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Global African Swine Fever
Research Alliance

Abstract Book

Contents

ORAL PRESENTATIONS

Epidemiology

Veterinary Anthropology? Searching for New Departures in Animal Disease Control

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Infectious animal diseases are often seen as dominantly in the epistemic and managerial jurisdiction of epizootology. In this talk I will propose that social sciences and humanities in general and social anthropology in particular can play a crucial role in both understanding and control of those diseases. Using African swine fever as an example, I will argue that the 'soft' anthropological knowledge-making method of ethnography, based on long-term participant observation, generates deep insights into why wild boar and its habitat became an ASF virus reservoir in Europe. Focusing on the Czech practice of game animal feeding, I will expose central-European hunting as complexly nested in metabolic environments of post-socialist industrialized monoculture, while recognising both, the effective and affective dimensions of feeding and how the two translate into the identity of the hunter and the prey-to-be. Taking the local hunting cultures seriously, I will demonstrate how social anthropology proves instrumental for analysis of and intervention in the fourth epidemiological cycle of African swine fever and speculate about its further potential to inspire fresh departures in animal disease control.

Provided as a Keynote Presentation

Participatory Modelling meets African swine fever – Systems Thinking in Action

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Since September 2020, African Swine Fever (ASF) has been present in wild boar in Germany. Despite intensive efforts, cases continue to emerge in affected regions of eastern Germany. Since June 2024, Hesse has also been affected, a federal state far from the previously identified epidemiological front. This development increases the risk of transmission to domestic pig populations, potentially causing severe socio-economic consequences.

Combating ASF is complex, also due to the necessary interaction between various stakeholders with differing priorities and perspectives, complicating collaboration and effective measures.
To better manage this complexity, systems thinking and participatory modeling can be applied, offering a holistic approach to analyze and solve complex problems. These methods identify and visualize system components and interactions while integrating diverse perspectives and expertise.

A series of participatory workshops using these methods took place in both ASF-affected and non-affected areas in Germany. The study aimed to highlight the complexity of ASF control in wild boar and identify starting points for more effective strategies. Participants from sectors including public institutions, forestry and hunting, nature and animal protection, and agriculture developed impact networks and stock-flow diagrams to represent and quantify dependencies. The workshops also explored unintended effects of current control measures and potential solutions.

The results demonstrated that improving communication and collaboration among stakeholders is crucial. Effective ASF response requires coordinated efforts and public awareness. Further research is needed to evaluate control strategies, emphasizing the importance of diverse perspectives to fully address ASF management challenges.

African Swine Fever: an update of the epidemiological situation in Italy.

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Italy experienced African Swine Fever (ASF) genotype I disease from 1978 to 2024, that involved domestic pigs, illegal-free range pigs and wild boars in Sardinia. In 2022, Italy notified the ASF genotype II disease. Currently ASF involves 4 clusters in 9 regions and 17 provinces. Infection is still present in the wild boar population in some of these areas and is sporadically notified in domestic pig farms.

In Italy, a national passive surveillance plan, for early detection in wild boar population and domestic pig farms in ASF free area, has been in force since 2020. In affected territories, the eradication plans require enhanced passive surveillance, improvement of biosecurity and management of the wild boar population. A national information system (VETINFO) enables portal tracking of all surveillance and eradication data of ASF at the central/regional/local level.

Currently, the largest and most worrisome cluster is the northwestern one, due to its contiguity with the most relevant areas for national pig production and its tendency to become larger: the spread of infection in the wild boar population is ongoing to the southeast (Tuscany, Emilia Romagna) and to the north (Ticino Park). Two epidemic waves occurred in domestic pigs in 2023 (Lombardy) and 2024 (Piedmont, Lombardy, Emilia Romagna). Latium data support the hypothesis that ASF is now eradicated. In Campania, the infection is still active in the wild boar population, but remains geographically confined. In Calabria, there is epidemiological silence, but surveillance data are sparse. Finally, in Sardinia, ASF-free status was achieved in September 2024, applying a strong eradication strategy based on increased passive surveillance.

The Italian epidemiological scenario for ASFV genotype II appears definitively challenging. ASF eradication in Sardinia is a goal achieved after some decades of endemicity.

Perceptions and experiences of hunters involved in the management of the first African swine fever outbreak in Sweden

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In September 2023, African swine fever was confirmed for the first time in Sweden in wild boar. Local hunters have been key actors both for the detection and the management of the outbreak: the disease was identified when hunters noticed ill and dead boars in the area, reported this and sent in samples to the Swedish Veterinary Agency. The hunters were thereafter mobilized to search for wild boar carcasses to identify the extent of the affected area. During the first months of the outbreak, more than 500 local hunters were involved in the search activity, and in total >90 carcasses (or parts thereof) were found. Local hunters were also mobilized for culling of wild boar in the infected zone. This paper presents results from interviews with groups of local hunters in the affected area who have been involved in the identification and management of African swine fever.

The interviews were semi-structured group interviews and revolved around three themes: perceptions and experiences of African swine fever before, during and after the outbreak. Main themes have been identified.

All the hunters described taking part in managing the outbreak, and that this had been very important to them, especially for the specific area where they usually hunt. The main motivation for their commitment was to make the area free of African swine fever to be able to hunt again. Several interviewees described hunting as extremely important for them - as a lifestyle. Hunting wild boar was described as a major and important part of their hunting, which they before the outbreak maintained through hunting with bait. While the outbreak of African swine fever and the related restrictions were described as stressful and emotionally difficult, the hunters had mainly positive experiences from their activity in managing the outbreak. They described deepening the relations in their own hunting group as well as getting to know other groups of hunters. They also described gaining new knowledge about their hunting ground and feeling proud of their commitment. However, some hunters also described feeling stigmatized and viewed as contagious by hunters from other areas, and sometimes being reproached by other non-hunting locals as responsible for the outbreak since they had fed the wild boar at the baiting stations.

Innovative barrier strategies and prioritization tools for controlling ASF spread in Italy

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The first case of ASF genotype II in Italian wild boar dates to January 2022, while the first outbreak in a domestic pig farm was notified in August 2023. Early identification of an epidemic infectious disease is a critical step toward implementing effective interventions. Key measures for controlling and eradicating ASF, as well as preventing its further spread, include the placement of barriers, depopulation, and removal of wild boar carcasses. Eradication of ASF in wild boar within the EU has been achieved where barriers were effectively implemented. The Italian strategy focuses on "perimeter fences", including both newly constructed barriers and pre-existing structures such as motorways with the aim of containing the disease and reducing the probability of its spread. However, the construction of barriers presents numerous challenges such as: construction details, differences in animal behavior, animal contact along fences, alignment with natural barriers, and integration with human infrastructure. Therefore, construction must be tailored to the epidemiological situation, particularly the progress of the epidemic front. Given the scope of activities required to secure motorway closures, a comprehensive survey of gates along these motorways was conducted to identify and classify critical points. A prioritization analysis was carried out to determine which sections should be addressed first to support the disease containment. The analysis utilized a standardized composite index (score) based on key factors: gate suitability for the presence of wild boar (based on land characteristics), width of the gap, and distance from positive cases (within a radius of 10 km around the gap). The variables were standardized and combined to develop a prioritization score. A monitoring system is now in place to verify and oversee the closure of motorway gates. Currently the system provides an important and innovative tool for controlling the disease and managing the advancement of the epidemic front.

Biosecurity and knowledge gaps identified in African swine fewer (ASF)-affected small scale pig farms in Serbia

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The first case of African swine fewer (ASF) in a domestic pig population in Serbia was confirmed in 2019. Since then, in the six-year period of disease presence, the largest number of ASF-outbreaks in the domestic pig population was detected in 2023 (in total 3078). Analysing the structure and size of affected units, the largest number was in the small scale pig farms category. The aim of this study was to identify the biosecurity and knowledge gaps as risk factors for ASF spreading in small scale farms. The study was conducted on 86 small scale pig farms with confirmed ASF outbreaks during the period 2023-2024. Farms visits and biosecurity control checks were related to ASF outbreaks as part of the official epidemiological investigation. The results clearly indicate that an important characteristic of small-scale farms in Serbia is low of level biosecurity and farmers misunderstanding the concept of biosecurity. The most common characteristics of these farms are low number of breeding animals and fattener production, non-professional farm management, home slaughtering and production of home-made meat products. In addition, natural mating with breeding boars is frequent. It should be stressed that humans were frequently recognized as the decisive and nature-independent factor that often unintentionally contributes to the ASF spread. The high density of small scale farms in combination with customs, traditions, and human mind-set implicated in pig production pose the biggest threat for ASF spread to domestic pigs in Serbia. Overall, biosecurity measures on the small scale pig farms were low and efforts should focus on strengthening external biosecurity, particularly measures related to human-related activities and visitors. Conclusions from field interviews suggested that small scale farmers have limited knowledge on the concept of biosecurity. Training programs should take into consideration socio-cultural aspects and the economic feasibility of the proposed measures.

African Swine Fever in the Pacific

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Following the emergence of African swine fever (ASF) in China in 2018, the ASF virus spread rapidly throughout the country and into southeast Asia. In March 2020, ASF emerged for the first time in Papua New Guinea (PNG), signalling its introduction into the Western Pacific. The index outbreak occurred in villages of the Mendi Munihu district of the Southern Highlands province with subsequent detections in five other Highland provinces (Enga, Hela, Western Highlands, Jiwaka and Simbu). Pigs represent an important cultural and currency animal in PNG and the emergence and ongoing circulation of ASF in the Highlands provinces, where pigs are highly valued, is a threat to food security, local economies and cultural practices. The extensive road network in disease areas and movement of pigs for traditional obligations were identified as major factors in the initial spread. The initial outbreak response and subsequent delimiting survey by PNG authorities paved the way for the current zoning and compartmentalisation strategy to contain the spread of ASF. Genotyping of ASFV positive samples collected between 2020 and 2023 indicated that they belong to p72 genotype II, IGR type II, CD2v serogroup 8, and for all but two samples the CVR subgroup was XXXII/CVR1. A 12-nucleotide deletion in the CVR was observed in the remaining two samples, encoding the tetramer repeat sequence of BNDBNDBAL, which has no associated CVR subgroup classification. Whole genome sequencing and phylogenetic analysis of the PNG samples revealed a high level of similarity (>99.8%) to the genotype II Georgia/07 reference sequence (NC_044959.2). Nonetheless, most PNG genomes clustered together, forming a distinct, country-specific monophyletic clade. Notably, two genomes were found outside of this clade and showed phylogenetic structure closer to other ASFV genomes reported from other countries in the Asia-Pacific region. Four mutations were found in over 75% of ASFV genomes from PNG that were not present in those analysed from other Southeast Asian countries. This work highlights the benefits of incorporating whole genome sequencing into ongoing ASFV surveillance to better reflect the natural population genetic structuring within the species as it spreads and evolves across the Asia-Pacific region.

Genomic analysis of putative *Potamochoerus* spp. x domestic pig hybrids in West Africa and Madagascar

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In many rural areas of Sub-Saharan Africa and Madagascar domestic pigs (*Sus scrofa domesticus*) coexist in sympatry with *Potamochoerus* spp. (buhspigs and red river hogs) and cases of hybridization between the two suid species are occasionally reported locally and in the literature. The occurrence of such hybrids could have important implications in terms of African swine fever (ASF) transmission and pig resistance to the virus. However, confirmation of the genetic introgression of those hybrids has never been sufficiently investigated to date. In this study, we investigated the genomic introgression of suspected domestic pigs (DP) and *Potamochoerus* spp hybrids reported in rural areas from Benin and Madagascar. A total of 96 samples of individuals representing *Sus scrofa domesticus* (n=37), *P. larvatus* (n=17), *P. porcus* (n=19) and putative hybrids (n=22) from different origins were collected and genotyped using 70K SNPs chip. The resulting genomic data were compared by principal component and admixture analysis. Our results revealed the absence of genetic introgression of *Potamochoerus* spp. genes in the selected putative hybrids. Moreover, they provided evidence that the two *Potamochoerus* spp. are closely related to each other but distantly related to domestic pigs. Moreover, our results revealed variable proportions of Asian genotype in local domestic pig populations (putative hybrids included) ranging from 0 to 25%, suggesting some of these putative hybrids are domestic pigs with atypical phenotypes. Our study suggests that *Potamochoerus* spp. are highly unlikely to hybridize with domestic pig species and that reports of putative hybrids from areas of sympatry are more likely to be the result of atypical pig phenotypes. These findings have implications for the management of ASF and the ability to develop DP breeds resistant to ASF virus.

Interrelationships of warthogs, *Ornithodoros* ticks and African swine fever virus in South Africa

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African swine fever virus (ASFV) causes a contagious and fatal disease of domestic pigs. In eastern and southern Africa, the virus circulates between warthogs that develop benign viremic infection and ticks of the *Ornithodoros* genus. In South Africa, the disease was first seen in the north of the country where domestic pigs had contact with warthogs, ostensibly through consumption of infected tissues or transmission through the intermediary of ticks. Outbreaks elsewhere were initiated by movement of infected pigs or pork products from the north. Consequently, a controlled area was declared in 1935 and regulations implemented to limit the spread. Over the last few decades, there has been an increase in informal pig farming in South Africa and widespread translocation of warthogs south of the controlled area. Outbreaks outside the controlled area have been increasing in domestic pig populations, in some cases with no link to the movement of infected domestic animals or products from the endemic area. We conducted sampling of warthogs and *Ornithodoros* ticks in wildlife reserves outside the traditionally infected regions and found serological evidence (>98%) of ASFV in extralimital warthogs further south in the country. ASFV was also detected in two of the four known tick species within the *Ornithodoros (Ornithodoros) moubata* complex in South Africa. One of these species was previously observed only on tortoises. Two of the three species of the *O. (O.) savignyi* complex ticks present in the country were collected and found negative for ASFV. Additionally, the only member of the subgenus *Pavlovskyella* known to occur in South Africa, *O. (P.) zumpti*, was collected from warthog burrows for the first time but was negative for ASFV as well. With these findings, we confirm the extension of sylvatic circulation of ASFV beyond the controlled area in South Africa.

An assessment of the economic impacts of ASF-induced quarantine imposition in smallholder pig value chains – a system dynamics approach

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Pig production in Uganda is constrained by endemic occurrence of African swine fever (ASF). Current measures taken by the Government of Uganda for controlling ASF outbreaks include quarantines followed by trade and livestock movement restrictions. Little is known about the actions taken by and the impact on different stakeholders in the pig value chain in response to ASF quarantines. This study describes actions taken by different stakeholders along the pig value chain, the perceived economic impact, and a cost–benefit analysis of different scenarios of ASF quarantine protocols.

Initial data were collected from ten focus group discussions (FGDs) using participatory epidemiology tools and two key informant interviews (KIIs) with District Veterinary Officers (DVO) of Kisoro and Moyo districts in Uganda. These data formed the basis for implementing a participatory group model building (GMB) approach to parameterize a system dynamics (SD) model of the pig value chain for scenario analysis. This involved guided discussions with 14 stakeholders from the pig value chain to jointly develop models, generate parameters, and validate output of model simulation.

The results from the FGDs and KIIs show that during ASF quarantine, pig value chain actors shifted their activities from formal places (livestock markets, slaughter slabs, pork butcheries and pork joints) to informal places (farmer homesteads). Farmers were perceived the most economically affected stakeholder group. Implementing biosecurity under different scenarios with the SD model, such as no ASF outbreak, ASF outbreak, and ASF outbreak with quarantine imposition highlights variations in profit margins across stakeholders.

The continued trade in pigs and pig products in informal marketplaces suggests that quarantine might not be effective for hindering activities that might spread ASF in these settings.

The perceived economic losses provide an insight into the negative economic impact of the quarantine for the different stakeholders.

Insights into the epidemiology of ASF in emerging scenarios: lessons learned in the Philippines and the Dominican Republic

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African swine fever (ASF) epidemiology is highly complex, in part due to the influence of anthropogenic and socioeconomic factors that may vary greatly in different settings. Country-specific context including the structure of the swine industry and value chain, available resources for control, and ecological factors all strongly influence ASF's spread and impact, especially in emerging scenarios of the disease. This complexity has been exemplified in both the Philippines and the Dominican Republic (DR), which have been affected with ASF since 2019 and 2021 respectively. Here, we present results from our extensive field work in these countries and identify important factors contributing to ASF's epidemiology. Data spanning multiple years of the outbreaks were received from DIGEGA and BAI, the official veterinary services of the DR and Philippines respectively, and collected from affected farms. Methods to evaluate data included spatiotemporal analysis, cluster analysis, and estimation of time-dependent reproductive ratio (Rt). To complement sometimes limited or hard-to-document field data, the experiences of swine veterinarians were formally captured and analyzed using semi-quantitative and qualitative activities at in-person workshops. In both countries, outbreaks continue to be geographically widespread and occur mainly in backyard farms. In the DR, Rt fluctuated around 1 from 2022-2024 and was highest on June 6, 2023 (Rt=3.23, 95%PI: 1.0-7.0). In the Philippines, Rt was above 8 in 2019, then sharply decreased and fluctuated around 1 from 2021-2022, suggesting a shift toward an endemic trend. Other key findings include the importance of human-mediated factors in ASF transmission and the need to identify appropriate incentives for swine actors. Overall, these findings provide key insights into the epidemiology of ASF in emerging scenarios and important information for developing ASF control strategies. We thank BAI, DIGEGA, USDA ARS, and USDA APHIS for their support of this work.

Range modeling and surveillance of Ornithodoros turicata: implications for detecting African Swine Fever virus in the US

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African Swine Fever virus (ASFV) is a re-emerging global swine disease that, if introduced to the US, would cause severe economic consequences. The widespread presence of feral hogs in addition to the presence of competent tick vectors, specifically *Ornithodoros turicata*, foster a greater risk of ASFV establishment, especially in Texas. The specific aims of this study were to assess the geographic distribution of *Ornithodoros* spp. along the US-Mexico border in Texas and in proximity to commercial and feral swine populations, to determine the potential distribution of *Ornithodoros* spp. in Texas, the spatial distribution of host species richness, and to identify localities that could be monitored for ASFv as part of a comprehensive surveillance system in the US moving forward. A systematic literature review and field collections were conducted to identify *O. turicata* localities. Ticks were collected from 15/16 surveyed counties and were identified using standard morphological keys and confirmed molecularly on a subset of ticks via amplification and sequencing of a 16S rRNA gene fragment. Ecological niche modeling was used to determine the suite of bioclimatic variables associated with the presence of *O. turicata*. Six variables of importance were identified: mean temperature of the warmest quarter, mean temperature of the coldest quarter, annual precipitation, precipitation of the wettest month, precipitation of the warmest quarter, and elevation. Subsequently a map was constructed of the potential distribution of this species, which stretched from southern California to Texas with an allopatric population in Florida. The majority of Texas with the exclusion of the easternmost quarter of the state appears to be highly suitable for this species. Establishing the current and projected distribution of *O. turicata* is essential to understanding the potential sylvatic cycle of ASFV and creating long-term surveillance zones if ASFV is introduced to the US.

*Abstract accepted but not presented at the meeting.

Quantitative risk assessment of African swine fever at the wild boar-outdoor pig farm interface

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African swine fever is a devastating disease of pigs that can be transmitted at the wild boar - domestic pig interface. Previous quantitative risk assessments focused on the introduction of African swine fever virus (ASFV) into a country. This study aimed to fill the knowledge gap in the local situation by assessing the risk of infection of a given outdoor farm from an infected wild boar population before infection is detected in wildlife. We developed a stochastic risk assessment framework considering three potential pathways of ASFV introduction into outdoor pig farms: (1) the movement of wild boar, (2) contaminated fomites due to outdoor activities of farmers, and (3) swill feeding. Each pathway was detailed and modelled through equations and a global sensitivity analysis was carried out to identify the main determinants of the risk. We estimated that if infection is spreading at prevalence 0.01 and undetected in wild boar, the probability of at least one pig to be infected in a farm keeping 50 pigs is 0.061 after 28 days. The contact with wild boars and the risk due to contaminated fomites accounted for 48.6% and 46% of the risk of new infection events, respectively, while the risk due to swill feeding was negligible in most simulations. The risk due to wild boar movement was particularly due to environmental contamination within farm facilities, while the risk related to fomites was due to both humans and dogs carrying contamination. The factors that most influenced the risk of infection were the probability of the farmer to perform activities in a contaminated area, the daily number of visits of wild boar to an outdoor farm and the rate of outdoor activities. Our findings highlights the importance of continuous surveillance activities to increase our capacity for earlier detection of ASF in the wild boar population.

Untangling the spatio-temporal spread of African Swine Fever virus using whole-genome sequences

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African Swine Fever virus (ASFV) has become a top priority for many veterinary health agencies worldwide. The current epidemic of the Genotype II ASFV, first detected in 2007 in Georgia, is having a devastating impact on the pig industry in Europe and Asia, where it has become established both in domestic pig and wild boar populations. Due to its high persistence and mortality rate, disease control policies require depopulation, fencing and other costly interventions, as a widely distributed and effective vaccine is not currently available.

