countries

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African swine fever (ASF) is an acute hemorrhagic viral disease of pigs caused by African swine fever virus (ASFV). Global spread of this double stranded DNA virus has serious impact on pig industry. Variance in genomic sequences have an important role in outbreaks and uncontrolled transmission of the virus. In the current report, the full-length genome of 2 Thai ASF were compared to a total 48 whole genome sequences of Genotype II strains related to Asian countries i.e. Georgia, China, Russia, Korea, Vietnam, India, Philippines. The FASTA format was downloaded from Genbank, where, some genomes were negative stranded i.e. Names, hence the direction of the sequences was corrected before alignment. While analyzing whole genome sequencing via MAFFT. Genome-wide diversity of ASFV genomes was explored by pairwise sequence similarity comparison and ORF distribution comparison. The complete, genomic sequences of these isolates were compared against Reference strain from Georgia (FR682468.2) ASFV genomes, annotated in the NCBI database. The obtained sequences were ranging from 171,046 bp to 192,664 bp long and encoded 187–190 open reading frames (ORFs). The isolates were grouped within genotype II and showed 99.941 to 99.956% nucleotide identity to the Georgia 2007/1 reference strain. Out of all Asian strains, about 25 open reading frames (ORF) showed single nucleotide polymorphisms (SNPs). Thai isolates showed distinct grouping with other Asian ASFVs in several genes including B646L, CP2475L, MGF 505-4R, K205R and NP419L. The results revealed variation in number of Tandem Repeat Sequence (TRS) in the intergenic region between I73R and I329L genes. Highly variable poly G or poly C were found in seven intron regions which contributed to the genome diversity. This study expanded our knowledge on the patterns of genetic diversity and evolution of ASFV, and provided valuable information for diagnosis improvement and vaccine development.

African Swine Fever Impacts and Control Measures on Pig Production in Vietnam since 2019: A Scoping Review

Le Thi Thu Ha, CIRAD

Pig farming holds a significant role within Vietnam's livestock industry and agriculture sector. However, the rapid and widespread dissemination of African Swine Fever (ASF) to all 63 provinces within five months of its initial detection in February 2019 has significantly severely harmed the pig farming industry. The ASF outbreak has not only had a negative impact on pig production but it has also exerted substantial socio-economic effects on Vietnam. This scoping review is conducted to gain a comprehensive understanding of the impacts of the ASF outbreak and the implementation of measures for prevention.

Out of 2,671 records that were systematically screened, thirty-seven met our inclusion criteria and were included in the study. Control measures and social impact were the two most common themes, highlighted in 28.98% and 26.09% of records, respectively, followed by economic/production impact (20.28%) and vaccine (4.34%). The study's findings emphasized the substantial impact of the ASF outbreak on pig production. When compared to the same period in 2018, the pig population decreased by approximately 25.5% in December 2019.

In terms of socioeconomics, it led to the expansion of the commercial and modern pig farming sectors while contracting the traditional sector at the national level. The ASF outbreaks had adverse effects on farm income, investment, earning potential, and overall quality of life. Various control measures were implemented; however, it was observed that households and small-scale farms had a limited understanding of ASF outbreaks and biosecurity practices compared to medium- and larger-scale farms.
Regarding the efforts to improve biosecurity implementation, two notable interventions have been developed and have shown positive outcomes: the FAO biosecurity model for small-scale pig farms and the Pighealth Security-X mobile app for biosecurity assessment of pig farms.

African Swine Fever Outbreaks in Vietnam during the 2019 – 2022 Period

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African swine fever (ASF) was first reported in Vietnam in February 2019, and to date, the disease is widespread and circulating in the pig population. In this study, the VNUA/HY/ASF1 strain isolated from the first ASF outbreak in Vietnam was used to infect 10 eight-week-old pigs orally with 103 HAD50 per animal. Results showed that all 10 pigs exhibited acute or subacute clinical signs and died of infection between 10 and 27 (19.8 ± 4.66) days post-inoculation (dpi). The first clinical signs occurred between 4 and 14 dpi. Viremia was observed in pigs between 6–16 dpi (11.2 ± 3.55). Molecular characterization of 319 samples of ASF-positive pigs collected in 35 different provinces of Vietnam from 2019 to 2022 showed that all ASF virus strains collected belonged to genotype II (p72 and p54), serotype 8 (CD2v), and variant I (CVR). Further analysis of the intergenic region between the I73R and I329L genes showed that the ASF virus strains circulating in Vietnam belonged to four different variants: IGR I, IGR II, IGR III, and IGR IV. The most common variant was IGR II (257/319; 80.56%), which was also present in samples from all provinces, followed by variant IGR I (54/319, 16.93%), IGR III (6/319, 1.88%), and IGR IV (2/319, 0.63%). Variant IGR IV was a new variant discovered for the first time in Vietnam and Asia.

Comparative evaluation of qPCR diagnostic tests for the detection of African swine fever virus DNA in oral swabs, swine oral fluids and whole blood

David Williams, CSIRO, Australian Centre for Disease Preparedness

Evaluation and validation of African swine fever (ASF) PCR tests is essential for reliable pathogen detection. Moreover, biological specimens should also be evaluated for "fit for purpose" when being considered for diagnostic testing. Our primary objective was to determine the relative sensitivity of ASF virus (ASFV) DNA detection in swine oral fluids (SOF), oral swabs (OS) and whole blood samples using the USA National Animal Health Laboratory Network (NAHLN) extraction protocol and ASFV qPCR assay. Four commercial ASF PCR assays were also evaluated: (i) IDEXX Real PCR ASFV DNA test; (ii) Ingenasa INgene qPPA; (iii) Applied Biosystems VetMAX ASF Detection Kit; and (iv) Indical Biosciences Virotype ASFV PCR kit. Experimental challenge trials using domestic swine were performed using genotype 2 ASFV to produce study samples. Pigs received either high (>300 HAD50/TCID50) or low (<200 HAD50/TCID50) dose ASFV. Samples were collected daily or every other day until clinical endpoints. OS samples were positive from 3-5 DPC, with positive rates ranging from 0-20% in the low-dose group to 100% in the high-dose group by 5 DPC. SOF samples from the low-dose pigs were positive at 5 DPC (50% of pigs), while 3 DPC SOF were negative. SOF samples from high-dose groups were weakly positive at 3 DPC (0-33%) and 5 DPC (66%). Nearly all OS and SOF samples (75-100%) from both groups were positive by 7 DPC, when pigs began to develop severe disease. A total of 364 blood, 158 SOF and 424 OS samples were tested using commercial PCR kits. High levels of sensitivity (>96%) and specificity (>98%) were found for all kits using whole blood. For OS samples, 2 of 4 kits had Se ~94% (Ingenasa and VetMax) and two had reduced Se of 88 and 83% (IDEXX and Indical, respectively); Sp was 98-100% for all kits. The Se for ASFV detection in SOF was lower for all commercial assays (92% Ingenasa, 87% VetMax, 85% IDEXX, 76% Indical); specificities ranged from 93-100%. The use of blood samples and any of the 5 PCR protocols evaluated in this study will most likely offer the most sensitive and rapid detection of ASFV in an outbreak situation. However, for surveillance purposes, blood, OS and SOF testing using the commercial kits evaluated here may provide a suitable approach particularly when testing sick animals with high viremia and oral shedding of virus.