The virus persistence in the environment is strongly affected by the ecological, climatic and environmental conditions. These can directly influence the infected wildlife ability to move, their carcass decomposition time, and the presence of vectors (*Ornithodoros* genus soft ticks). On the other hand, anthropic factors can also play a role. For example, transport of contaminated material over long distances can lead to new outbreaks in unaffected areas. The interplay between local and long-distance transmissions resulted in the current global epidemic.

The objectives of this research are (i) understand the global spatial-temporal dynamic of ASFV, and (ii) understand which environmental, anthropic, and ecological factors are associated with its spread at the local level.

We analysed a dataset of 228 ASFV whole-genome sequences from Africa (n=15), Asia (n=66) and Europe (n=147). We ran a phylogenetic analysis to understand the timing and provenance of new introductions. Further, using phylogeographic models, we examined the local dynamic of the outbreak in a Northern Italian wild boar population (n=53 sequences), and estimated the epidemic front-line wavefront expansion. Finally, we analysed a number of environmental and anthropic factors that might affect the outbreak dynamics.

Using the viral genomes combined with the above-described data, we were able to provide crucial insights to improve disease control in ASFV affected areas, and for active surveillance in uninfected areas.

Counting the cost: Economic scars of African swine fever outbreaks on smallholder pig farmers in Plateau state, Nigeria

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Pig farming in Nigeria, mainly constituting smallholder farms, provides opportunities for increased meat production and income generation for many poor families. African swine fever (ASF), however, continues to hinder the development of the sector. There are gaps in understanding the economic implications of outbreaks at household level. This study evaluated the economic consequences of ASF outbreaks at household level for smallholder pig farmers in Nigeria. Multistage sampling technique was used to select households (HH) from 35 villages in two pig-producing local government areas of Plateau state, where ASF is endemic. A total of 437 pig farmers with at least two years' experience were interviewed using semi-structured questionnaires administered per household in July and August 2023. Data including demography, pig production and management system, pig business, contribution to the household income, ASF experience, as well as ASF outbreak costs and impact on the household were collected. Results show that 41% of the farmers report pig farming as the major source of income, contributing 20-40% of income to their households. Most (73%) farmers had breeding pigs from mostly indigenous breeds, with an average herd size of 5 pigs. Fifty-six percent of the participants reported experience of ASF with outbreak duration of mostly 2-7 days. 85% of the affected households stated that the outbreaks induced pig mortalities with subsequent financial loss. A major loss to 82% of farmers was the drop in value of pigs to as low as 20% of pre-outbreak price. Payment of school fees was at the top (42%) of household needs impacted by ASF outbreaks. About 50% of the farmers have downscaled after ASF and only 22% report their herd sizes are back to the previous size before the outbreak. Combating ASF is critical for sustainable pig production and income stability for smallholder households

Diagnostics

African swine fever: leveraging molecular and biological virus knowledge to enhance diagnostics and control

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African swine fever (ASF) is a critical threat to the global swine industry, with no effective vaccine available in Europe. Control relies on herd culling, leading to significant economic losses and emphasizing the need for improved diagnostics. Despite advances, challenges remain, particularly the ASFV genome's low variability and large size, which complicate typing of emerging isolates and tracing outbreaks. These issues are further compounded by the increasing endemicity of ASFV in Europe and Asia, where less virulent strains with nonspecific clinical signs obscure field detection, highlighting gaps in understanding the virus's persistence and infection dynamics. To address these challenges, the European Reference Laboratory (EURL) for ASF developed a multi-gene sequencing approach targeting six variable ASFV regions. Analysis of over 797 sequences, alongside genotype II data from GenBank, identified 28 genetic groups circulating in Europe since 2014. This has enhanced tracing and epidemiological understanding of ASFV spread. A significant finding was the detection of non-haemadsorbing (non-HAD) genotype II ASFVs, first identified in 2017 in the EU and linked to mutations in the EP402R gene. Thirteen additional non-HAD isolates, collected from wild boar in Lithuania, Latvia, Estonia, and Poland, revealed seven distinct mutations in the CD2v protein. Biological characterization of eight isolates demonstrated varying virulence: four were attenuated, two were virulent, and two were moderately virulent. Virulent strains regained the HAD phenotype after in vivo passages while retaining EP402R mutations. Non-HAD ASFVs highlight the complexity of ASFV infection dynamics, as they cause clinical forms ranging from acute to subclinical infections and can revert to HAD phenotypes. These findings underscore the importance of robust molecular and biological surveillance to address the combined challenges of genome variability, endemicity, and infection dynamics, ultimately improving diagnostic accuracy and ASF control efforts.

A recombinant salivary lipocalin protein (rtTSGP1) assay validation as a diagnostic tool

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The medical and economic importance of ticks has been recognized due to their ability to transmit diseases to both humans and animals. Tick species of the genus *Ornithodoros* are known vectors for diseases, such as African swine fever (ASF). This disease is undoubtedly the most serious disease of livestock spread by *Ornithodoros* ticks, posing a threat to domestic pig populations. Surveillance of domestic pig exposure to soft tick vectors would aid in developing effective prevention and control strategies against ticks. We aimed to evaluate a method based on the detection of antibodies to the salivary lipocalin protein, enabling the incorporation of information on exposure to tick bites into risk assessments for ASF introduction. Since sera from domestic pigs known to have been exposed to tick bites are not readily available, the assay was validated using sera from warthogs sampled in selected South African National Parks, both within and outside ASF endemic areas. Sample sites were selected based on the known distribution range of different argasid species and the ASF status of local warthog populations. Craig et al. (2020) indicated >98% seropositivity for ASF in warthogs and confirmed warthog-tick interactions by evaluation of blood meals at all study sites. An indirect ELISA based on a recombinant salivary lipocalin protein (rtTSGP1) of *Ornithodoros (Ornithodoros) moubata* ticks developed by Diaz-Martin et al. (2011) including antigen solution and controls, were obtained from Parasitología, IRNASA (CSIC), Salamanca, Spain. The test was carried out using an in-house method to pre-screen warthog sera from Kruger National Park for use in validation of the diagnostic test. Results indicated inclusivity (ability to detect other species within the *O. (O.) moubata* complex), exclusivity (ability to exclude known species of *Ornithodoros* ticks falling within another subgenus) and selectivity (ability to accurately detect and quantify the target despite other biologicals present). The modified rtTSGP1 assay is seen to be fit for purpose and a suitable for inclusion as a diagnostic method in South Africa.

A Novel DIVA Approach for ASFV: Utilizing MGF100-1L Serology to Differentiate Wild-Type and Cell-Adapted Infections

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African swine fever virus (ASFV) remains a critical threat to the global swine industry, necessitating innovative control measures. In this study, we developed a Differentiating Infected from Vaccinated Animals (DIVA) assay targeting the MGF100-1L protein, which is absent in a cell-adapted ASFV strain lacking several multigene family (MGF) genes. Seven deleted genes from the ASFV genome's right variable region were evaluated for reactivity against sera from convalescent pigs. Among these, MGF100-1L demonstrated strong immunoreactivity and was subsequently expressed as a recombinant protein for use in an enzyme-linked immunosorbent assay (ELISA). The optimized assay, with a cut-off value of 0.284, differentiated naive and infected pigs with 100% accuracy. Pigs infected with the cell-adapted ASFV strain exhibited no significant change in ELISA readings over 27 days post-infection. However, upon challenge with the wild-type virus, a significant increase in MGF100-1L reactivity was observed by 21 days post-challenge. These findings establish MGF100-1L as a reliable DIVA marker for ASFV. This assay offers a practical tool to distinguish infections with wild-type ASFV from those involving cell-adapted variants lacking specific MGF genes. The implementation of such a diagnostic strategy could significantly enhance ASFV surveillance and control, aiding in the global effort to mitigate the disease's impact.

Tongues and other alternative samples for the detection of African swine fever virus by PCR-testing: fit for purpose and approval?

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Several different alternative samples have been described for the detection of African swine fever virus (ASFV) by PCR-testing. To overcome practical and logistical challenges for the implementation of the continuous surveillance described in (EU)2023/594, samples which can be easily collected from dead pigs without opening the carcass are of specific interest. Therefore, the diagnostic sensitivity of tongue exudate, tongue tissue, nose swabs and ear punches was investigated and compared to target and alternative samples mentioned by the EURL.

During necropsy of 11 pigs that died after ASFV challenge in different experimental studies, EDTA-blood, spleen, tonsil, tongue, blood swabs, nasal swabs, ears and the femur (for the collection of bone marrow) were collected. Part of the tongue tissue was prepared directly, the rest of the tongue was frozen overnight (-20°C). After thawing, the tongue exudate was collected using a swab. Two ear punches (Ø 4mm) were combined to one sample/pig. All samples were prepared and PCR-tested following standard protocols at WBVR.

ASFV genomic material was detected with Ct-values <34 in all collected samples, resulting in a diagnostic sensitivity of 100% for all sample types. All samples mentioned by the EURL (EDTA-blood, spleen, tonsil, bone marrow and blood swab), as well as the nose swabs and tongue exudate swabs showed Ct-values <28.5. The mean Ct-values of EDTA-blood, spleen, bone marrow and tonsil were <22, the mean Ct-values of blood swabs, nose swabs and tongue exudate swabs were <25, the mean Ct-values of the ear punches and tongue tissue were <29.

ASFV can be detected in several different samples which can be easily collected from dead pigs without opening the carcass. Considering these alternative samples fit for purpose and approving them might help EU-member states in the implementation of the continuous surveillance described in (EU)2023/594 and other diagnostic programs for the detection of ASFV.

Assessing the feasibility of using oral swabs and ear biopsies for African swine fever virus detection during field outbreaks and active surveillance in Nigeria

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African swine fever (ASF) is a highly lethal and devastating disease of pigs caused by ASF virus (ASFV). To date, ASFV has been classified into 24 genotypes with the emergence of low virulent and recombinant ASFV strains, complicating the epidemiology of the disease. Additionally, there is no known cure for the disease, and available ASF vaccines are ineffective. Therefore, continuous surveillance is critical for early detection, mitigating spread, and limiting the impact of the disease. However, active surveillance, which entails collecting blood samples from thousands of pigs, can be challenging and requires expertise, hence the need to assess alternative sample types that are easy to collect and effective for ASFV detection. The use of sample types such as oral swabs and ear biopsies has been reported in experimental settings for ASFV detection, but not during field outbreaks or active surveillance. This study assessed the feasibility of using oral swabs and ear biopsies for ASFV detection during field outbreaks. In this study, 31 ASF suspect samples were collected over a one-month period from Jos abattoir, Plateau State, Nigeria. Four samples were collected from each pig showing clinical signs suspected to be ASF, namely oral swab, ear biopsy, blood, and tissue (spleen and lymph nodes). The samples were analysed by real-time polymerase chain reaction; samples with Ct values ≤ 40 were considered ASFV positive. Results revealed ASFV was detected in tissue (n=17, 51.5%) and ear biopsies (n=13, 41.9%), oral swabs (n=13, 41.9%), and blood (n=7, 22.8%). Recorded Ct values for positive samples were tissue (19.58-26.08), ear biopsies (22.28-28.59), oral swabs (24.50-30.73), and blood (17.47-26.08). The laboratory results showed the feasibility of oral swabs and ear biopsies as alternative sample types for ASF diagnosis during outbreaks and surveillance in Nigeria, although more studies are required.

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Improved real time polymerase chain reaction (qPCR) assays for the detection, characterization and quantification of African Swine Fever Virus

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Rapid and reliable detection of African Swine Fever Virus (ASFV) is paramount to control the spread of this virus and critical for prevention strategies. Currently, qPCR for ASFV DNA in clinical or environmental samples is mainly based on the detection of a single gene target: the p72 protein (B646L). WOAH (formerly OIE) and USDA regulatory assays and most commercially available assays target p72. p72 gene mutations of various ASFV strains can influence PCR sensitivity. Moreover, infection with different ASFV strains with variable pathogenesis or modified live vaccines can alter PCR detection efficiency. To improve the reliability and sensitivity of ASFV detection, additional gene detection assays were developed and evaluated. PCR assay development for the detection of the CD2v gene (EP402R) and the polymerase X gene (pO174L) gene underwent primary screening using plasmid DNA before employing viral DNA. Finally, successful assays were used to screen samples and virus stocks derived from multiple ASFV genotypes. All assays had similar to slightly improved analytical sensitivity when compared to the p72 assays, with the detection limits of 3-30 copies. Inclusivity screening of various ASFV strains from genotype I, II, V, X, and XIII, demonstrated similar assay performance. Clinical sensitivity using field samples and samples from experimentally infected pigs was 100%. Assays were also successfully multiplexed with the USDA p72 assay (Zsak et al., 2005).

This study developed and evaluated two sensitive and specific, quantitative qPCR assays for the detection of ASFV, based on CD2v and polymerase X genes. The novel assays can be used alone or multiplexed with the p72 assay. Multiplexing of the CD2v and p72 can provide improved sensitivity and help identify CD2v deficient isolates or distinguish infected from vaccinated animals when a CD2v deletion vaccine is used. The incorporation of detection of the polymerase X gene could help improve detection when CD2v deficient vaccines or isolates are circulating.

Dynamics of ASFV Genes Expression in vitro: Impact on Innate Immunity Pathways and Virulence

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The ongoing outbreak of African Swine Fever (ASF) has already devastated the swine industry in China (300 million animals slaughtered in 2018-2019 and colossal economic losses) and threatens pig farming worldwide. Virulent strains of ASFV cause a severe and lethal illness, while attenuated strains are associated with mild symptoms. Attenuated strains typically are characterized by deletion of more than 20 genes, particularly in the Multi Gene Family (MFG) of genes located at the genome extremities. The precise role and mechanisms of action of these genes are, however, only partially characterized. Whether other differences (beyond these deletions) exist between virulent and attenuated ASFV strains remains an open question.

Our aim is to characterize the innate immune response to virulent and attenuated ASFV strains in infected macrophages. We used two main approaches: thanks to a medium-throughput transcriptomic pipeline, we characterized the viral transcriptome of several virulent and attenuated strains, and the host response of infected macrophages. A confocal imaging approach allowed us to follow innate immune pathway activation in infected and bystander cells.

Our results indicate that the two types of strains exhibit global differences in genome replication dynamics and transcription. Over 20 viral genes are expressed earlier in virulent strains, which may contribute to their pathogenicity. The host response was exacerbated in cells infected with attenuated strains compared to virulent ones, with higher levels of ISGs, of some innate immunity sensors, and of the chaperone HSP70.2. Further, our results suggest that attenuated strains induce stronger and earlier nuclear translocation of IRF3 and STAT2. These differences in viral gene expression and activation of innate immune pathways could explain the lower pathogenicity of attenuated ASFV strains, and in the long run, may help to better understand the protection offered by Live Attenuated Vaccines.

High *ex vivo* ASFV recombination rate in PAM

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Although homologous recombination is known to play an important role in the evolution of other large DNA viruses such as herpes and poxviruses, very little is known about this process in ASFV. Until recently, some indirect experimental evidence, including the success of the CRISPR/Cas system in producing recombinant viruses with high frequency, and some *in silico* data suggested that homologous recombination may contribute to the genetic diversity of ASFV.

In the course of our research, we successfully isolated a recombinant ASFV Lv17/WB/Rie1-ΔCD-ΔGL from *ex vivo* porcine alveolar macrophages (PAMs) by cross-infecting two viruses, (Lv17/WB/Rie1-ΔCD and Lv17/WB/Rie1-ΔGL containing eGFP and mCherry markers), providing direct experimental evidence for homologous recombination between ASFVs.

The use of a number of our fluorescently labeled genotype I and genotype II viruses has allowed us to study the process of ASFV homologous recombination in greater detail. Recombinants were always detected when PAMs were simultaneously infected with our genotypeI-II viruses carrying different fluorescent markers located at different loci (the smallest distance tested was ~20 Kbp), strongly suggesting the possibility of the emergence of cross-genotype ASFVs. The emergence of recombinant viruses was confirmed by isolation and whole-genome sequencing of some progeny viruses. In PAMs, more than 20% apparent recombination frequency can be achieved between distant loci at high MOI co-infection. The number of recombinants in the crosses depended on the multiplicity, genotype, distance between the two loci and the synchrony of the coinfection.

Our *ex vivo* studies and the emergence of field recombinants in China should raise caution about the use of live ASFV vaccines.

The molecular highlights of African Swine Fever virus in Italy

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African swine fever (ASF) is a highly contagious disease caused by the ASF virus (ASFV), posing a significant threat to the global swine industry. Recognized as a notifiable disease by the World Organisation for Animal Health, ASF remains a critical concern. This study investigates the molecular characteristics of ASFV strains circulating in Italy during the 2022-2024 epidemic, using multi-gene and next-generation sequencing (NGS) approaches.

The molecular characterization of circulating ASFV strains in Italy was performed using a multi-gene and NGS techniques.

All ASFV-positive samples tested belonged to genotype II. The multi-gene approach identified four genetic groups among the Italian strains. The majority of samples (72%) belonged to genetic group 3, the most prevalent in Europe, followed by group 19 (15.9%). Additionally, two new genetic groups were identified: group 25 (9.1%) and 26 (3.0%). Genomic sequencing revealed large deletions and translocations in a small number of strains. Five samples exhibited deletions in the 5' region: a ~4340 bp deletion in four samples and a 2162 bp deletion in one. Another strain showed a truncation of 1950 bp at the 3' end. Additionally, 24 strains displayed a 5137 bp deletion, with 22 of these also having a ~2 kb truncation in the 3' region. Two strains showed a translocation from region 1-2244 to positions 188,631-190,584. *In vivo* characterization of one deleted strain showed disease progression similar to the wild-type strain, with severe ASF symptoms in inoculated pigs.

This study outlines ASFV circulating in Italy during the 2022-2024 genotype II epidemic, identifying two new genetic groups and naturally deleted ASFV strains. These findings highlight the complexity of ASFV in Italy and present challenges for its epidemiological study and diagnosis. The insights gained are essential for advancing ASFV control and management strategies.

Generation of chimeric African swine fever viruses between attenuated strains through *in vitro* and *in vivo* intergenotypic gene complementation

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African swine fever (ASF) is a fatal febrile disease in domestic pigs and Eurasian wild boars, caused by ASF virus (ASFV). ASF continues to spread across the globe causing a significant impact on the global pig industry. Recently, highly virulent chimeric ASFV (chASFV) with recombined genomes of the genotype I and II strains have been reported in the field, while the origin of the chASFV strains is unknown. In order to understand the mechanism of genetic recombination of ASFVs, we attempted to experimentally generate chASFVs both *in vitro* and *in vivo* employing two distinct attenuated ASFV strains: OUR T88/3 (genotype I) and AQSΔB119L (genotype II). When IPKM cells were co-infected with the attenuated ASFV OUR T88/3 and AQSΔB119L strains, three genetically distinct chASFV strains were isolated. After pigs were inoculated with individual isolates, all pigs exhibited symptoms of acute ASF and either died or were euthanized. When four pigs were co-infected with ASFV OUR T88/3 and AQSΔB119L, they developed symptoms of acute ASF and died or were euthanized. Three ASFV strains harboring chimeric genomes were successfully isolated from splenic homogenates from each pig. Our research indicates that chimeric viruses with diverse genomes can be experimentally generated both *in vitro* and *in vivo*.

Detection of ASFV in inspected carcasses cleared for public consumption in Kampala, Uganda

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African Swine Fever (ASF) is a highly fatal and contagious disease of pigs caused by the African Swine Fever Virus (ASFV), a DNA arbovirus of the family Asfarviridae. ASF is a highly devastating disease, with mortalities approaching 80-100 % among domestic pigs, thus constraining the pig industry and leading to poverty and food insecurity. Mitigating the dangers associated with ASF requires a robust surveillance program for early detection of potential sources of infection. Therefore, in this study, we set out to analyze the quality of pork inspection at the slaughterhouses by screening for ASF-positive carcasses among those pronounced healthy by official meat inspectors in three major city abattoirs in Kampala, Uganda.

Five hundred carcasses were randomly selected from three abattoirs (Wambizzi, Lusanja, Budo) for 3 months (May, June, and July 2024). Spleen samples were collected and tested by real-time PCR to detect the ASF virus.

Data shows that of the 500 carcass samples analyzed, 98 (19.6%) carcasses from the abattoirs studied were positive for ASFV. None of these pigs had clinical signs and lesions that included hemorrhages in the skin of ears, legs, and flanks, markedly enlarged and darkened spleen, and enlarged and diffusely hemorrhagic gastro-hepatic and renal lymph nodes.

This data suggest an increased number of asymptomatic forms of ASFV in Uganda. Because these cases cannot be easily screened out by the naked eye of the meat inspector, there is an increased risk of spreading the virus in the community through purchased pork.

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Evaluation of disease progression of three different doses of the highly virulent ASFV Ghana2021 in domestic pigs

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African swine fever virus (ASFV) is the causative agent of African swine fever (ASF), a haemorrhagic disease affecting pigs and wild boars. The severity of ASF varies from subclinical to highly lethal, depending on the virus strain infecting and the characteristics of the host. The disease is endemic in Africa but is rapidly becoming a growing global concern, with continuing outbreaks in Eastern Europe, Asia and the Caribbean.

In Asia and Europe, only one ASFV genotype is circulating in a specific area with genotype I previously present in Sardinia and genotype II in Europe and Asia. In contrast, several genotypes circulate simultaneously in sub-Saharan Africa, where the disease is endemic. ASFV isolates have been commonly characterised using different technologies, including genetic sequencing (partial gene sequences of whole genomes), serological analysis, and *in vitro* growth kinetics. However, there is incomplete knowledge about the clinicopathological forms of ASF disease associated with isolates circulating in Africa. Characterising ASFV strains, both *in vitro* and *in vivo*, is key for understanding the virulence of outbreak strains and the correct implementation of biosecurity measures. Additionally, the development of challenge animal models with well-characterised ASFV strains present in different areas of the world offers a unique opportunity to test vaccines aiming for a global market.

This study evaluated the progression of ASF disease in domestic pigs intramuscularly inoculated with increasing doses of Ghana2021 ASFV genotype I (102, 103 and 104 HAD50). Different parameters were assessed, including clinical signs, haematology, viraemia, and pathological findings, among many others.

The three doses of the virus caused acute infections, with animals reaching the humane end-point between days 5 and 7 after infection. All infected animals developed clinical parameters and lesions consistent with ASF, corroborated by ancillary laboratory findings. After deeper analysis of blood parameters and tissue lesions, dose-dependent differences among the groups were found. For example, there was a slight delay in detecting virus in blood, as well as the occurrence of clinical signs (including temperature) and less aggressive tissue lesions in the group inoculated with the lowest dose (102 HAD50). These findings highlight the high virulence of the Ghana2021 ASFV strain and underscore the need for well-designed *in vivo* challenge experiments to better characterise circulating ASFV strains and assess cross-protection efficacy in vaccine development.

Two Sardinian ASFV isolates with a large genomic deletion presented an attenuated phenotype *in vitro*

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African swine fever virus (ASFV) causes a devastating disease affecting domestic and wild pigs. ASF was first introduced in Sardinia in 1978 and until 2019 only genotype I isolates were identified. A remarkable genetic stability of Sardinian ASFV isolates was described, nevertheless in 2019 two wild boar strains with a genomic deletion of 4342 base pairs were identified (7303WB/19, 7212WB/19). In this study, we performed *in vitro* experiments to unravel the phenotypic characteristics of these deleted strains. Porcine macrophages were infected with virulent 26544/OG10 or deleted 7303WB/19, 7212WB/19, alongside mock-infected controls. The ability of these isolates to replicate in macrophages was first assessed, using a high (1) or a low (0.01) multiplicity of infection (MOI). Cell supernatants were collected at 0, 24, 48, 72 hour post infection (hpi) and then titrated. Subsequently, interaction of ASFV with these cells was investigated using flow cytometry and multiplex ELISA. Macrophages were infected using a MOI of 1 and after 24 h the intracellular levels of viral proteins and the levels of key immune cytokines (IL-1α, IL1β, IL-6, IL-10, IL-12, IL-18, TNF) were assessed. Our data revealed that both 7303WB/19 and 7212WB/19 presented a lower growth kinetic in porcine macrophages compared to virulent Sardinian 26544/OG10. In addition, flow cytometric analysis showed that both 7303WB/19 and 7212WB/19 presented lower intracellular levels of both early (p30) and late ASFV (p72) proteins. Infection with either deleted or nondeleted ASFV strains did not trigger release of pro-inflammatory or anti-inflammatory cytokines from macrophages. Overall, we observed the deleted virus isolates in Sardinia only in 2019, at the end of a strong eradication campaign, and our data suggested that it might possess an attenuated phenotype. *In vivo* studies should be performed to analyse the phenotype of these deleted isolates, to better understand their role in ASFV persistence in Sardinia.

Identification of ASFV genes involved in innate immunity control

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ASFV codes for more than 150 proteins, many of them related to immune evasion. A link has been suggested between innate immunity control and ASFV virulence, since virulent strains are able to modulate type-I IFN and several cytokine/chemokine pathways. In this study, we have identified several genes potentially involved in different innate immunity pathways modulation by using a screening based on genomic comparison, luciferase and qPCR assays. Two ASFV genes, MGF110-1L and MGF110-2L, were selected as potential genes involved in innate immunity regulation. We have determined that MGF110-1L has two transmembrane zones and a signal peptide while MGF110-2L presents a signal peptide that is predicted to be secreted by using protein structure prediction software. Moreover, we have determined their intracellular localization by FACS and confocal microscopy, showing that MGF110-2L located both inside and on the cell surface while MGF110-1L showed a reticular localization pattern.

To gain a deeper understanding of their function, we have generated recombinant viruses lacking any of these genes from the virulent Arm/07/CBM/c2 strain by using CRISPR/Cas9 technology. *In vitro* studies showed a loss of control of IFN production by the virus when either of these two genes are missing. Besides, an *in vivo* experiment immunizing pigs showed that Arm-ΔMGF110-2L was partially attenuated, indicating not only the role of MGF110-2L in virulence, but also suggesting a correlation between factors that control the IFN-I production and the attenuation of the strain. Further studies are ongoing to better understand the molecular mechanims of these proteins during ASFV infection.

Cytotoxic cells in African swine fever immunity: a dual marker of protection and pathogenesis

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The African swine fever (ASF) pandemic in pigs is currently causing enormous economic losses to the swine industry worldwide. The lack of prophylactic treatments hampers its control, and the insufficient knowledge regarding the immunological mechanisms underlying protection hinders rational vaccine design. While antibody-mediated mechanisms associated with protection remain poorly characterized, there is increasing evidence of the involvement of cytotoxic cellular responses in ASF immunity. However, their contribution to ASF control remains elusive. While some studies show the presence of cytotoxic cells in pigs with severe disease, others have associated them with a protective status. Indeed, we have demonstrated the induction of a broad cytotoxic recall response in pigs immunized with the live attenuated vaccine prototype BA71ΔCD2. In the present study, we aimed to better characterise ASF virus (ASFV)-induced cytotoxic responses in ASF immunity. First, we demonstrate that ASFV-infected pigs with overt clinical signs and lesions showed a high percentage of perforin-producing NK and T cells. The levels of some of such cell subsets positively correlate with viremia, indicating their contribution to the ASFV-induced systemic uncontrolled immune response. Second, we show the presence of perforin-producing ASFV-specific CD8ab+ CD45RA- CCR7- effector memory T cells upon *in vitro* stimulation in PBMC from immune pigs inoculated with BA71ΔCD2. Importantly, high levels of these cells significantly correlate with protection, as demonstrated when comparing protected and non-protected pigs after a suboptimal vaccination regimen. In contrast, no correlation was observed with either ASFV-specific antibodies or IFNg-producing cells, indicating that cytotoxic T cells are a critical arm of the adaptive cellular response against ASFV. This study further demonstrates the central role of cytotoxic cells during ASFV infection, highlighting the importance of their early appearance before a systemic infection is established, thus being an important immune mechanism to be targeted in the rational development of effective ASF vaccines.

Pericarditis in pigs immunized with live attenuated African swine fever virus followed by highly virulent challenge

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An effective vaccine and in-depth knowledge about the relationship between African swine fever virus (ASFV) infections and host responses are lacking. To study host responses, research institutes collaborated in the international project 'ASF-RASH': African Swine Fever pathogenesis and immune responses in Resistant And Susceptible Hosts. This provides knowledge that is essential for vaccine development and implementation, and further ASF research.
For this project, two animal studies have been carried out at Wageningen Bioveterinary Research, the Netherlands. In both, the pigs received zero, one or two intramuscular doses of one or two live attenuated strains, followed by an oronasal challenge with a virulent ASFV strain (all genotype II). Animals that survived the immunization with the attenuated strain(s) and the subsequent challenge were considered protected (survivors). At the end of the study survivors showed mostly no clinical signs. However, in all survivors a chronic pericarditis was observed at necropsy, with differences in severity. Other organs showed no relevant macroscopic lesions. Histological findings revealed infiltration of mainly lymphocytes and plasma cells in the pericardium(/epicardium) and adjacent myocardium. Non-survivors showed clinical signs, and necropsy revealed occasional acute hemorrhages in the heart, next to other ASFV related pathology.

The cause of the pericarditis in relation to immunization and/or challenge will be further investigated using immunohistochemistry for ASFV protein, immune and cell markers. The observed pericarditis may render protected animals more susceptible to secondary infections and result in decreased performance. These findings emphasize the relevance of necropsies and macroscopic pathology in ASFV studies.

Vaccines

Safety evaluation of the ASF vaccine produced by NAVETCO under field conditions in Dominican Republic

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The objective of the study was to evaluate the safety of the NAVET-ASFVAC vaccine produced by the Vietnamese company NAVETCO under typical commercial production conditions in the Dominican Republic. A prospective cohort study was performed in which a cohort of 500 pigs, approximately 10 weeks old, were vaccinated once intramuscularly with NAVET-ASFVAC. A matched unvaccinated cohort of 500 pigs were included as controls, kept under the same management and treatment practices, in a separate shed on the same farm. A subset of both cohorts of pigs received intensive monitoring for 30 days post vaccination to document body temperature, weight gain, presence of vaccine virus in blood, and presence of antibodies in serum. All animals in both cohorts were serologically negative for ASF virus at the beginning of the study. During the first month after vaccination, all monitored health parameters were similar between vaccinated and non-vaccinated pigs including body temperature, weight gain, frequency of the presentation of concurrent diseases, and overall mortality. More than 91% of the vaccinated animals were serologically positive for the ASF vaccine virus. ASFV DNA of the vaccine virus was present in the blood of 53.9% of vaccinated pigs at 30 days after vaccination but had decreased to 6.6% of pigs at 100 days. The animals continued to be clinically monitored daily until 100 days after vaccination. Again, during this extended observational period, there were no substantial differences in the frequency of the presentation of concurrent diseases or in animal mortality between the groups.

Optimization of oral vaccination strategies against ASFV

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The African swine fever virus (ASFV) is the causative agent of African swine fever, a devastating disease that significantly impacts the pig industry and wild boar populations, with severe socio-economic consequences. Despite a near pandemic spread, no licensed vaccine is as yet available on the European market. Oral immunization of wild boar is of particular interest for Germany and other European countries for containment of infectious spread and biosafety. In pursuit of an authorized vaccine, vaccine candidates for intramuscular vaccination of domestic pigs have undergone initial investigation in several studies. However, comprehensive data on the efficiency and efficacy of oral vaccination are still missing. In this study, we conducted a comparative trial to assess efficacy and efficiency of four vaccine candidates for oral administration. The animals were challenged with the highly virulent ASFV strain Armenia08 at d28 post vaccination. Clinical signs, serological responses and viral replication were detected. To further optimize oral vaccine application, an additional trial was carried out, exploring whether increased inoculation doses, the addition of adjuvants, or modifications of the bait matrix could improve protective immune responses. This was assessed by monitoring of humoral and cellular immunity. The findings of these studies support the potential of oral vaccine application in swine as a promising vaccination strategy. Further studies on safety and efficacy of the vaccine candidate are essential for the development of an effective prevention and control strategy for ASFV.

A gene-modified genotype II live attenuated African swine fever virus induces cross-protection against genotype I but not against genotype IX

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At least twenty-three African swine fever virus (ASFV) genotypes are present in Africa whereas only genotypes I and II have spread to other continents. Vaccine development has been directed mainly to genotype II, and the ability of these vaccines to induce cross-protection against other genotypes is unknown. We previously described a modified live vaccine (MLV) candidate for ASF, based on genotype II, in which K145R, EP153R and DP148R genes were deleted, and the gene coding for CD2v protein was modified to prevent binding of the virus particles and infected cells to red blood cells (non-HAD). This reduced both viremia levels and persistence in blood post-immunization. We showed that this MLV induced dose dependent protection (83 – 100%) against homologous genotype II ASFV challenge (doi:10.1080/22221751.2023.2265661). Here, pigs were immunized and boosted intramuscularly with GeorgiaΔDP148RΔK145RΔEP153R-CD2v_mut and subsequently challenged with either a genotype I ASFV or a genotype IX ASFV. An immunization and boost dose of 5x10E4 TCID50, induced 70% protection against genotype I, but no protection was achieved against genotype IX. These results indicate potential for the use of a single vaccine in regions where genotypes I and II are co-circulating.

The Russian Attenuated African Swine Fever Virus with MGF360 and MGF505 Deleted Protects against Two Heterologous Strains of Serotype 8, but with Different Effectiveness

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African swine fever virus (ASFV) has led to major economic losses to pork producers by the spread of African swine fever (ASF) worldwide. In Russia, there are no developed or registered vaccines against ASFV genotype II, which is associated with numerous ASFV outbreaks in populations of domestic pigs and wild boars in the country. Several LAV candidates obtained in different countries (O'Donnell V., et al, 2015; Borca, M.V., et al, 2020; Tran, X.H., et al, 2022), as well as the licensed vaccine AVAC ASF live (AVAC VietNam, Vietnam), were based on genetically similar strains of ASFV genotype II with a deletion of the three genes of the multigene family MGF360 and three genes of the multigene family MGF505. We introduced deletions of the 6 MGF360 and MGF505 genes of the ASFV virulent Stavropol_01/08 strain, isolated in Russia in 2008. We show here that this deletion did lead to full attenuation of the ASFV virulent Stavropol_01/08 strain. Animals intramuscularly inoculated with 4-6 lg HAD50 of ΔMGF360/505_Stav developed a strong immune response and short period of viremia (at 3-7 day post-inoculation). Recombinant ΔMGF360/505_Stav strain provides protection of pigs against the ASFV parental Stavropol_01/08 strain (3 lg HAD50). The optimal dose of virus for immunization was selected using animals from local small pig farms.

In contrast, we found only partial protection (40%) of the ΔMGF360/505_Stav-immunized pigs against challenge with the ASFV heterologous Rhodesia strain. And the surviving animals had a prolonged fever and their condition was depressed for most of the experiment.

Thus, the ASFV recombinant ΔMGF360/505_Stav strain is the first live attenuated vaccine (LAV) in Russia that induces protection in pigs challenged with highly virulent epidemiologically relevant strains genotype II and serotype 8.

Insight into the molecular mechanisms leading to reversion to virulence of LAV against African swine fever virus

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African swine fever virus (ASFV) infects domestic pigs and wild boar and is currently the biggest animal pandemic affecting more than 40 countries in four continents and the greatest threat to the global swine industry. Currently, the most realistic vaccination strategy is live attenuated vaccines (LAV), where attenuated prototypes with high protection rates are usually generated by manipulating genes involved in virulence. However, safety issues associated with these prototypes remain a challenge to be addressed. Recently our group has generated a prototype from the virulent Arm/07/CBM/c2 strain in which both the A238L and EP402R ASFV genes were deleted, generating a fully attenuated prototype in pigs, which further induces 100% protection against a circulating virulent strain. However, regulatory-required back-passage tests, consisting of inoculating virus from blood/tissues from the vaccinated animals (8-10 days after vaccination) into naïve pigs, eventually resulted in the death of the newly blood-inoculated animals, indicating that the prototype had somehow reverted to virulence. Due to the importance of these results in terms of safety, and in order to analyze the causes, viruses were isolated from different tissues of the animals and viral DNA subjected to next generation sequencing (NGS). By doing so, we have identified mutations on two genes probably involved in the viral phenotype change. The molecular mechanisms of this reversion to virulence, currently under study, would help to answer important issues that commit the safety of LAVs vaccines and could help to develop new, safer LAVs in the near future.

Immunogenicity of an African Swine Fever Virus Multiepitope Protein Nanoparticle-based Subunit Vaccine

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The Ohio State University

Although African swine fever virus (ASFV) has not been detected in the United States (U.S), it continues to be an emerging threat and has the potential to result in dire consequences to the multi-billion-dollar U.S. pork economy. The continuing spread of ASFV into the Caribbean necessitates the development of vaccine interventions. Immunogenic epitopes of ASFV have been identified and used in vaccine trials, but they have provided only limited protective immune responses. We hypothesize that combining known partially protective immune epitopes with a nanoparticle vaccine delivery platform will enhance subunit vaccine protection. Here, we use *in-silico* modeling to construct a synthetic, ASFV protein containing multiple conserved immunogenic ASFV epitopes with the aim of inducing robust, protective immune responses utilizing a single protein vaccine antigen with nanoparticle vaccine platform. The candidate vaccine, along with nanoparticle entrapped adjuvant, was delivered intramuscularly to pigs, and T- and B-cell responses were evaluated following initial (DPV 22) and booster (DPV 42) vaccine dosages. Nanoparticle entrapped multiepitope protein vaccinates showed a positive multiepitope-specific IgG antibody response earlier than unentrapped antigen vaccinates. Furthermore, we detected a significantly higher multiepitope-specific IgG antibody response at DPV42, compared to mock and DPV22 titers. The nanoparticle entrapped multiepitope vaccine also induced a greater frequency of antigen-specific cytotoxic T-cells and T-helper cells in vaccinates compared to mock and unentrapped vaccinates. In summary, our nanoparticle entrapped multiepitope vaccine induced enhanced antigen specific T- and B-cell responses, both of which have been identified as important correlates of protection against ASFV. These promising preliminary immunological data suggest that our nanoparticle entrapped multiepitope protein vaccine could provide protection against ASFV challenge, which will be evaluated in future studies. This study demonstrates the promising potential for a novel ASF nanoparticle vaccine and provides a strategy for engineering cross-protective vaccines against various strains of viral infectious diseases.

Using CRISPR as a broad spectrum pan-genus therapeutic to Rapid Respond to Viral Threats: A Case study for African swine fever and beyond.

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Seek Labs

CRISPR/Cas systems have evolutionarily evolved to be an effective method to protect prokaryotes against viruses by detecting and destroying the DNA or RNA genomes of invading bacteriophages. Using our CRISPR platform we designed and developed a therapeutic system consisting of CRISPR-Cas9 with multiplexed guide RNAs to target conserved ASFV genomic sequences as an effective treatment for ASF. This innovative approach is designed to specifically target and cleave multiple viral nucleotide sequences, reducing viral replication and limiting disease burden in infected hosts. By targeting multiple conserved sites in the ASFV genome the CRISPR treatment is not genotype specific and could potentially be used to universally target all current circulating strains of ASFV. Here we report that following intramuscular (IM) injection of a lethal dose of ASFV, swine treated with our anti-ASFV CRISPR therapeutic had reduced viral loads, were less symptomatic with a majority (57%) demonstrating complete clearance of the virus and achieving full recovery from ASF. Interestingly, all surviving animals developed robust antibody-based adaptive immunity from the initial exposure to ASF virus, and when rechallenged with a lethal dose of ASFV, were fully protected and survived the trial. Taken together, these trial outcomes indicate that our CRISPR-Cas9 targeted treatment for ASF could be used to combat all circulating strains of ASFV and be given after the initial outbreak to save the farm, avoiding the massive culling of infected farms. Moreover, this innovative approach could be adapted to disrupt other viral pathogens by simply selecting the optimal Cas for DNA or RNA genomes and reprogramming with computationally prioritized guide RNAs complementary to the target sequences.

African Swine Fever (ASF) Prevention and Control Program and Vaccine Implementation: Current Experience of the Philippines

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DA Bureau of Animal Industry

Since the detection of the first case of African Swine Fever (ASF) in Asia in 2018, the Philippines has been vigilant in guarding its borders from the introduction of the ASF virus. The Philippine swine industry is one of the biggest contributors to the country's agricultural growth. In 2019, the country's total swine inventory was estimated to be at USD 12.78 million. The Philippine government through the Department of Agriculture - Bureau of Animal Industry (DA-BAI) immediately formed the ASF Task Force (ASFTF) to manage and oversee disease prevention and control activities including risk communication.

The National ASF Prevention and Control Program (NASFPCP), pursuant to the Department of Agriculture Administrative Order issued in 2021, was created and has been guiding the implementers (national, subnational, and local) in the control and prevention of ASF. The ASF Program has five key components that include surveillance, biosecurity, movement management, local government engagement, capacity building and awareness, and recovery.

Other related policies issued that support the effective program implementation include zoning and movement plan, granting of cash assistance (indemnification) to encourage early reporting, surveillance, biosecurity, strategic communication, and recovery from the outbreaks, among others.

To complement the above strategies to control and prevent the spread of the disease, the Department of Agriculture (DA) recently issued the guidelines on the controlled use of the vaccine. To date, vaccination of the susceptible swine population through controlled roll-out is being conducted in eligible farms using ASF Live Attenuated Vaccine registered by the Philippines Food and Drug Administration under the Certificate of Product Registration with Monitored Release (CPR-MR). Administration of the vaccine by controlled use is being implemented in identified provinces in Luzon.

The recombinant ASFV genotype I/II strain emerged in Vietnam resists the immunity induced by the ASFV genotype II vaccine strains

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African swine fever virus (ASFV) continues to spread in Vietnam, causing severe economic losses to the pig industry. Vietnam licensed two live attenuated vaccines based on the ASFV strains ASFV-G-ΔI177L and ASFV-G-ΔMGF to control the ongoing ASF outbreaks.

In 2023, newly emerging highly virulent recombinant ASF viruses (rASFV I/II) containing genetic elements from both p72 genotype I and II ASF viruses were reported from Northern Vietnam. This study evaluated whether the two live attenuated vaccine strains were able to protect the pigs against the emerging rASFV I/II strain VNUA/rASFV/TN1/23. All pigs vaccinated with ASFV-G-ΔMGF strain when challenged died within 8 dpc. Only three out of five pigs that received the ASFV-G-ΔI177L strain developed acute clinical signs when challenged and succumbed to ASF at 19, 18, and 9 dpc, respectively. The two remaining pigs survived but started to show signs of chronic ASF infection.

In conclusion, the findings from this study show that both vaccine strains licensed in Vietnam are unlikely to effectively protect pigs from the emerging highly virulent rASFV I/II strain. This complicates the ongoing efforts to control ASF in Asia and globally and emphasizes the urgent need for a novel vaccine that can protect pigs from the rASFV I/II strain.

POSTER PRESENTATIONS

Epidemiology

Farmer perceptions and actions following the first outbreak of African Swine Fever in Sweden

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In September 2023, the first case of ASF in Swedish wild boar triggered regional control measures. In addition, an ASF certification programme was initiated in December 2023 to improve herd biosecurity and disease prevention. To capture the pig farmers' perceptions and what measures they had implemented, two surveys were conducted (September 2023 and March 2024) via online questionnaires distributed via the Swedish pig producers' organization.

The first survey received 155 responses (response rate 36%). Most of the respondents had received general information about ASF and how to protect their farm. A majority thought the information was easy to understand, relevant and sufficient. If given the necessary resources, 58% would like to implement measures such as fencing, and heavily reduce the wild boar population. Most of the farmers had a positive outlook on the future.

The second survey received 113 responses (response rate 27%), with the majority seeing the risk of ASF reappearing in Sweden as high. While many farmers sought biosecurity advice from veterinarians, 43% had not implemented the suggested measures. Very few of the respondents had signed up for the new ASF certification programme, 13% answered that they will join during 2024, and 19% that they will join in 2025. A few did not plan to join, and 34% stated that they will join if in an ASF-restricted zone. Of those that did not plan to join the programme, 46% deemed it too expensive, 19% that the risk is small, and 19% stated that they will cease production if they end up in an infected zone.

In conclusion, farmers were concerned about new ASF outbreaks, but a majority identified cost as a substantial hurdle for improving biosecurity. The results highlight the importance of effective communication, context-specific biosecurity advice and economic support to address the challenges posed by ASF.

Understanding the Epidemiology of African Swine Fever (ASF) in Mindanao, Southern Philippines Towards Its Control

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African Swine Fever (ASF) is a highly contagious and deadly viral disease affecting pigs. Research from 2020 to 2023 aimed to determine the epidemiology of ASF in Mindanao, southern Philippines, focusing on prevalence in small-holder farms and abattoirs, identifying risk factors, assessing small-holder pig farmers' knowledge, attitudes, and practices (KAP), and estimating financial losses. Nearly 5,000 pigs from 150 villages and 53 abattoirs in 5 regions in Mindanao were randomly sampled. Serum samples were tested for ASF antibodies using ELISA, while blood samples were tested for viral DNA using PCR. Over 1,500 pig owners were interviewed. Low seroprevalence estimates of 0.6% (20/3,158) and 0.1% (2/1,538) were detected among pigs in small-holder farms and abattoirs, respectively. Potential ASF risk factors included the proximity of piggeries, absence of footbaths, contact with other animals, purchase of pork and pork products, lack of fly control, allowing visitors inside the pig farm, use of outside service boars, outside sourcing of pigs, swill feeding, and improper disposal of dead pigs. Respondents exhibited average knowledge and favorable attitudes and practices for ASF control. The financial impact was significant, with each of the four surveyed provinces losing nearly $1 million from ASF, with losses of $138,000, $19,000, and $700 per affected municipality, barangay, and farmer, respectively.
The study concludes that ASF seroprevalence is low among pigs in small-holder farms and abattoirs in Mindanao during the survey, with some identified important risk factors. Small-holder pig raisers have average knowledge but favorable attitudes and moderate practices for ASF prevention and control. The direct financial losses due to ASF are high. The study recommends intensifying preventive and control measures against ASF among pigs in Mindanao, including, among others, more awareness campaigns, improved farm biosecurity, enhanced border control, further research, sustained active disease surveillance, and assistance for farmers for alternative income generation.

Epidemiology of African Swine Fever Among Selected Pork Products and Slaughtered Pigs in Davao de Oro, Philippines

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African Swine Fever (ASF) is a highly fatal swine disease that entered Mindanao, Philippines and significantly impacted the swine industry. Transmission of virus to vulnerable pigs through contaminated pork and by-products has long been known, and thus is crucial in prevention and control strategies. Therefore, this study was conducted to determine the epidemiology of ASF among pork products and live pigs in selected wet markets and abattoirs in Davao de Oro, and assess the lived experiences of vendors, consumers, and meat inspectors through in-depth phenomenological survey. A total of 168 slaughtered pigs and local pork products were sampled in three municipalities of Davao de Oro. Samples were tested for viral DNA and antibodies using PCR and ELISA. Thirty vendors, consumers and meat inspectors were interviewed for their in-depth lived experiences on ASF. Overall, 4.76% of the sampled pork products were positive to ASF DNA, wherein all positive products were 'longganisa'[1], and, 21.4% of the slaughtered pigs were ASF-antibody positive, with 3.93% overall prevalence to ASF DNA. The phenomenology survey revealed five themes, including its socioeconomic impact, control and preventive measures, the LGUs actions toward the disease, viral transmission, and misconceptions. In conclusion, prevalence is low among pigs slaughtered in Mindanao; some pork products in the wet markets are contaminated with ASF virus; and respondents are aware of the impact and control measures against ASF, and are actively participating in its control; however, information reinforcement is still needed. The present preventive and control measures against ASF should be sustained and intensified in the province, including surveillance and regulation in the movement and sale of pigs and pork products and intensifying awareness campaigns regarding ASF, to completely contain and possibly eradicate the disease in Davao de Oro, and consequently in Mindanao.

[1] A type of skinless sausage

Risk Assessment of ASFV Transmission in Pork Products and Molecular Detection in Visayan Warty Pigs in the Philippines

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African swine fever (ASF) is a contagious hemorrhagic disease affecting pigs with high mortality rate and severe socio-economic losses. Due to the virus' potential ability to remain infectious in suitable conditions and environments, it is important to identify risk factors that may contribute to its transmission. Currently, ASF surveillance in the Philippines relies on pig blood samples, providing limited data on transmission risks from contaminated pork and potential wildlife reservoirs. In addition, ASF transmission risk evaluation currently includes positive cases, population density, and pork production volume, but the potential roles of contaminated pork commodities and wild pigs remain unexplored. In this study, a total of 384 raw and 384 processed pork products from selected wet markets were collected and detected the ASFv VP72 gene using real-time polymerase chain reaction (rt-PCR), and the overall positivity rates were 10.16% and 10.68%, respectively. Based on the regression analysis employed, positive samples were associated with factors like zoning status, season, 'Longganisa' preparation, selling different meat types, pork batch duration, and market practices like cleaning and disinfection. Furthermore, a risk assessment identified six provinces with high ASF transmission risk due to positive pork samples and high ASF incidence, while four provinces had consistently low risk. The difference in the meat contamination level between low and high-risk provinces emphasized the importance of including this factor in ASF spread assessment. Additionally, molecular detection of ASFv was conducted in fecal samples from Visayan warty pigs (*Sus cebifrons*) in one conservation center in the Philippines. Among fifteen (15) wild pig fecal samples tested, five (5) were positive for ASFv, confirming the virus's presence in wild pig populations[2]. This finding suggests that wild pigs may serve as an additional reservoir for ASFV, posing a risk for further spread to domestic swine and pork commodities. Overall, ASFV contamination in raw and processed pork products and its detection in wild pigs can pose a threat to the swine industry, and market practices may further lead to ASFv persistence in these commodities which may contribute to ASF spread. These findings highlight the need for comprehensive ASF surveillance and monitoring of pork products and wild pig populations, stricter handling practices throughout the food supply chain, and targeted resource allocation to high-risk areas to control ASF spread in the Philippines.

Keywords: ASFv, pork products, wild pigs, risk assessment, rt-PCR

[2] A report from the conservationist at the centre where the samples were taken, presented at the GARA Gap Analysis in Manila, 5-7 December 2023, confirmed that the pigs at the conservation centre all died of acute ASF. These rare and highly vulnerable pigs that are threatened with extinction are incapable of becoming a reservoir for ASFV due to small population size and inability to survive infection with ASFV. This must be recognised to ensure that no irresponsible measures that threaten the survival of the bearded and wqrty pigs should be implemented at any time.

Bridging Gaps in Risk Communication and Community Engagement for African Swine Fever Management in Asia and the Pacific

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African swine fever (ASF) poses catastrophic threats to domestic and wild pig populations, with significant socio-economic impacts across Asia and the Pacific since its detection in 2018. Despite containment efforts, risk communication and community engagement gaps have exacerbated the disease's rapid spread. The FAO Emergency Centre for Transboundary Animal Diseases (ECTAD), in collaboration with the Bhutan National Center for Animal Health and the Philippine Bureau of Animal Industry, conducted studies using knowledge, attitudes and practices (KAP) surveys in selected communities in Bhutan and the Philippines to address these gaps.

Findings revealed widespread awareness of ASF among surveyed stakeholders. Nevertheless, misconceptions about ASF's non-zoonotic nature and environmental persistence were prevalent, while biosecurity adoption remained limited. In Bhutan, surveyed farmers expressed concerns about government interventions to eradicate ASF, which may stem from a limited understanding of the disease's severity. Innovative approaches, such as the "biosecurity champions" initiative in Bhutan and social media campaigns in the Philippines, demonstrated how localized and culturally sensitive communication strategies could foster compliance, address misinformation and enhance stakeholder engagement. The survey outcomes also highlighted the need for attitudinal interventions.

Our study offered chances to address challenges and explore practical intervention designs and scalable strategies. These approaches embraced participatory development communication. Thus, they required active participation and implementation. Core recommendations focused on integrating community-driven advocacy with structured risk communication strategies tailored to key stakeholders. Strengthening feedback mechanisms for the real-time adaptation of messages and leveraging digital and traditional media to reinforce biosecurity behaviours were emphasized. This participatory approach aimed to bridge the gap between technical knowledge and grassroots implementation, fostering resilience against ASF outbreaks while protecting livelihoods and pig populations.

The findings highlight the critical role of cultural context and participatory communication in managing ASF and offer valuable lessons for controlling other transboundary animal diseases.

A Strategic Approach to Disease Mapping Based on Insights from the African Swine Fever Outbreak in Sweden

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Risk mapping is a vital tool in epidemiology, providing a systematic approach to understanding and mitigating the risk of disease introduction and spread. This method is particularly significant for managing wildlife diseases, where the complexities of wild animal movement and behaviour challenge traditional surveillance and control measures. African swine fever (ASF), a contagious viral disease affecting wild boar and domestic pigs, exemplifies these challenges. The disease has severe economic impacts, including high mortality rates, trade restrictions, and the absence of effective vaccines. In Sweden, ASF introduction via wild boar is primarily linked to human-mediated activities, such as improper disposal of contaminated food waste.

This study examines the 2023 ASF outbreak in Sweden, as a case study to explore how the selection and resolution of risk factors influence risk mapping. Factors such as wild boar density, human activity, and waste management practices were analysed to assess their impact on identifying high-risk areas. The findings underscore the need for high-resolution, region-specific risk maps to address variations in ecological and human factors. Notably, the role of municipal waste collection centres, initially overlooked in risk assessments, was identified as a significant factor in the outbreak.

The study highlights the importance of selecting relevant risk factors, assigning appropriate weights, and adapting assessments to local conditions to enhance predictive accuracy. Additionally, periodic updates to risk maps are essential to account for changes in environmental and ecological conditions, such as those driven by climate change. By improving the spatial resolution and incorporating comprehensive datasets, risk mapping can better inform targeted interventions, strengthen biosecurity measures, and mitigate the economic and ecological impacts of ASF outbreaks. This approach emphasizes the need for a multi-dimensional, adaptive strategy in managing wildlife diseases.

Epidemiology of African Swine Fever in Pork Products and the Lived Experiences of Selected Stakeholders in Davao de Oro, Philippines

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African Swine Fever (ASF) is a highly fatal viral swine disease that significantly impacted the Philippines since its incursion. This project was conducted to determine the prevalence and risk factors of ASF among pork products and slaughtered pigs and to assess the lived experiences of selected stakeholders in ASF-affected areas in the province of Davao de Oro. A total of 84 pork products were assessed for ASF viral DNA using molecular test. Additionally, 84 slaughtered pigs were tested for ASF viral DNA and antibodies using conventional polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. In-depth interviews were also conducted with 30 stakeholders: pork vendors, consumers, and meat inspectors. An overall 4.76% ASF prevalence was found in pork products, with all positive samples derived from 'longganisa', and prevalence rates of 3.93% and 21.4% for ASF viral DNA and antibodies in slaughtered pigs, respectively. The lived experiences survey revealed five critical themes on ASF, including the socio-economic impact of ASF, control and preventive measures against ASF, the local government unit's actions against the disease, viral transmission, and common misconceptions about ASF. While stakeholders demonstrated awareness of the disease and its mitigation strategies, there is a critical need for continued information dissemination and education to reinforce biosecurity practices. This study highlights the low yet persistent presence of ASF in pork products and slaughtered pigs, posing significant risks to the province's swine industry. Enhanced and sustained surveillance, strict biosecurity measures, and targeted educational campaigns are thus essential for controlling ASF and mitigating its socioeconomic impacts.

Assessment of Risk Factors for African Swine Fever in Gauteng Province

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African swine fever (ASF) is an infectious viral disease of porcine (pig) species, that might cause up to 100% mortality in infected domestic pigs and European wild boar. In sub-Saharan Africa, ASF has been endemic in some countries since 1926. The control area in South Africa was established in 1935, encompassing Limpopo, the northern regions of North West, KwaZulu-Natal, and the northeastern parts of Mpumalanga, as stipulated in the Animal Diseases Act of South Africa (Act 35 of 1984). However, in 2012 the first outbreak occurring outside of the control zone in South Africa was reported in Gauteng. These outbreaks are suspected to involve pig-to-pig transmission within the domestic cycle.

This study assessed the potential risk of ASF spread within Gauteng, focusing on areas currently known to be ASF-free and those exposed to ASF outbreaks between 2016-2022. In 2020 and 2021, interviews of 137 pig farmers were conducted in Gauteng on a voluntary basis. Data were collected through interviews and questionnaires, addressing farm characteristics, herd management, biosecurity practices, pig trade and ASF awareness. Farmers were categorized based on their experience with ASF and disease status of their respective area.

Data collected indicated gaps in ASF knowledge, basic biosecurity as well as concerns about free-roaming pigs and informal trade in the area where these risk factors may increase ASF spread. The study identified challenges due to the COVID-19 restrictions and the voluntary participation of farmers and it was not possible to carry out a complete census of the areas selected.

According to the conclusion of the study, farmers in Gauteng province need to be trained on ASF epidemiology and control, pig management as well as encouraged to practice safe feeding and adopt appropriate husbandry and biosecurity practices. This study highlighted the importance of knowledge sharing to prevent risk of outbreaks.

African Swine Fever and Smallholder Farmers

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African swine fever (ASF) disease outbreaks have gained global importance in terms of the running of livestock farms. The 2012 and 2019 - 2024, ASF outbreaks in Gauteng are linked to the ASF domestic cycle, which consists of pig-to-pig transmission. Horizontal transmission between pigs is due to free-roaming pigs as well as pig-derived product movement, swill feeding, and the lack of adequate biosecurity measures. Smallholder farmers are faced with intensified vulnerabilities due to challenges in infrastructure, financial constraints, and limited access to veterinary services. The South African government has measures like quarantine and movement restrictions in place during outbreaks; however, compliance remains problematic. The objective of this study was to determine the potential risk of ASF spread within Gauteng with reference to Ekurhuleni municipality. A coordinated approach involving community engagement, education, and policy support proves vital in mitigating ASF outbreaks in Gauteng, creating awareness in known communal/peri-urban pig-keeping areas. Basic biosecurity measures such as the confinement of pigs, ensuring the safety of feed, and preventing the introduction of the virus via people and fomites can prevent ASF. Collaboration with stakeholders along the pig value chain is critical to identify culturally acceptable and economically viable biosecurity options to achieve better management of ASF. Interviews with pig farmers were conducted in Gauteng on a voluntary basis and the sharing of knowledge was accomplished through the involvement of extension officers, who play a key role in assisting farmers in accessing important information, which is then translated into advice and guidance for better farming practices.

WiBISS: A Tool to Estimate Losses Avoided to Pig Producers with Early ASF Wild Boar Vaccination – A Northern Italy Case Study

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African Swine Fever (ASF) is a lethal hemorrhagic viral disease affecting domestic pigs and wild boar, causing substantial economic losses to the global swine industry. Preventing ASF outbreaks in livestock farms requires strict biosecurity and control measures which result in high expenses and have not stopped the spread of the virus. This study focuses on analyzing the economic losses that could be avoided by introducing various wild boar vaccination scenarios in Northern Italy, where ASF virus genotype II has been present in wild boar since its first occurrence in 2022 and is continuously progressing.

We developed several interconnected models based on cellular automata, forming a tool named WiBISS (Wild Boar Immunization Simulation System), to simulate the impact of vaccinating wild boar populations on ASF control and its effects on trade restrictions that influence economic losses for farmers. The model integrates epidemiological data from the WAHIS database, vaccination data from the EU funded project VACDIVA and from the scientific literature, and economic data on pig production.
By varying key parameters such as vaccination radius, time to vaccinate, and vaccination rate, the results indicate that the maximum percentage in epidemic reduction is achieved with a vaccination rate of 75% and a vaccination radius of 40 kms.

This work highlights the practical implications of having a comprehensive tool to establish optimal vaccination strategies using mathematical and geospatial analysis techniques, thus protecting the swine industry from the impact of ASF.

Keywords
African Swine Fever, Disease modelling, Cellular automata.

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Re-analysis of Nigeria-RV502 ASFV Suggests a Relationship with a Vaccine Candidate

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Introduction: African Swine Fever is a contagious disease caused by the African swine fever virus (ASFV) the only member of the *Asfarviridae* family. The virus is deadly in domestic pigs and wild boar, and there is presently no globally accepted vaccine against the disease. The virus is classified into 24 genotypes based on p72 genotyping, all 24 genotypes are domiciled in Africa (east Africa), with only genotypes I & II escaping from the continent. The virus has a genome length ranging from 170kb to 194kb, and the variation in the genome size is due to multigene family (MGF) genes with more than 150 structural proteins. Nigeria first recorded the presence of the virus in 1997 (genotype I) and recently in 2021, genotype II was reported.

Method: Genotype II whole genome sequence: Nigeria-RV502 (OP672342.1) reported from Nigeria in 2023, accessed from NCBI and re-analyzed with a total of 210 whole genome sequences of the ASFV collected from NCBI, the sequences were aligned using MAFFT V.7 The online version, and a phylogenetic tree was constructed using iqtree v. 2.2.0 (GTR+F+I+G4 model chosen according to BIC) and visualised with iTOL.

Results: Out of the 210 whole genomes analyzed ASFV genotype II reported in Nigeria-RV502 (OP672342.1) forms a clade with the vaccine candidate ASFV Lv17/WB/Rie1 reported in Latvia in 2017 from a wild boar with GenBank accession numbers OR806651.1 and OR806652.1 with bootstrap value 74. These suggest that Nigeria-RV502 may have originated in Latvia, probably imported by farmers to be used as a vaccine or transported via travelling.

An Overview of African Swine Fever Outbreaks in South Africa (2016 to 2024)

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Since 2016, African swine fever virus (ASFV) emerged as a critical challenge for the pig industry in South Africa resulting in an increase of the number of outbreaks across the country. The first incursion of the disease into the ASF-free areas of South Africa occurred in 2016 with cases of ASF reported in the Free State and Northwest provinces. The disease subsequently spread to the northern Cape, Gauteng, Mpumalanga, Eastern Cape, Western Cape and finally KwaZulu-Natal. Persistence of ASF in these geographic regions is facilitated by the lack of strict biosecurity measures and the unrestricted movement of livestock. The socioeconomic impact is most severe in smallholder farmers, threatening food security. At least nine genotypes of ASFV have historically been present in South Africa. Surprisingly, the incursions of ASF into the ASF-free areas of the country were caused by viruses classified as genotypes I and II, which were previously not known to occur in South Africa. Genetic characterisation of the viruses recovered from outbreak in domestic pigs has identified three genotype I variants, each associated with similar but distinct geographical distributions and epidemiologies. Conversely, the ASFV genotype II first reported in South Africa in 2019 has remained genetically stable and is now largely restricted to the coastal provinces of South Africa. The failure to curb the spread of the disease has resulted in the establishment of a domestic pig epidemiological cycle in peri-urban settings in areas which were previously considered free of the disease and has necessitated amendments of the ASF Control Strategies in South Africa.

Duplicate of the first abstract under Epidemiology Posters

African swine fever in Sardinia: a happy ending story

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African swine fever virus (ASFV) is the etiological agent of the devastating disease African swine fever (ASF), for which there is currently no licensed vaccine or treatment available. On the Italian island of Sardinia, the disease has been endemic since 1978. The virus has been absent from circulation since April 2019 and in 2024 the Island completed the ASF genotype I eradication from all the involved populations (domestic pigs, wild boar and illegal free ranging pigs). Considering the illegal free-ranging pigs as the key populations for disease transmission, several control measures were put in place against these animals and to control the disease in the other two groups. A total of 159 culling actions were put in place against illegal free-ranging pigs and 5,645 animals were culled between 2017 and 2022. In addition to these measures, a robust control network was established on domestic pig farms to improve compliance with the requirements for pig identification and registration. In particular, proper record-keeping in the holding registers for all newborn piglets, along with timely notification to the competent authority, was enforced. Ultimately, the main eradication goal was achieved by applying the EFSA exit strategy for the wild boar population. Targeted passive surveillance, involving the active search for carcasses within a specific timeframe and in high-risk areas, was implemented to provide timely evidence of virus eradication. A specific protocol for passive surveillance was developed, outlining different strategies depending on the type of terrain, the number and experience of people involved, the presence of dogs (trained or untrained), and the prevailing climate. The eradication of a disease is a

complex process which requires a sensitive surveillance system. Otherwise, the design of a sufficiently sensitive surveillance system requires a solid understanding of the epidemiology related to the local eco-social context, especially in the absence of virus detection. A fruitful collaboration between politics and healthcare is absolutely necessary to achieve the main goal.

AFRICAN SWINE FEVER IN LATIN AMERICA AND CARIBBEAN: Innovative approach for awareness campaigns.

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The ASF emergency in the Americas constituted a great challenge for all countries due to the relevance of pig production for small-scale farmers and the potential impact on their livelihood means if affecting their animals.

Appropriate risk communication actions are essential for stakeholders' behavioral changes in their attitudes and practices, and more specifically tourists and farmers.

Social media and platforms are a reality for communication strategies worldwide, allowing customization according to audiences while maximizing the impact of the messages to promote active compliance and risk practices reduction.

YouTube campaigns based on cartoon characters and simple messages proved highly appealing and attention catching for tourists and small farmers; by defining geographic locations and mandatory first and second hot messages, national campaigns proved highly effective at low cost.

Lessons learnt showcased the tool for further use on other diseases and health priorities to promote good practices and attitudes adoption and compliance along a variety of audiences.

Status of African swine fever post outbreak survivorship among domestic pigs in Nigeria

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African swine fever (ASF) is an acute hemorrhagic disease of pigs causing varying degrees of mortality depending on virulence of the circulating virus. In some studies, survival has been associated with certain breeds of pigs. However, this is not well documented in Nigeria despite suggestions that survivor pigs may potentially be involved in the persistence of the disease among pigs. This study was therefore aimed at documenting survivorship following ASF outbreaks in pig farms in Nigeria as well as the breeds which survive. A cross-sectional survey was carried out in 37 communities across 9 pig producing states in Nigeria between April and September 2019. We administered semi-structured questionnaires to 335 pig farmers to get information about pig breed survivorship in communities with previous records of ASF outbreaks. Data variables included experience of ASF outbreak, pig breeds, herd size at time of outbreak, number that died and survived as well as action taken on the survivors by farmers. Results were summarized using descriptive statistics in R. Out of 166 farmers who reported having outbreaks, 76 farmers had 100% mortality and 90 had survivors. Given the wide range of survivors (1-50 pigs/farm), the total average survival rate of pigs was 19.27%. We also found that the breed most associated with survival from the visited farms was the Nigerian indigenous pig breed (57.95%) followed by farms with mixed breed populations (25%), then exotic breeds (17.05%). 79 (87.78%) farmers sold their survivors to reduce losses, 4 (4.44%) slaughtered for personal consumption but only 7 (7.78%) kept theirs on the farm. There is a need for further investigations into the survival strategy of the local breed to ascertain if they are suitable for production as ASF tolerant breeds.

Estimating the sensitivity of the surveillance system to provide evidence of African swine fever free-status in the final stage of disease eradication

Federica Loi and Stefano Cappai

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Early disease detection and estimation of the sensitivity of the surveillance system are mandatory to provide evidence of disease eradication. Quantifying the sensitivity of early detection surveillance allows important aspects of the performance of different systems, approaches, and authorities to be evaluated, compared, and improved. This work tested a new approach to provide evidence of African swine fever (ASF) absence, quantifying the overall sensitivity of the surveillance system, in terms of population coverage, temporal coverage and detection sensitivity. ASF is a devastating disease, resulting in the high mortality of domestic and wild pigs. ASF has been endemic in Sardinia for more than 40 years, and the last ASFV detection dates to 2019. A practical interpretation of the strategy was implemented based on the failure probabilities of wrongly declaring the freedom of an area even if the disease agent is still present but undetected. Sensitivity analyses were performed using different design prevalence combinations to examine the impact of the assumptions under the null hypothesis. The results suggest that the surveillance system was able to detect virus circulation at a design prevalence below 1% and 2%. High values of negative predictive values (>95%) demonstrated that we could be confident affirming that the Sardinian domestic pig farms and wild boar population are free from ASFV from 2019 and 2021, respectively. The results of the analysis indicated that the confidence in the surveillance system was very sensitive to the design prevalence assumptions under the null hypothesis. Indeed, the role of passive surveillance as target was confirmed in terms of efficacy in detecting the virus, and amount of sample needed. Otherwise, its sustainability during several years is currently under discussion.

Balance of an eradication campaign of a sporadic outbreak of African swine fever that occurred in Portugal in an area with field persisting *Ornithodoros erraticus* tick reservoirs

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In April 1999, a sporadic outbreak of African swine fever (ASF) occurred in the Southern Region of Alentejo in Portugal, although the country was officially declared free of ASF in 1993. The outbreak was detected on an Iberian pig farm (Farm 1) and later identified in an adjacent farm (Farm 2) and in another farm (Farm 3) located 5 km away, owned by the same producer as Farm 1.

Investigations revealed heavy infestations of soft ticks (*Ornithodoros erraticus*) on Farms 1 and 2. Ticks collected via CO_2 traps tested positive for the ASF virus. After ruling out human transmission, contaminated garbage food, or direct contact with domestic pig and wild boar, the veterinary services determined the outbreak likely originated from the repopulation of premises that had been depopulated for several years but harboured persistently infected soft ticks. Farm 2 was infected via fence-line contact, while Farm 3 was likely exposed through the movement of animals or fomites from Farm 1.

Quarantine measures were immediately implemented after the laboratory results confirmed the infection, and protection (3 km) and surveillance (10 km) zones were established. A census of all farms within these zones was conducted together with epidemiological questionnaires and serological surveys (3,653 sera from 128 herds), with no further positive results. The three infected farms, as well as all farms within the protection zone, were depopulated. In total, 1,445 pigs were culled, and 102 producers received compensatory payments. Additionally, in this zone the premises of Farms 1 and 2 were demolished, as no effective method for disinfesting *O.erraticus* ticks is currently available.
The prompt action by official services, supported by local authorities and diagnostic laboratories, led to the successful eradication of the sporadic outbreak within just two weeks.

Semi-quantitative risk assessment of African swine fever introduction into Trinidad and Tobago through legal importation of swine products

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Spread of transboundary diseases like African swine fever (ASF) continues to be a challenge to the global swine industry. The Caribbean region remains under threat of ASF spread after its introduction into the Dominican Republic and Haiti in 2021 and further movement of the virus across Europe and Asia. This study aims to identify countries that pose a high risk of introduction of ASF virus through annual importation of swine products into Trinidad and Tobago (T&T). Data were collected from the Central Statistical Office of T&T in February 2024 which included types and amounts of swine products imported and exported per year and origin of products from 2018 to 2022. Disease notifications and surveillance data were retrieved from World Animal Health Information System (WAHIS). Probability scores were generated for detection and surveillance of ASF at country of origin, as well as detecting survivability of ASF virus in the imported product. Using a model built in R, the adjusted probability was calculated that ASF virus will be present in swine products originating from each country. The results show a relatively low probability of importation of ASF virus from swine products into T&T from the United States and Canada. Limitations of this model exist where country reporting to WAHIS and details of locally imported swine products are not precise. The risk assessment should be regularly revised and updated as conditions used in the model are not constant. The findings of this study can be used by officials in making risk-based decisions concerning imported animal products. This will lead to improved prevention and control strategies that will reduce the risk of entry of ASF virus into Trinidad and Tobago.

Keywords: African swine fever; importation; swine products; semi-quantitative risk assessment; Trinidad and Tobago

Epidemiological investigation of an African swine fever outbreak on a commercial farm in Uganda

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Introduction: In Uganda, the increasing pig production is contributing to food security and household income for many people. The majority of pigs are kept by smallholder farmers, but a few commercial farms exist in the country. African swine fever (ASF) is endemic in Uganda and remains a big threat to the pig industry. This study describes the epidemiological investigation of two ASF outbreaks at a large commercial farm in central Uganda in 2021 and 2022.

Methodology: The study comprised farm observation, analysis of farm records, interviews with farm staff to assess the pig movements and biosecurity protocols within the farm, and laboratory testing of biological and feed samples. Samples of whole blood from suspected ASF cases and in-contact pigs, and tissues (spleens and lymph nodes) from dead pigs were collected in recommended sterile containers. Feed samples were collected from trailers while being offloaded into silos and from mills at the feed manufacturing company during loading. Collected samples were transported to Collage of Natural Sciences in Makerere University for laboratory analysis. Using PCR-Real time, genomic DNA was extracted from blood, tissue and feed samples to test for presence of ASFV. We further analyzed the spatial and temporal pattern of ASFV positive samples in the two outbreaks.

Results: The investigations showed that the farm had high levels of external and internal biosecurity. Laboratory results showed 1,121 and 241 biological samples positive for ASF-virus (ASFV) in the 2021 and 2022 ASF outbreaks respectively. Furthermore, 6 feed samples tested positive for ASFV in 2022.

Discussion: The results indicate that ASFV could have been introduced to the farm through contaminated vehicles and feeds respectively.

Conclusions: The study underlines the high risk for introduction of ASF at a commerical pig farm in an ASF-endemic setting, and the importance of everyday adhernce to exisiting biosecurity routines.

National Biosecurity Plan for the swine value chain in the Dominican Republic: Modernization and resilience

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The National Biosecurity Program (BNP) was born from the need to reinforce and improve biosecurity conditions in swine farms in the Dominican Republic and modernize the productive structure of the value chain, reducing the prevalence of ASF in the country and mitigating the risk of dissemination. It currently covers about 82% of the country's pork production with more than 600 registered commercial farms.

The methodology applied in the swine biosecurity program includes epidemiological analysis to select and prioritize the intervention areas, viral circulation sampling to validate the non-presence of the ASF virus in the intervened farms, training, application of the biosecurity checklist in commercial and non-technical farms, through which 17 criteria and 75 variables are evaluated (See Figure 2). The properties must meet a minimum score of 175 points (70%) and 100% compliance with the "Non-Negotiable" criteria as requirements to achieve certification as a Biosecure Property.

Currently, the National Biosecurity Program (PNB) of the Dominican Republic has 609 farms registered; 582 biosecurity evaluations have been carried out, the results of which have improved criteria with greater vulnerability, such as the construction of perimeter fences, the establishment of parking areas outside the perimeter fence, the prohibition of the entry of vehicles outside the farm, and providing equipment and boots for the exclusive use of farm employees and visitors.

The control of the entry of genetic material, the implementation of cleaning, washing and disinfection protocols, and the implementation and monitoring of sanitary and biosafety procedure manuals, among other aspects are included in the plan. The farms enrolled in the PNB Program have improved and raised their biosecurity conditions by 35% compared to January 2024, which has allowed 4 farms to be certified as Biosecure Farms and another 20 farms are close to their certification.

In conclusion, 82% of the technified swine farming in the Dominican Republic is part of the BNP, whith the first 4 technified properties as Biosecure Properties. There have been no positive cases of ASF in the properties that are part of the BNP. The establishment of hygiene and disinfection protocols in each area of the production system has been implemented and improved in 36% of the farms that are part of the BNP. The use and installation of bird netting to prevent the entry of birds has been improved and implemented in 48% of the farms. Biosecurity is the best tool to protect swine farming in the Dominican Republic.

Assessment of carbon dioxide traps for collecting *Ornithodoros* ticks from warthog (*Phacochoerus africanus*) resting sites in Subsahara Africa.

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In East and Southern Africa, African swine fever (ASF) virus is maintained in an ancient sylvatic cycle involving warthogs (*Phacochoerus* spp) and soft ticks of the genus *Ornithodoros*.Confirmation of the occurrence of the ASF sylvatic cycle in Sub-Saharan Africa has traditionally relied on the manual collection of ticks from warthog resting sites Although alternative methods such as carbon dioxide (CO2) traps have been used successfully elsewhere, to date this method has never been tested in the context of the sylvatic cycle of ASF in Africa. Therefore, the goal of our study was to evaluate the effectiveness of carbon dioxide (CO2) traps in different warthog habitats by comparing the number of ticks collected by traps as opposed to using the traditional manual method. Therefore, a total of 61 warthog resting sites (31 in natural burrows and 30 in anthropized house decks) were sampled in a wildlife game reserve for the presence of *Ornithodoros* ticks with consecutive implementation of the manual and CO2 trap method during two seasons (wet and dry). The number of ticks collected with CO2 (n=2024) was significantly higher than those collected with the manual method (n=871, $p < .001$). Moreover, the number of ticks collected from decks (n=1967) was significantly higher ($p < .001$) than the number collected from warthog burrows (n=928). Our results suggest that CO2 traps are highly efficient for collecting *Ornithodoros* ticks from warthog habitats in our study area. They also suggest that warthogs can adapt to different levels of habitat transformation and human presence without any detectable impact on the tick-warthog association. The standardised use of this method could facilitate investigations on the distribution of tick-related ASF cycles in sub-Saharan Africa and improve our understanding of the epidemiology and ecology of ASF and other *Ornithodoros* tick-borne diseases.

Understanding the spatial transmission of the ASF virus under different ecological and production conditions

Hadi Taghvafard, Gert-Jan Boender, Thomas Hagenaars, Jose Gonzales Rojas

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During the past years, the African Swine Fever Virus (ASFV) has been spreading from Eastern Europe towards Western Europe. In this regard, there is a crucial need for understanding the mechanisms contributing to the spatial spread of the ASFV (i) between wild boars, (ii) from wild boars to domestic pig farms and vice versa, and (iii) between domestic pig farms. To this end, a robust analysis of the spatial transmission of ASF from the existing epidemics is necessary. Transmission kernel models provide such an analysis summarizing in a distance-dependent probability function the spatial transmission of diseases in different populations. We fitted five different types of transmission kernels to epidemic data of five European countries with different ecological and production systems. The estimated parameters characterize spatial transmission in these countries and can be used to build transmission risk maps and evaluate different control strategies (e.g., vaccination) suitable for each of the studied countries. Our analysis shows that (part of) the estimated kernel characteristics are specific for each studied country. To understand these differences between countries, several epidemiological causes for these differences are hypothesized and further analysis strategies are proposed for generalization of the transmission kernel characteristics.

From national parks to international borders: Unveiling the silent threats, high-risk zones and seasons of African swine fever in Uganda

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Ministry of Agriculture Animal Industry and Fisheries

African Swine Fever (ASF) remains a major threat to pig farming in Uganda. Routine surveillance is essential in characterizing epidemiology ASF as detailed by the FAO/OIE progressive control pathway. In this study, we utilized routine surveillance data from the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) to spatio-temporally map and identify the associated risk factors of ASF.

A total of 368 samples were analyzed at NADDEC for ASF using PCR or ELISA. The prevalence, as well as risk factors were assessed. Odds ratios (OR) were calculated using binomial regression to identify potential predictors of ASF outbreaks.

The overall prevalence of ASF was 25.54%. The Northern region had the lowest prevalence (9.6%) and the West the highest (36.4%). Seasonal variation was notable, with a higher prevalence during the dry season (37.4%) compared to the wet (14.9%). Districts neighboring national parks had a prevalence of 46.6%, compared to 17.4% in districts without proximity to parks. Similarly, districts at international borders recorded a prevalence of 41.2%, while non-border districts reported 22%.

The central, eastern, and western regions were 2.1, 4.5, and 5.4 times more likely to experience ASF than the northern region. The dry season was 3.4 times more likely to have ASF outbreaks than the wet season. Districts neighboring national parks were 4.1 times more likely to report ASF, while districts at international borders had 2.5 times higher odds of ASF outbreaks than those away from parks and borders respectively.

High-risk districts, such as those the western region, districts near national parks, and border districts, as well as the dry season, require targeted intervention strategies such as enhanced biosecurity and education. Focused surveillance in these areas could play a pivotal role in mitigating the impact of ASF outbreaks.

Advances in molecular epidemiology of the African swine fever virus in Rwanda and Tanzania

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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. The domestic pig industry in Rwanda and Tanzania is highly threatened by ASF, with several outbreaks reported yearly to the World Organisation for Animal Health. Despite the endemic status, no ASFV from Rwanda has been genetically characterized, and few complete genomes of ASFV from Tanzania have been described. This study reports, for the first time, the ASFV genotypes causing outbreaks in Rwanda and the complete genome sequences of ASFV from Tanzania. The ASF confirmation was performed by polymerase chain reaction followed by molecular characterization. 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. The first complete genome sequence of ASFV genotype XV was also described. In addition, the first Tanzanian complete genome of ASFV genotype IX and three ASFV strains belonging to genotype II collected during ASF outbreaks in domestic pigs in Tanzania were determined. The extension of the geographical range of genotype II in eastern Africa is of concern. This genotype was reported in Tanzania at the Tanzania-Malawi border in 2011, followed by a relentless spread of the virus northwards along major highways threatening neighboring countries. In addition, the ongoing spread of ASFV genotype IX across Africa poses a risk of spreading beyond the continent and potentially impacting the domestic pig industry globally. The results of this study provide insights into the genomic structure of ASFV circulating in Rwanda and Tanzania and can be used to monitor changes within the ASFV genome and improve our understanding of ASF transmission dynamics for improved prevention and control.

An Overview of the U.S. Swine Health Improvement Plan

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The United States Swine Health Improvement Plan (US SHIP) is a United States Department of Agriculture (USDA) funded pilot program to establish a voluntary health certification program for swine. The concept for the program was born out of a case study conducted by Iowa State University in 2018; it has since developed into a widely adopted pilot program, with 36 participating states and more than 12,000 enrolled swine sites. The program is intended to be a cooperative initiative between State, federal, and industry partners and is modeled after the US poultry industry's National Poultry Improvement Plan (NPIP). The key program goals are to improve the health of the US swine herd, increase swine traceability, and facilitate maintenance and resumption of trade following an African swine fever (ASF) or Classical swine fever (CSF) incursion. Participants are certified based on compliance with Program Standards in the areas of biosecurity, traceability, and sampling and testing. The Program Standards have been adopted, and can be updated, via a vote of the delegate body at the annual house of delegates meeting. The pilot program is administered at a local (State) level with national coordination and oversight. USDA is currently in the rule-making process to migrate the pilot program concepts into a USDA-codified animal health program.

Biologging as a surveillance tool for ASF monitoring in wild boar: first insights from a Sardinian Case Study

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The early detection of wildlife diseases is critical for mitigating their spread and protecting animal health. This project focuses on developing and implementing a real-time monitoring system for free-ranging wild boar in Sardinia, with a specific emphasis on African swine fever (ASF). Although biologgers have been shown to detect ASF-induced behavioral changes under controlled conditions, their deployment in natural settings poses significant challenges, including energy optimization, reliable data transmission, and functionality in rugged, remote terrains. Sardinia's ecological and epidemiological landscape provides an ideal context for testing these advancements, given the central role of wild boar in ASF dynamics.

We tested accelerometer-equipped eartags and collars capable of detecting sickness behaviors and transmitting data to a cloud-based platform for near real-time analysis. By addressing the challenges of biologger deployment in natural environments, this project aims to demonstrate their potential as early warning tools for wildlife disease surveillance. The resulting framework integrates activity and movement data to offer wildlife managers and researchers actionable insights, contributing to improved ASF management and advancing disease surveillance systems.

Community African swine fever Biosecurity Interventions in Kosovo

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Food and Agriculture Organization

In order to improve the knowledge on African swine fever and improve the biosecurity among backyard and small-scale pig producers in Kosovo, we used an approach termed the “Community African swine fever Biosecurity Interventions” (CABI) by the Food and Agriculture Organization (FAO).

Under the CABI Kosovo 62 backyard pig farmers were involved. Between 18 and 31 October 2024 all farmers were surveyed to assess their pig production, knowledge on ASF and farm biosecurity. Afterward trainings were held with the following themes: ‘Feeding and Breeding of pigs’ (16 – 20 December 2024); ‘Pig diseases and ASF’ (20 – 22 January 2025) and ’Biosecurity and cleaning and disinfection’ (27 – 29 January 2025). Furthermore, pig farmers are supplied with ‘Biosecurity intervention kits’ consisting of items needed to improve their biosecurity, including boots, brushes, detergents and disinfectants. This is followed by visits to each of the pig farms to support the pig producers in the adoption of new procedures. At the end of the intervention farmers will be surveyed again to measure the change in ASF knowledge and farm biosecurity between 24 February – 15 March 2025.

While the current abstract only outlines the methodology, by April the analysis of the surveys will be completed to present the changes in ASF knowledge and farm biosecurity as well as summarizing lessons learnt from the intervention conducted among backyard and small scale pig farmers in Kosovo.

(All references to Kosovo should be understood to be in the context of United Nations Security Council resolution 1244 (1999).)

Estimation of the sensitivity of the surveillance system used to inform actions against African swine fever in northern Italy

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African swine fever (ASF) is a devastating infectious disease of suids that is spreading worldwide, causing huge economic losses. Approximately three years ago, ASF affected the wild boar susceptible populations in Italy. In this context, understanding the accuracy of the surveillance applied across the Italian territory is crucial for informing disease control measures. The main goal of this work was to develop specific tools aimed at measuring the sensitivity of the surveillance applied within the northern Italian ASF cluster. For the purpose, data on wild boar carcasses, including those culled or killed in road traffic accidents, were retrieved from the official Italian database. A four-month period (August-November 2024) was considered to establish baseline estimates. The Wild Boar Management Units were identified as epidemiological units. The methodology employed a scenario tree approach to evaluate the surveillance system, assuming that different components of a population have varying probabilities of infection and detection. Thus, among the identified groups, found dead wild boar (carcasses) were identified as the highest-risk group compared to hunted animals. Additionally, within the found dead group, animals killed in road traffic accidents were considered to have a lower probability of illness. Relative risks (RR), were calculated using a Poisson model and adjusted for age and seasonality, to quantify the difference in the probability of ASF positivity among the three wild boar' categories. Surveillance sensitivity was then calculated using a binomial distribution for each group of animals tested and for each risk factor, using the actual probability of infection. Currently, sensitivity values for WBMU are updated monthly, incorporating surveillance data from the most recent four-month surveillance period. These values are then used to modulate depopulation and wild boar management activities for the purpose of eradication.

Enduring the epidemic: Disease tolerance and pig breed selection in Uganda's African swine fever hotspots

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The pig population in Uganda almost doubled from 2016 to 2021 by increasing from 4.0 million to 7.1 million respectively, despite African swine fever being endemic in the country with frequent reported outbreaks. This study participatorily analyzed Uganda's pig production systems and farmers' livestock and pig breed preferences among smallholder pig farmers. Data were collected from 210 farm households using a simple random sampling method from purposively selected villages and parishes in Masaka and Tororo districts, which are ASF hot spots in Uganda. Semi-structured questionnaires were used to collection data on farm and farmer demographics; production systems, and farmers' pig breed preferences. Framers ranked their livestock and pig breed preferences and production constraints. Data analysis was descriptive for farm and farmer characteristics, and indices of weighted averages of all rankings of each livestock, production constraints, and pig breeds were computed. A sharp contrast in production system used and breed preferences between Masaka and Tororo districts was revealed. Farmers' choices of breeding boars and sows in the two districts were influenced mostly by disease tolerance, an adaptive trait. This study's findings can aid in creating stakeholder-participatory breeding programs to enhance pig production, ASF control, and household income in Uganda.

Key words: Breed preference, indices, weighted averages, extensive, semi-intensive, endemic.

Two complete African Swine Fever Virus Genomes Isolated from the 2023 Outbreak demonstrate the First Report of Genotype II in Benin

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OBJECTIVE: The aim of this study was to characterize the isolates from an outbreak in Benin

METHODS: Two isolates (BEN-AACB2 and BEN-OPNB1) were collected from the 2023 outbreak in swine in southern Benin (Department of Atlantique), which were thought to have perished from ASF. DNA was extracted and DNA Libraries were constructed for illuminia using the Nextera XT kit and Nanopore, with the Rapid BC Kit v 14 (ONT), and R10.4 flow cells. *De novo* assembly was performed using SPAdes and Genomes were annotated using the default settings of TheTransporter.

RESULTS: Each sample's minion reads and two sets of illumina paired-reads resulting in a 180,642 (BEN-OPNB1) and a 184,758 (BEN-AACB2) nucleotide length contig each with a GC content of 38.6%. Both genomes were Genotype 2 (historic genotype II) and Biotype 2. Both genomes exhibited the 14 gene deletion that has been observed in the Georgia variants causing outbreaks in western Africa and analysis of the 3' end of the genome revealed both genomes were more similar to the Ghana 2022 isolates than Nigeria-RV502 as they did not contain the reverse complement of the 5' region.

CONCLUSION: This study presents insight on African swine fever dynamics in Benin. It constitutes a guide for the country to establish action plan for better managing the disease for the pork industry protection. To the scientific community, research should focus on developing local vaccine to rapidly tackle the emergence of the strain circulating.

Keywords: African swine fever, status, Biosecurity, control, Benin.

Network analysis of the pig transport data in the Netherlands: towards a dashboard for tracing during the high-risk period

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Animal movement is a key factor in spreading infectious diseases. After the detection of a notifiable disease, a contingency plan should be activated to mitigate the risk of spread from the index case. Also, animal movements during the critical timespan between the disease introduction and detection, the so-called “high-risk period” (HRP), should be inventoried. We describe the conceptualization and first steps towards implementing a dashboard using network analysis which can be used for forward and backward tracing of animal movements during the HRP of an outbreak. We used data of pig transports in the Netherlands, between 2019-2022. The network was set up as a dashboard application in Shiny in R. The user selects the date range (HRP), index premises, and depth of the network (shortest number of steps out/in-going concerning the focal node), and filters for incoming or outgoing movements. The resulting network shows only the more intuitive and relevant parameters for the dashboard's goal including the attributes: indegree, outdegree, betweenness, depth, geographic location, and farm type. The Dutch authorities were asked to test the dashboard using an African swine fever outbreak simulation. The dashboard was received as an intuitive dashboard with a clear visualization, although some of the network attributes were not known before. The authorities indicated that the main added values of the dashboard were the quick and easy access to deeper levels of the animal movements, rather than only focusing on the direct contacts during the current tracing, and the map showing the geographical location of all connected premises. The dashboard is an intuitive tool with added value for tracing and prioritizing capacity during the HRP of an outbreak. Further improvements such as including data on farm visitors and feed trucks, and the implementation in ASF and CSF contingency plans are under discussion.

Enhancing African swine fever control strategies in South Africa: contributions to disease introduction and spread by smallholder pig farmers

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In South Africa, an alarming increase in the intensity of African swine fever (ASF) epidemics has been observed over the last decade and continues to overwhelm established disease control efforts by veterinary services. New research has determined that these events are linked to an emergent pig-pig transmission cycle driven by high-risk pig management practices often associated with resource-poor production systems. In the heterogenous non-commercial pig sector, local ASF transmission dynamics are poorly understood, and risks remain unquantified.

It is believed that the South African pig farming sector can be divided into multiple farm profiles with distinct husbandry, biosecurity and trade practices which vary significantly between regions. This study aims to address knowledge gaps relating to small-scale pig farm demographics, production practices and trade, to determine the contribution of these variables to the pig value chain.

In 2024, 770 semi-structured electronic questionnaires were administered by veterinary services officials throughout Mpumalanga Province, South Africa, to evaluate the knowledge, attitudes, and practices of smallholder pig farmers. Survey themes explored potential determinants of disease, with a strong focus on identifying environmental and anthropogenic drivers of ASF outbreak occurrence. Supplementary swine census and movement data were accessed through government authorities and industry. Descriptive and multivariate analyses were used to evaluate relationships between management practices and the risk of introduction or spread of ASF as well as to define farm profiles based on their distinguishing characteristics.

This study has improved our understanding of ASF transmission dynamics and risk factors in Mpumalanga Province, with applications to the rest of South Africa. The information collected will inform a spatially explicit agent-based disease transmission model for ASF epidemics, currently under development. This model will provide decision-makers with tools to inform and support ongoing national disease surveillance and control strategies.

Diagnostics

Development of molecular and antigenic-based rapid tests for the identification of African swine fever virus in different tissues

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African swine fever (ASF) is a severe haemorrhagic infectious disease affecting suids, thus representing a great economic concern. The disease is caused by a large and complex double-stranded DNA virus (genus *Asfivirus*, family *Asfarviridae*). Many epidemics occurred during the last century, from the first records in Kenya (1907), Sardinia (1978-current), until the recent outbreak from Eastern to Central and Southern Europe. Considering the importance of early diagnosis, rapid point of care testing (POCT) for ASF is in high demand. In this study, we developed two strategies for the rapid and onsite diagnosis of ASF, based on Lateral Flow Immunoassay (LFIA) and Recombinase Polymerase Amplification (RPA) techniques. The first one is based on sandwich-type LFIA exploiting a monoclonal antibody directed to the p30 protein of the virus (Mab). ASFV protein is captured by Mab anchored onto LFIA membrane and a secondary antibody to detect Mab-p30 complex, if formed. The initial use of the same antibody for protein capture and complex detection showed a significant competitive effect for antigen binding. To solve this problem, minimizing reciprocal interference and maximizing the response, an experimental design was applied. This approach allowed us to develop the most sensitive test achievable with the available materials. The second one, based on RPA assay, required the employment of primers to the capsid protein p72 gene, an exonuclease III probe and was performed at 39 ◦C. Using a plasmid encoding target gene a limit of detection of 5 copy/µL was achieved. The devloped LFIA and RPA were applied for ASFV detection in the usually analysed animal tissues such as kidney, spleen, and lymph nodes. For sample preparation we used a simple and universal virus extraction protocol, followed by DNA extraction and purification for the RPA. Regarding LFIA, the in-field addition of 3% H2O2 is sufficient to limit matrix interference and prevent false positive results. The two rapid methods are performable in just 25 min (RPA) and 15 min (LFIA). An high diagnostic specificity (100%) and sensitivity (93% and 87% for LFIA and RPA, respectively) was obtained for samples with high viral load (Ct < 27). False negative results were observed for samples with low viral load (Ct > 28) and/or also containing specific antibodies to ASFV, which decreased antigen availability and were indicative of a chronic and poorly transmissible infection. The simple and rapid sample preparation and the diagnostic performance of the LFIA are key points to suggest its broad applicability for POC diagnosis of ASF.

A new RT- PCR for of Classical Swine Fever Virus detection in Swine and Wild boar, allowing for parallel testing with the ID Gene ASF qPCRs.

Anna Greatrex, Léa Despois, Loïc Savarit, Emilie Bianchini, Adrien Limozin, Loïc Comtet, Philippe Pourquier

Classical swine fever (CSF) is a contagious viral disease affecting domestic and wild swine, caused by the CSF Virus (CSFV).

Swift implementation of control measures relies on reliable and accurate diagnostics to detect and prevent CSFV spread. Innovative Diagnostics introduce hereafter a rapid and specific RT-qPCR, the ID GENE™ Classical Swine Fever Virus duplex, for detecting CSFV RNA. Its protocol is compatible with our ID Gene® African Swine Fever Duplex (IDASF) and ID Gene® African Swine Fever Triplex (IDASFTRI) kits, enabling parallel testing for CSFV and ASFV from the same extracts. This is particularly valuable as both diseases share similar clinical patterns.

The test yields result in 65 minutes (rapid amplification protocol). Analytical specificity was confirmed with 21 CSFV strains (FLI), 11 CSFV isolates (ANSES), and 62 other pathogens, including BVD virus and PRRS virus. The Limit of Detection (LDPCR) used a synthetic RNA fragment, and the Method Detection Limit (MDL) involved swine samples (blood,serum,spleen) spiked with a modified live virus vaccine (PESTIFFA - Boehringer). Diagnostic specificity and sensitivity were assessed using 66 samples.

The ID GeneTM qPCR reliably detected all CSFV strains with 100% inclusivity and exclusivity, showing no cross-reaction with other pathogens. The LDPCR was 4 copies/PCR (95%), and the MDL on swine blood was 4.103 copies/ml using the ID Gene™ Mag Fast Extraction Kit. Diagnostic sensitivity and specificity were both measured at 100%.

The RT-PCR kit demonstrates excellent performance and user-friendliness, including a single reaction mix with an internal control. For differential diagnosis, samples can be concurrently processed with our ASF PCRs (IDASF/IDASFTRI) on the same plate. The kit has received approval in Germany from the Friedrich-Loeffler-Institute (FLI C-106). When combined with the ID Screen® CSF E2 Competition, Innovative Diagnostics provides a comprehensive solution for CSF diagnosis and vaccination monitoring.

The ACDP Swine Diseases PCR and African Swine Fever Serology Proficiency Testing Program

Kristen McAuley, Mai Hlaing Loh, Julie Cooke, James Hollier, Jude Wilson, David T. Williams

The CSIRO Australian Centre for Disease Preparedness (ACDP) plays a vital role in infectious disease control through its ISO 17043-accredited Proficiency Testing (PT) programs and diagnostic reference materials (RM). This role contributes to strengthening laboratory capability and capacity across the Asia-Pacific region by ensuring the accuracy and reliability of diagnostic testing for high-risk pathogens, including African Swine fever (ASF). Aligned to its WOAH African swine fever Reference Laboratory, ACDP provides and coordinates a swine disease PCR PT program consisting of stable, inactivated, lyophilised samples of ASF virus, classical swine fever virus, porcine reproductive and respiratory syndrome virus and swine influenza virus. Laboratories from seventeen countries in the Asia-Pacific region enrolled in this program in 2024. From 2025, ACDP will also provide an ASF serology panel for detection of antibodies to ASF virus, comprising antisera derived from pigs experimentally infected with genotype I and genotype II ASF viruses. The ACDP PT program helps to maintain cutting-edge diagnostic capabilities critical for the prevention, control, and eradication of ASF and other swine disease that pose significant threats to pig production and wild suid populations in the Asia-Pacific region. The ACDP PT program is also available to laboratories from other regions of the world.

Laboratory validation of Real-Time PCR assays for detection of African swine fever in faecal samples

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African swine fever has significant impacts on pig industry worldwide, amplified by the absence of safe and efficacious vaccines, making a prompt and reliable diagnosis of paramount importance. Diagnosis targets virus-rich tissues: lymph nodes, bone marrow and spleen. Recent studies proposed non-invasive sampling of oral-nasal swabs and faeces for a faster diagnosis, requiring data on test performances. In this study, two WOAH-recommended ASFV Real-Time PCR (qPCR) assays were validated based on WOAH guidelines, providing analytical performances for swine faeces. Analytical specificity (ASp) was assessed *in silico* and *in vitro* with main swine enteric pathogens. Analytical sensitivity (ASe) was assessed by determining the limits of detection (LOD) using ten-fold diluted Armenia/07. Interference on ASe was analysed by spiking Armenia/07 in faecal suspension. Three manual extraction kits were used: High Pure PCR Template Preparation Kit [Roche] (Roche, Switzerland), QIAamp® Fast DNA Stool Mini Kit [Mini Stool] and AllPrep PowerViral DNA/RNA Kit [AllPrep] (QIAGEN, Germany). All DNA extracts were amplified with the WOAH-recommended methods (King and UPL) and the ID Gene™ African Swine Fever Duplex Kit (Innovative Diagnostics, France). The higher ASe (50 HAD50/ml) was detected using the Roche kit in combination with King and ID GENE™ but not with UPL (5·102 HAD50/ml). The ASe decreased of 1 Log testing faeces with Roche kit and King qPCR and the ID GENE™ showed the lowest Ct values compared to other methods. The ASe was further reduced of 1 Log with the Mini Stool kit and King qPCR. This study confirmed the robustness of the WOAH-recommended Real-Time PCR protocols with a complex matrix as faeces and showed the ID GENE™ provided comparable results. Validation tests are on-going with automated extraction methods. Further diagnostic sensitivity assessment is necessary to provide robust data on the inclusion of faeces and other non-invasive samples in ASFV diagnostic algorithm.

Field comparison of ASFV target tissues and non-invasive samples in wild boars during the genotype II Italian epidemic

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African swine fever (ASF) is a highly lethal viral disease affecting domestic and wild pigs, caused by a dsDNA virus (*Asfarviridae* family, *Asfivirus* genus). Virus detection typically targets organs such as the spleen, kidneys, lungs, tonsils, lymph nodes, and bone marrow from deceased animals. However, collecting these tissues in the field can be complex and may contaminate the surrounding environment. Recent studies based on experimental infections have focused on non-invasive (NI) samples (faeces, blood, oral, and nasal swabs) for easier field collection and faster diagnosis.

This study compared ASFV detection in target organs (spleen and kidney) and NI samples from wild boars during passive surveillance in an endemic area in North-West Italy in 2023-2024. NI samples were collected along with target organs from 172 dead wild boars, resulting in 1017 samples processed by Real-Time PCR. Preliminary analysis to quantify DNA copies was performed on a subset of samples using Digital PCR.

Sixty-six (38%) wild boars tested positive in target organs, and sixty-eight (40%) had at least one positive NI sample. Sixty-two (36%) wild boars showed Ct values below 30 in at least one NI sample, with nasal swabs generally having lower Ct values than other NI matrices. Two wild boars with negative target organs had at least one positive NI sample. Using target organs as the gold standard (a boar was positive if any organ tested positive), the sensitivity (SE) and specificity (SP) of the NI tests were: faeces SE=88.9% (78.4-95.4 95%CI), SP=100.0% (96.5-100.0); nasal swabs SE=92.4% (83.2-97.5), SP=99.1% (94.9-100.0), oral swabs SE=86.4% (75.7-93.6), SP=99.1% (94.9-100.0); blood swabs SE=93.9% (85.2-98.3), SP=98.1% (93.4-99.8). All NI tests showed very good agreement (Gwet's AC1>0.81) with the gold standard

This is the first field comparison of non-invasive samples and target organs in wild boars, suggesting the potential use of nasal and blood swabs.

Evaluation of a Portable Point-of-Care Test to Detect African Swine Fever Virus using EDTA blood and serum samples.

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African Swine Fever Virus (ASFV) poses a significant global threat to swine health and the pork industry. Effective monitoring and biosecurity measures are critical for controlling outbreaks.

The ZYTCA Ulfa™ ASFV test is a compact hand-held point-of-care diagnostic device that weighs only 500g and delivers results in 40 minutes. It is designed to process blood samples directly after a simple threefold dilution. The device employs loop-mediated isothermal amplification (LAMP) and uses a colloidal gold-based immunoassay for rapid and easy result interpretation. This integrated approach allows for quick, on-site diagnosis, enabling timely responses to ASFV outbreaks.

This study, conducted at CISA-INIA, evaluates the performance of the ZYTCA Ulfa™ ASFV test, using ASFV-positive/negative pig whole blood and serum samples. The primary objectives were to assess the test's analytical sensitivity and specificity, benchmarked against the WOAH-validated real-time PCR (Fernández et al., 2013).

We used 45 EDTA-blood samples from pigs experimentally infected with ASFV isolates of varying virulence levels and genotypes (I, II, IX, X, and XXIII). These samples represented a range of clinical presentations, from acute to chronic infections. To further validate performance across the infection timeline, an additional 50 paired serum and EDTA-blood samples were analyzed.

The ZYTCA Ulfa™ ASFV test demonstrated an overall analytical sensitivity of 86% (38/44) and sensitivity of 92.3% (36/39) when taking out weak-positive samples (Ct > 35). Analytical specificity was high at 95% (20/21), and the test effectively detected ASFV across different clinical stages, achieving 100% detection in acute cases (7–36 dpi), 50% in subacute cases (7–119 dpi), and 83% in chronic cases (10–126 dpi).

These results highlight the ZYTCA Ulfa™ ASFV test's potential as a robust, field-deployable diagnostic tool for ASFV detection for animals presenting acute stage infection.

Evaluation of a commercial real-time PCR kit to detect African Swine Fever Virus using various clinical samples and direct amplification using porcine serum.

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Real-time PCR is a gold standard for diagnosing African Swine Fever Virus (ASFV) due to its high sensitivity and specificity, enabling reliable detection of ASFV DNA in a wide range of sample types.

The UlfaQ™ ASFV Test is a commercial real-time PCR assay designed to amplify the ASFV *P72* gene using primers and double-quenched probes, with an internal control for result validation. It exhibits limit of detection (LoD) of 2 copies/reaction, ensuring high sensitivity. In addition, it includes a polymerase protection reagent that enables direct amplification of serum samples, eliminating the need for DNA extraction. This innovation significantly accelerates the workflow and reduces operational complexity.

Three independent clinical validation studies were conducted using 240 pig samples. These included diverse sample types such as whole blood (n=85), serum (n=83), cell-cultured ASFV virus (n=19), homogenized tissues (n=22), blood swabs (n=10), nasal/throat swabs (n=3), environmental swabs (n=2), and purified DNA (n=16). Samples were collected across China (Studies A and B) and the UK (Study C, conducted at the Pirbright Institute) and were benchmarked against WOAH-approved PCR methods for ASFV confirmation. Additionally, the direct amplification protocol was evaluated using four serum samples spiked with ASFV P72 plasmids and compared to the standard protocol.

The studies demonstrated that the UlfaQ™ ASFV Test has a diagnostic sensitivity of 97.6% (120/123) and specificity of 94.9% (113/119) across various sample types. Furthermore, the comparison between the direct and standard amplification protocols showed no significant differences in Ct values, highlighting the assay's robustness and accuracy even with simplified workflows.

In conclusion, the UlfaQ™ ASFV Test offers a reliable, sensitive, and efficient diagnostic solution for ASFV detection. Its ability to perform direct amplification of serum samples, combined with robust performance across clinical sample types, makes it a valuable tool for ASFV diagnosis in both laboratory and field settings.

Development of a Novel Indirect ELISA for the Serological Diagnosis of African Swine Fever Targeting p11.5 Protein

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African swine fever (ASF) is a highly lethal viral disease in pigs, with a mortality rate approaching 100%. As a result, it is designated as a notifiable disease by the World Organisation for Animal Health. The current vaccines have not yet been widely used globally due to safety concerns. African swine fever virus (ASFV) control and eradication solely depend on good farm biosecurity management and rapid and accurate diagnosis. In this study, we developed a novel indirect enzyme-linked immunosorbent assay (ELISA) using recombinant ASFV p11.5 protein as the target antigen. Compared to a commercially available serological ELISA, our assay demonstrated a relative sensitivity of 93.4% and specificity of 94.4% (N = 166). Furthermore, to compare the performance of the serological ELISAs, we conducted the assays on a panel of sera collected from pigs and boars experimentally infected with different ASFV isolates. The results indicated the greater sensitivity of the newly developed assay and its ability to detect anti-ASFV antibodies earlier after virus inoculation.

Co-circulation of Two Genotypes and Serogroups of African Swine Fever Virus in Nigeria

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African swine fever (ASF) is one of the most important diseases of domestic and wild pigs that is a threat to food security globally. The disease is associated with severe socio-economic consequences that threaten the livelihood of small scale farmers and the profitabiity of large scale farmers in Nigeria. The virus has been characterised into 24 genotypes based on p72 genotyping and all are found in Africa. The genome size ranges between 170 – 194kb with more than 150 structural proteins. Genotype I was first reported in Nigeria in 1997 and by 2020, genotype II was reported in the country. Therefore continous understanding of the epidemiology and dynamics of the circulating viruses is relevant to the control and future eradication programme. A total of 37 samples (tissue and blood) were submitted (passive surveillance) to the Biotechnology Centre of the National Veterinary Research Institute (NVRI), Vom from from March 2023 – October 2024 from 13 pig producing states. The B646L and CD2v genes that encode structural proteins p72 and CD2v, respectively were utilised to delineate circulating genotypes. The result obtained showed that of the 13 states studied, 12 had genotype I and II co-ccirculating while only one state had genotype I alone circulating. Similarly, serogroup 2 and 8 were circulating. The practical implication of the genetic variability of the Nigerian viral isolates is the need for continuous sampling and analysis of circulating viruses which will provide epidemiological information on the evolution of the virus for informed strategic control of the disease in the country.

Development of Long-Read Targeted Whole Genome Sequencing for African Swine Fever Virus

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African swine fever virus (ASFV) is a contagious pathogen that can cause high mortality in domestic swine and wild boar (*Sus scrofa*) populations. ASFV is a large double-stranded DNA virus with genome sizes ranging from 170-190 kilobases (kB), belonging to the family *Asfarviridae*, genus *Asfivirus*. ASFV outbreaks are mitigated through strict quarantine measures and culling of affected herds, resulting in massive economic impacts to the global pork industry. Current detection and genotyping methods provide little genetic information on circulating viral strains. Due to the reduced cost and availability of sequencing, it is vital to have a targeted whole genome sequencing protocol in place for the rapid complete genetic characterization of ASFV for outbreak and surveillance situations. In this study, a panel of 19 primer pairs that span the genome of ASFV was developed to amplify ~10kB amplicons. The primer pairs were further optimized for batch primer pooling and thermocycling conditions, enabling the whole genome amplification of ASFV with 5 different primer pools. The ASFV primer pools were tested on viral DNA extracted from blood and spleens collected from pigs experimentally infected with ASFV genotype II viruses. The amplicons were sequenced on the Oxford Nanopore MinION platform. The targeted whole genome sequencing protocol for ASFV resulted in an average coverage greater than 1000X for ASFV with 99% of the genome covered. The ASFV targeted whole genome sequencing protocol has been optimized for genotype II ASFVs; optimization for other genotypes is in progress. With this protocol the full genome of ASFV including analysis can be obtained within 36-48 hours from beginning to end. This targeted whole genome sequencing protocol will be an important tool to assist in early pathogen detection and whole genome characterization of this high-consequence porcine virus in outbreak and surveillance situations globally.

Differences in Clinical Scoring May Influence the Outcome of Experimental African Swine Fever Studies; Harmonization Needed?

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Survival rates and clinical scores are critical metrics in experimental African swine fever (ASF) studies. Alongside these, the timely application of humane endpoints (HEPs) is essential to safeguard animal welfare. However, research institutes often employ varying clinical scoring systems and HEP criteria, leading to differences in study outcomes. These differences pose challenges when comparing results, particularly in studies such as vaccine efficacy trials. This abstract highlights how variations in clinical scoring and HEP application can affect study outcomes.

As part of an ongoing EU project, a multicenter study was conducted at the Friedrich Loeffler Institute (FLI), Germany, and Wageningen Bioveterinary Research (WBVR), the Netherlands. The study evaluated the efficacy of three live-attenuated vaccines administered orally. While both institutes used clinical scoring systems with 9 (FLI) and 10 (WBVR) parameters that largely overlapped, a key difference lies in the HEP application regarding body temperature. Wageningen Bioveterinary Research did not include a temperature-based HEP, while FLI applied a HEP if the body temperature exceeded 40.5°C for three consecutive days.

At WBVR, 5 out of 30 vaccinated pigs survived the challenge and completed the study. If FLI's temperature-based HEP had been applied, 3 animals would have survived. Conversely, at FLI, 4 out of 60 pigs survived under their scoring system, but survival rates may have improved if WBVR's criteria had been used.

These findings underscore the impact of differing clinical scoring systems and HEP criteria on study outcomes. They highlight the need to harmonize these practices in ASF studies to ensure consistent results and uphold animal welfare.

Detection of Recombinant African Swine Fever Virus via Confiscated Pig Products into South Korea

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Since 2018, we have been engaged in the surveillance of pork products seized from travelers at airports, ports and international mail shipments, specifically targeting African Swine Fever Virus (ASFV) as part of our strategy to promptly identify and assess the risks associated with potential incursions. The samples, including processed pork products, are dispatched for analysis to the national ASF reference laboratory within the Animal and Plant Quarantine Agency (APQA), where we conduct tests for the presence of ASFV using the WOAH TaqMan qRT-PCR methodology. In this study, we identified 26 recombinant strains of ASFVs with a mix of genotype I and II within the pork samples from 2023 to 2024. All positive samples were of Chinese origin (with one case involving a product from China brought in from Vietnam). The PCR results showed CT values ranging from an average of 26.32 to 34.24. These recombinant strains, which exhibit genetic similarities, are categorized as p72 genotype I based on B646L gene; however, p54 gene (E183L) and the intergenic region (IGR) spanning the I73R and I329L genes were derived from genotype II viruses, but the virus has not been isolated. Following the Covid-19 pandemic, the number of international travelers has increased annually, largely influenced by the global prominence of the Korean Wave (K-pop, K-dramas, etc.). Many travelers, often unaware of the legal restrictions, are found to be transporting illicit pork products. Given the potential risks posed by these recombinant ASFVs through their illegal introduction into the domestic swine industry, stringent border control measures and continuous ASFV surveillance are essential for the development of effective containment strategies.

Development and preliminary evaluation of a highly sensitive method for detecting African swine fever virus in oral fluids from naturally infected raised pigs in Northern Vietnam

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Early detection and early slaughter through quarantine are essential to prevent the spread of the African swine fever virus (ASFV). Highly accurate testing is effective for early detection, but it is still difficult to establish a system, especially in the Global South. Pooled oral fluid tests have been used for simple pathogen monitoring, but compared to blood tests, the virus concentration in oral fluids is low, resulting in false negative and missing true positive cases. In this study, we collected oral fluids from sub-clinical raised pigs in northern Vietnam and attempted a highly sensitive ASFV survey using a newly developed virus concentration and detection method. In a spike test result, the developed method showed up to 100 times greater sensitivity than a reference method. To compare and evaluate the performance of the developed method, a total of 68 pooled oral fluid samples were collected, 63 from northern Vietnam and 5 from southern Japan. Using real-time PCR, 9/68 (13.2%) were positive by the reference method, and 23/68 (33.8%) by the developed method. Using real-time LAMP, 1/68 (1.5%) were positive by the reference method and 6/68 (8.8%) by the developed method. Therefore, the developed method improved the sensitivity of ASFV detection from oral fluids and enabled early diagnosis of pigs before the onset of the disease. The developed method has the potential to enable simple and highly sensitive diagnosis of ASF, which can contribute for its early diagnosis and early containment by rapid screening in clean and suspected farms.

Development of a Highly Sensitive Point-of-Care Test for African Swine Fever That Combines EZ-Fast DNA Extraction with LAMP Detection: Evaluation Using Naturally Infected Swine Whole Blood Samples from Vietnam

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While early detection and early containment are key to controlling the African swine fever (ASF) pandemic, the lack of practical testing methods for use in the field are a major barrier to achieving this feat.

We describe the development of a rapid and sensitive point-of-care test (POCT) for ASF and its evaluation using swine whole blood samples for field settings.

In total, 89 swine whole blood samples were collected from Vietnamese swine farms and the POCT using a combination of crude DNA extraction and LAMP (loop-mediated isothermal amplification) amplification was performed.

The POCT enabled crude DNA to be extracted from swine whole blood samples within 10 min at extremely low cost and with relative ease. The entire POCT required a maximum of 50 min from the beginning of DNA extraction to final judgment. Compared to a conventional real-time PCR detection, the POCT showed a 1 log reduction in detection sensitivity, but comparable diagnostic sensitivity of 100% (56/56) and diagnostic specificity of 100% (33/33). The POCT was quicker and easier to perform and did not require special equipment.

This POCT is expected to facilitate early diagnosis and containment of ASF invasion into both regions in which it is endemic and eradicated.

A Brisk Response Mobile Biocontainment Laboratory and Genome Sequencing Project to Support Rapid, Efficient Diagnostics and Surveillance of ASF Outbreaks in the Philippines: Current Updates and Challenges

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Infectious diseases remain the major cause of losses in animal production, significantly impacting food security. In the Philippines, outbreaks of African Swine Fever (ASF) have led to substantial production losses and rising meat prices. Despite the technological advances in veterinary diagnostics, the country still lacks the capabilities to fuilly characterize pathogens. Moreover, local farmers from rural areas have limited access to the testing laboratories and veterinary resources. These factors limit the producers, veterinarians and policymakers in decision making for the industry. Through a five-year public-private collaboration called the “Brisk Response through In-location Diagnostics and Genome Sequencing” or the BRIDGES project, the first mobile biocontainment laboratory (MBL) in the country was created to address the lack of rapid and efficient diagnostics response and surveillance of ASF at the point of need. The project also supported the initial efforts in whole genome sequencing and analysis of ASF samples from different parts of country. As the project reaches its fourth year, the project was able to produce whole genome sequences ASF samples from all over the country, thus placing the Philippines as a contributor to global genomic databases. On the other hand, despite lack of engagement from key opinion leaders in the industry, the project has continued to advocate for the use of the MBL by partnering with veterinary colleges from state universities and research institutions in the country to establish a biorisk manual specific for conducting diagnostics of ASF in a mobile laboratory setting. The BRIDGES project’s initiatives represent a significant step in the animal disease surveillance to protect livestock thus ensuring a sustainable food supply.

Emergence of African swine fever in Sri Lanka, 2024

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African swine fever virus (ASFV) continues to spread globally, causing severe economic losses to pig farmers. Sri Lanka is a small tropical island located in the Indian Ocean. Swine industry in Sri Lanka is considered a highly profitable livestock sector gaining increasing popularity. In September 2024, increased numbers of deaths in pig farms across the country and in wild boars were reported. The gross lesions were suggestive of ASF and therefore samples were sent to the Animal Virus Laboratory (AVL), Polgolla, Sri Lanka. At the AVL, most of the samples tested positive for ASFV genomic material by real-time PCR (RT-PCR), and the samples including formalin-fixed tissues were sent to the WOAH Reference Laboratory for ASF at the NCFAD, Winnipeg, Canada for confirmation.

At the NCFAD, the RT-PCR positive samples were subjected to conventional PCR to amplify the full-length p72 gene (B646L), p54, CD2v, CVR and IGR regions followed by Nanopore sequencing. All p72 and p54 sequences from Sri Lanka aligned with genotype II viruses and CD2v sequences with the serogroup 8 viruses. All CVR sequences were BNDBNDBNA and IGR sequences were variant 2 suggesting that the outbreak was caused by a single introduction of ASFV into the country. The RT-PCR positive samples were also inoculated onto primary porcine alveolar macrophages and hemadsorption (HAD) positive isolates were obtained. Whole genome sequencing of the isolates is ongoing, and the results will be discussed. Formalin-fixed tissues were subjected to hematoxylin and eosin (H&E) straining and Immunohistochemistry (IHC). Widespread loss of lymphocytes, inflammation, and extensive staining of ASFV structural protein pA137R were visible in all the tissues.

Poor biosecurity in small-to medium-scale pig farms and the presence of ASFV in wild boars will make control and eradication of ASF from Sri Lanka extremely challenging.

Simultaneous Detection of Antigen and Antibodies of African Swine Fever in a Novel Combo Lateral Flow Assay

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African Swine Fever (ASF) is an infectious disease of swine, caused by an enveloped double-stranded DNA virus. Infection with ASFV correlates with a wide range of clinical syndromes from unapparent disease to haemorrhagic fever with high fatality rates. To date, the active transmission of the ASFV across the globe, and the lack of licenced vaccines available worldwide, left early diagnosis as the main available tool for control. To properly identify infected animals, point-of-care (POC) diagnosis is crucial. Lateral flow assays offer advantages that make them suitable for this POC application. In this work, we validated a combo test for the combined antigen and antibody detection in field.

The new combo assay was composed by the combination of a strip for antigen detection (as in INgezim® ASFV CROM Ag 2.0) and a strip for antibody detection (as in INgezim® ASFV CROM Ab 2.0) within a single combo cassette. To evaluate the performance of the combined detection, 332 positive and 193 negative blood samples were evaluated. Samples were collected from field during surveillance campaigns performed in Latvia, Lithuania, Czech Republic, and Serbia. These samples were previously characterized by PCR and serology (ELISA and/or IPT) and separated into groups according to their Ct value.

The combined detection improved the percentage of positive results in all the PCR-positive groups tested. Notably, when no viral load was detected by PCR, this combined detection allowed the identification of 93 antibody-positive animals.

The new combo assay (INgezim® ASFV Combo CROM Ag/Ab) was shown to be a valuable tool for ASF surveillance. Our results support the idea that combined antigen/antibody detection give valuable information for an improved control of ASF, allowing the identification of more infected animals. The greatest improvement was found in wild boar due to the different surveillance approach (carcases/hunting vs signs control).

The administration doses of high virulent African swine fever virus in domestic pigs influences the early diagnosis in clinical, tissues, non-invasive and environmental samples

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In the absence of an effective global vaccine against African swine fever virus (ASFV), disease prevention depends on strict surveillance implementation, including fast and accurate diagnosis and biosecurity measures. While the infection generated in pigs infected with highly virulent ASFV strains genotype II is rapid and lethal, the early disease stages have not yet been extensively addressed from a diagnostic perspective. This work studies the effects of high, moderate and low doses of the ASFV Georgia 2007 pandemic strain by intranasal route to modulate the disease and viral progression in domestic pigs. Three groups of 6-week-old domestic pigs, with 20 animals per group, were used. All pigs were intranasally inoculated using 104 haemoadsorbing units (HAU) per pig in group A, 102,5 HAU in group B and 10 HAU in group C. The qPCR and LAMP tests were used to follow the infection dynamics in clinical, tissues, non-invasive and environmental samples to rapidly detect the onset of infection. At 3 days post infection (dpi), in the absence of clinical signs, ASFV DNA was detected in samples from group A, mainly in blood. In group B, only one pig was positive in blood, sera and spleen. At 4 dpi, the ASFV DNA was detected in samples analyzed from group A, while, some sera, nasals swabs, spleen, among others, continued negative from group B, despite the clinical signs. Only one pig from group C was positive during the trial. High ASFV DNA load was detected in the air and the walls in groups A and B at 5 and 6 dpi, respectively. We will discuss the application of the large panel of matrices used in this study for the rapid diagnosis of ASFV by qPCR and LAMP assay, including in a pen-side format. Special emphasis will be placed on the type of sample that can be collected non-invasively, as well as the environmental samples application for ASFV diagnosis.

Novel African Swine Fever DIVA serological assay based on the detection of antibodies against pEP153R and eGFP

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African Swine Fever (ASF) is one of the most significant infectious diseases affecting both domestic pig and wild boar populations. Currently, outbreaks have been reported worldwide, and disease management relies on stringent biosecurity measures and surveillance through diagnosis, emphasizing the urgent need for an effective and safe vaccine for ASF control. Thus far, the most promising strategy for vaccine development is based on the modification of the ASF Virus (ASFV) genome to enhance its safety and DIVA (Differentiation between Infected from Vaccinated Animals) characteristics. In this context, several promising vaccine candidates based on mutants of Lv17/WB/Rie1, a wild-type live attenuated genotype II ASFV strain, have been generated under VACDIVA project. The objective of the present study was to develop a companion serological assay for some of these vaccine candidates in which the EP153R gene is deleted and replaced by the eGFP reporter gene.

To achieve this objective, we produced the recombinant pEP153R and eGFP proteins, and demonstrated their antigenicity in domestic pigs and wild boar. Based on these antigens, we designed and developed a serological ELISA DIVA test that also includes a highly immunogenic viral protein, p72, as a control. A first evaluation of the assay was performed using experimental serum samples. A total of 112 samples from 6 domestic pigs (DP) and 87 samples from 8 wild boar (WB), inoculated with the parental virus, were analysed. The results showed that 100% of the animals seroconverted against p72 and pEP153R, although with a delayed onset between both antibody responses, and all resulted negative against eGFP. On the other hand, 207 samples from 16 DP and 96 samples from 8 WB immunized with VACDIVA candidate vaccines were analysed. All vaccinated animals were negative against pEP153R, and positive against p72 and eGFP, with a similar seroconversion profile. Additionally, serum and alternative samples from field animals were analysed for a final validation of the assay.

The newly developed DIVA assay was demonstrated to be a useful companion tool for vaccine candidates based on modified genotype II ASFV strains, in which the EP153R gene has been deleted and the eGFP reporter gene has been inserted. This approach could potentially improve surveillance during prospective vaccination campaigns.

Exploring Viral Metagenomics in ASFV-Positive Samples from the Philippines Reveals Co-Infection with Other Swine Pathogens

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Swine act as a natural reservoir for numerous pathogens, including emerging and re-emerging viruses. Under field conditions, co-infections involving multiple pathogen species frequently occur more than single infections. Such co-infections may also play a key role in mitigating or exacerbating disease severity. While the impact of co-infections on various swine pathogens is increasingly recognized, the role of co-infections with African swine fever virus (ASFV) remains largely unexplored. To investigate the complexity of viral infections in swine, ASFV-positive DNA and RNA samples were sequenced in a high-throughput metagenomic approach for an *a priori* discovery of co-existing swine viruses. After *de novo* assembly, viral contigs with near-complete to full-length coverage and sufficient depth were considered for further analysis. In total, 63 out of 75 (84%) DNA samples and 2 out of 14 (14.29%) RNA samples contained at least one viral species other than ASFV. The most prevalent DNA viral family was *Anelloviridae*, which included four identified species: torque teno sus viruses 1a, 1b, k2a and k2b. These were previously described as an enhancing factor in co-infection with other viruses. The viral families following that were *Circoviridae*, represented by porcine circovirus 2, and *Parvoviridae*, with porcine parvoviruses 3, 4, and 6, both of which are major DNA pathogens that influence the productivity in pig farms. Meanwhile, the viral family detected in RNA samples was *Sedoreoviridae*, with the species rotavirus A, a pathogen associated with morbidity and mortality from acute diarrhea in young pigs. To our knowledge, this is the first report to explore co-infection in ASFV-infected swine using an unbiased approach. These findings provide baseline data on swine viral diversity for future research, including surveillance studies and investigations correlating viral co-infections with clinical signs and disease severity.

Whole-Genome Sequencing of African Swine Fever Virus in Serbian Samples 2019–2023

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African swine fever (ASF) is a highly fatal viral hemorrhagic disease with devastating economic impacts on global swine production. In Serbia, ASF was first detected in domestic pigs in 2019, followed by its reemergence in wild boar in 2020, where it has persisted. This study aimed to assess genomic changes in the ASF virus by performing shotgun metagenomic sequencing on wild boar and domestic pig samples. Spleen samples from hunted or found dead wild boar and dead domestic pigs were processed. Five samples were selected based on nucleic acid concentration and purity and sequenced using Illumina technology at Novogene Ltd. (UK). Sequenced reads were trimmed with BBduk v.38.84 and *de novo* assembled using metaSPAdes v.3.13.1 to generate contigs, which were mapped to the ASF reference genome Georgia 2007/1 using Minimap2 v.2.0. as plugins in Geneious Prime software suite v. 2022.1.1. Genome alignments were performed with MAFFT v.1.5 and phylogenetic analysis was conducted using MEGA X. Five high-quality genomes were obtained and deposited in NCBI GenBank (accession numbers OR660695–OR660699). Phylogenetic analysis, incorporating seven additional genomes, revealed three subgroups: strains NC044959, ON108571, and LR722599 formed the first subgroup; MT847622, MT847623, and LR899193 formed the second; while sequences from this study clustered with MK543947 in the third subgroup. Mutational analysis revealed both major and minor mutations. Major mutations contributed to enhanced strain classification and potential impacts on protein functionality. In contrast, minor mutations included extended poly-C and poly-G regions in MGF 110 and 360 gene families and ACD genes, as well as single deletions in intergenic regions. Silent mutations and poly-C/G insertions, although functionally unclear, were also observed. This study provides critical insights into ASF virus genetic diversity and evolutionary dynamics in Serbia, contributing to the global understanding of its transmission and persistence. These findings underscore the importance of genomic surveillance in controlling this devastating disease.

Biological Containment for African Swine Fever (ASF) in BSL-3 Laboratory and Animal Facility of the Italian National Reference Laboratory: A Continuous Journey in Enhancing Biosafety and Biosecurity Measures

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African Swine Fever Virus (ASFV) is a highly contagious and lethal virus affecting swine populations, leading to significant economic losses globally. Handling ASFV in laboratory and animal facility settings necessitates stringent biosafety and biosecurity measures to prevent accidental or intentional release and environmental contamination. Over the years, the Italian National Reference Laboratory (NRL) has developed a robust biological containment framework for ASF laboratories and animal facilities.

The initial step involved creating a comprehensive main document, a biosafety and biosecurity manual, supported by several linked Standard Operating Procedures (SOPs). This containment framework integrated detailed structural and procedural requirements based on current legislation (2003/422/EC, Legislative Decree 08/81), risk assessments, and internal audits. Internal audits were conducted annually to ensure the proper application and efficacy of the biosafety and biosecurity system, preventing any accidental or intentional release of ASFV-infected material outside the BSL-3 containment area. Recently, the Commission Delegated Regulation (EU) 2020/689 repealed the Commission Decision 2003/422/EC, which had provided minimum and supplementary requirements for ASF laboratories. This regulatory change created a gap that has yet to be fully addressed. In response, and following the suggestions of WHO in the document “Strengthening Laboratory Biological Risk Management” (January 2024), the NRL has reviewed and further improved its biological containment tools for ASF laboratories and animal facilities. The new structural and managerial requirements were developed by considering both current and repealed legislation, extensive biosafety manuals and guidelines published by national and international agencies, and the NRL's extensive experience with ASF. Consequently, the NRL has updated its biosafety and biosecurity manual and linked SOPs.

Thanks to this complex biological containment framework and a major shift in mindset towards leveraging transversal capabilities, the Italian NRL has achieved significant improvements in the biosafety and biosecurity within its ASF BSL-3 containment area.

Hybrid Sequencing Approach for African Swine Fever Virus Whole Genome Assembly

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African Swine Fever (ASF), currently the biggest threat to the swine industry, is caused by the African Swine Fever virus (ASFV), a large double-stranded DNA virus with a genome size of 170-190 kbp. Its genome is characterized by homopolymer and repetitive regions, making it challenging to assemble using next-generation sequencing data alone. To generate high-quality whole-genome sequences, we developed an optimized laboratory workflow and bioinformatics pipeline for the hybrid sequencing of ASFV samples, generated from Illumina and Nanopore.

65 ASFV positive whole blood samples were extracted using bead-based DNA extraction, followed by the removal of methylated DNA (mammalian DNA) and an amplification step. For Illumina sequencing, samples were isothermally amplified via multiple displacement amplification (MDA), while for nanopore sequencing samples, PCR amplification was used. In brief, the hybrid workflow first involves long-read *de novo* assembly of host-depleted reads, followed by read polishing through mapping ASFV-mapped short reads to the *de novo* assembled contigs.

The number of long reads generated per sample ranged from 18,000 to 900,000, with at least 95% of the reads passing the filtering threshold. Illumina throughput averaged 68 million reads, with a 92% pass rate. The hybrid assembly resulted in large contigs, ranging from 180-190 kbp in length, with a breadth of coverage of 95-100% at 10X depth, and a mean depth of coverage ranging from 28X to 9850X. Homology searches using NCBI blastn revealed close similarity with ASFV genotype II sequences from Asia and Europe.

In total, 65 high-quality ASFV sequences were generated using our streamlined protocol, demonstrating its scalability and suitability for routine ASFV biosurveillance efforts.

Role of *Calliphora vomitoria* larvae as possible reservoirs and mechanical vector of African Swine Fever virus: preliminary results.

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African swine fever (ASF) is a highly contagious viral disease infecting wild and domestic pigs. Transmission occurs through direct and indirect contact with infected animals and contaminated fomites. Moreover, some tick species, i.e. *Ornithodoros*, can carry and transmit ASFV. Given the high resistance of ASFV in the environment and its proven ability to survive cadaveric decomposition, some necrophagous insects are supposed to play an important role as potential mechanical vectors and/or reservoir for ASFV.

In this study, necrophagous flies of the Calliphoridae family were fed on ASF-infected spleens, in order to assess whether ASFV is able to survive and replicate in their digestive tract and to be potentially transmitted through insect stages.

New-born larvae of blue bottle fly (*Calliphora vomitoria* Linnaeus, 1758) (n=478) were divided into 3 groups. Group A (n=203 larvae) were reared until the pupa stage on ASFV-infected spleen while group B (n=203 larvae) were bred for 48 hours on ASFV-infected splenic tissues and afterwards on virus-free tissues; group C (control) (n=72 larvae) were reared on virus-free splenic tissues during all stages. Pools of five insects underwent 10 times washing before DNA extraction and isolated DNAs, from insects and washes 1, 5 and 10, were tested for ASFV detection by real-time PCR. Positive samples underwent virus isolation (VI).

Overall, ASFV was detected in 7 larvae pools and 4 larvae pools from group A and B, respectively. All pupae pools were negative for ASFV DNA. All washes analyzed were negative for ASFV DNA too. However, VI attempts on ASFV PCR-positive pool samples from group A and B resulted negative. These preliminary findings suggest that larvae of *C. vomitoria* fed on ASFV-infected tissues could host the virus, which does not replicate nor survive through the stages, thus their potential role as reservoirs or mechanical vectors of ASFV could be excluded.

Survival at +4°C, +20°C and +37°C of Italian genotype I and II African Swine Fever strains

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ASF is a major threat to the swine industry worldwide. The environmental contamination during an outbreak is a critical issue in limiting the disease spread. ASF persistence and spread is a function of the virus survival to physical and chemical factors. Data on ASFV resistance in swine tissues and inanimate materials have recently increased, but baseline data on residual infectivity ASFV at different temperature are scarce. This study generated data on survival of ASFV strains of genotype I and II detected in Italy at 3 different temperatures (+4°C, +20°C, +37°C) for 15 days. The BA71/V genotype I as a reference strain, a genotype I virus isolated in Sardinia in 2008, and a genotype II strain isolated in wild boar in Italy in 2022 were selected. At 0, 7, 15 days, 3 independent virus aliquots were exposed and tested for residual infectivity by virus titration on cell cultures. Difference in virus titres according to the strain, temperature and time point were statistically analysed. All strains tested remained infectious at 15 days post exposure at +4°C and +20°C. A difference was observed at +37°C at 15 days, with genotype I being inactivated after 12 days. Comparing survival curves of each ASFV, a statistically significant difference was observed among temperatures and time points, indicating a higher survival rates at +4°C and +20°C than +37°C. The decrease in virus titres at +37°C was statistically significantly dependent on the strain and the time. Differences were detected at each time points for genotype I, between 7 and 15 days for genotype II and between 0 and 7 days for BA71/V. This study partially confirms literature data on ASFV prolonged survival temperature, suggesting a difference in survival between genotype I and II that needs additional tests to assess the role of survival characteristics on ASFV transmission.

Generation of tools for the study of p32-host cell interaction

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Centro de Biología Molecular Severo Ochoa

African Swine Fever Virus (ASFV) contains a large, double-stranded DNA genome, which encodes for 150-200 viral proteins, including 68 structural and 100 non-structural. The functions of many of these proteins are still unknown, thus impairing the development of vaccines and tools to control the spread of the virus. Here, we have focused on the viral protein p32, encoded by the CP204L gene. Multiple functions have been suggested for this protein, although the molecular mechanisms regulating cell and p32 interaction are not yet elucidated. It is noteworthy that genetic sequence encoding p32 from virulent Arm/07/CBM/c2 (Genotype II), or the attenuated NH/P68 strains (Genotype I), seems to be different, and this fact also occurs among other viral genotypes. In order to better understand the role of p32 in the antigenic signature of different ASFV genotypes and /or in virulence, we plan to generate stable COS-1 cells using the lentiviral system by expressing CP204L, either from Arm/07/CBM/c2 or NH/P68 strains. However, several problems were found to maintain these p32-stably COS cells, indicating possible toxicity of the viral protein. Hence, we have set up the generation of p32-stably-expressing cells by using a new conditional expression system, which allows to maintain low levels of the viral protein unless a specific stimulus is added. In this way, we also intend to generate a general platform to study other proteins that could also be key to viral infection and to understand the molecular mechanisms of ASFV virulence and genotypes' differences.

Pathological characteristics of domestic pigs infected with the virus strain causing the ASF in South Korea

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African swine fever (ASF) was first reported in South Korea in 2019, and as of December 2024, a total of 49 cases in domestic pig farms have been confirmed in the country. The forty-nine viruses from the domestic pig farms were categorized as p72 genotype II based on B646L gene and CD2v serogroup 8 encoded by EP402R gene. We conducted animal experiments to estimate their pathogenicities and pathological characteristics at the national ASF reference laboratory within the Animal and Plant Quarantine Agency (APQA). We chose four ASFV strains with potentially reduced pathogenicity considering the disease outbreak situation, clinical signs and gross lesions, epidemiological association and genetic analysis among viruses obtained from pig premises from 2022 to December 2023. For the experimental *in vivo* studies, the strains, Korea/Pig/Hongcheon/2022, Korea/Pig/Pocheon1/2023/, Korea/Pig/Pocheon2/2023, Korea/Pig/Cheorwon/2023 have been inoculated intramuscularly at titer of 103HAD50/ml per group of five pigs. All inoculated pigs died 7-10 days post inoculation after showing fever, depression, anorexia with the common pathological lesions of enlarged hemorrhagic lymph nodes and splenomegaly with infarction. These results support the hypothesis that the pathogenicity among ASFV isolates in South Korea still remained unchanged. With growing concerns about the emergence of new ASFV strains with less or low pathogenicity in the country, these animal studies will help inform quarantine and surveillance policies.

ASFree M.e.a.t. (meet export agreement on trading): safeguarding the Italian cured pig products from African Swine Fever

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The Italian export of processed meats represents about 56% of all Italian pork meat exports. The marketing of safe pork products ("safe commodities") contributes to managing the risk of disease spread. The ASFV tenacity and the conditions under which it remains viable over time in aged pork products dated back to the late 80s. These studies assessed the presence of genotype I that is no longer circulating in Italy, using detection methods less sensitive than those available today. The ASFree M.e.a.t project aims at updating the existing knowledge on ASFV persistence in aged pork products and investigates the efficacy of High Pressure Processing (HPP) in ASFV inactivation to provide health guarantees to importing countries regarding the absence of ASFV in Italian aged products. ASFree M.e.a.t. consortium is constituted by 4 Italian institutes and will produce cured pig products including ham either by artificial contamination and by *in vivo* experimental infections of pigs. Six types of Italian salami were selected as representative of the exported products. Two batches of 26 Milano salami each were experimentally contaminated according to the FSIS guidelines, with 1% w/v with a BA71/V and cured for two months. At 1/3, 2/3 and the end of aging 3 salami were sampled for ASFV detection by qPCR, Digital PCR and virus isolation. At the end of curing, positive ASFV salami will be treated with HPP and negative results will be confirmed by *in vivo* trials by feeding pigs with HPP treated salami. Preliminary data by qPCR showed that Milano salami sampled at 2/3 of aging are ASFV positive, with Ct values ranging between 30 and 35. A third batch of Milano salami is aging for HPP efficacy. This project is pivotal to provide updated information on the risk of ASF spread and introduction of cured pig products following HPP treatment.

An African Swine Fever Vaccine-Like Variant with Multiple Gene Deletions Caused Reproductive Failure in a Vietnamese Breeding Herd

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This longitudinal study highlighted the incursion of a novel ASF vaccine-like variant into a non-vaccinated breeding herd. Retrospective epidemiology suggested a high replacement rate and improper biosecurity measures might have introduced the disease into the herd. Affected young gilts displayed no to mild symptoms, whereas gestational sows experienced reproductive disorders such as abortion, miscarriage, and stillbirth. Remarkably, severe ulcerative dermatitis in udders was observed in lactating sows 1–2 weeks postpartum. The ASF outbreak was significantly associated with reduced reproductive performance compared to the pre-outbreak period ($P < 0.001$). Whole genome sequence analysis revealed several virulence-associated gene deletions and the presence of a marker gene in the left variable region, consistent with the ASFV-G-ΔMGF vaccine strain. Molecular detection and immunohistochemistry on necropsy samples indicated viral antigens distributed in macrophage-like cells of the reproductive organs and affected udders. Microscopic findings implied massive necrotizing vasculitis with fibrinoid degeneration compatible with immune complex-induced lesions. In conclusion, naïve sows are highly susceptible to the novel ASF vaccine-like variant that gilts can bring into the farm, highlighting biosecurity failures.

Establishment of a Single-Cell RNA Sequencing Platform for the Investigation of Immune Responses Against ASFV Under High Containment Conditions

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African swine fever virus (ASFV) causes a devastating hemorrhagic fever in suid species and has become a global threat to the pig industry and conservation efforts alike. Understanding the host response is of pivotal importance in order to design new vaccines that induce improved and protective immune responses.

Immunological studies are especially challenging given the limited availability of scientific tools to investigate immunity in veterinary species. While there are numerous reagents available for domestic pigs, the application of these species-specific reagents is complicated in more distantly related suid species. Moreover, research involving ASFV necessitates high containment laboratory conditions, which often restrict the use of traditional methodologies such as flow cytometry. Here, we established a chip-based single-cell RNA sequencing (scRNAseq) pipeline that requires no further equipment and enables the investigation of immune responses at the single cell level while following regulatory biosafety protocols.

We employed this scRNAseq pipeline in a comparative study of highly virulent ASFV infection in two highly susceptible Eurasian suids, domestic pigs and wild boar, and two resistant African species, Red River hogs and warthogs. PBMCs were isolated as a relatively accessible sample matrix both pre- and post-infection with the highly virulent ASFV strain ‘Armenia08’. Grouped samples of up to six animals per time point were loaded onto the chip. The isolated RNA of 10,000 cells per time point was subsequently used for sequencing. Bioinformatic approaches then enabled the identification and investigation of various leukocyte populations and their responses in all species.

Overall, our study demonstrates the applicability of scRNAseq analyses for the investigation of immune responses in suid species, particularly in contexts where conventional approaches prove unfeasible.

A Model of Protective Immune Responses Against African Swine Fever Virus Infection in Immunized Pigs

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African swine fever virus (ASFV) causes a fatal hemorrhagic disease in domestic pigs and wild boars. Currently, the development of robust preventive strategies against ASFV is of utmost importance. Live-attenuated vaccines demonstrated promising efficacy in the recent studies, however, they can shift the balance of the immune response from protection towards immunopathology. For that reason, it is essential to distinguish between protective and detrimental immune responses following immunization and challenge infection. In this study, we used a farm versus SPF pig setting along with a systems immunology approach to generate a temporally resolved model of protective innate and adaptive immune responses to the virus. The obtained data highlight an important role for the early type I IFN response during both immunization and challenge phases. Its protective role is associated with inducing innate immunity, causing antigen-presenting cell (APC) activation and promoting adaptive immune response. We also demonstrated that a tightly regulated pro-inflammatory response, as indicated by serum cytokines and blood transcriptome data, correlated with better clinical outcomes. In addition to that, early ASFV-specific memory helper and cytotoxic T cells detectable in the blood after challenge were associated with protection. Our findings also indicate a protective role for plasma cells, as their transcriptional signatures, as well as antibody titers against certain viral proteins were elevated in protected animals after the challenge phase. Defined correlates of protection can be further applied for a rational vaccine design and efficacy testing.

Molecular Profiling of Cytokine Gene Expression: Insights into the Immune Response of Pigs Infected with Local Isolates of ASFv in the Philippines

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ASFV infection is known to elicit a strong immunological response, which frequently leads to an overexpression of cytokines, which are important mediators that control immune cell activation and mobilization. ASF vaccine trials are now being carried out in the Philippines before these vaccines are used; however, despite tremendous efforts, no ASF vaccine has been approved for commercial use as yet. This might be partially explained by the limited knowledge and understanding of the immunological processes underlying ASFV infection. There is still little research on ASF immunity, which indicates a significant knowledge gap that must be filled in order to create an effective ASF vaccine.
This study used 25 whole blood samples taken from pigs infected with local isolates of ASFV to investigate the function of cytokines and other immune mediators during ASFV infection. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to assess the expression levels of different cytokine and chemokine mRNAs.

Pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-13, IL-17A, IL-21, IL-33, and IFN-γ were significantly upregulated, suggesting a strong inflammatory response to ASFv infection. The observed upregulation suggests an intense activation of immune pathways aimed at combating the viral infection. On the other hand, the study discovered a notable downregulation of IL-5 mRNA, which would indicate possible strategies used by ASFv to alter the host immunological response and circumvent immune defenses.

Furthermore, the study highlighted the upregulation of key chemokines, such as CCL2, CCL3L1, CCL4, CXCL2, CXCL8, and CXCL10, suggesting an enhanced recruitment of immune cells to infection sites. Interestingly, CCL5 exhibited downregulated expression, indicating specific modulation of chemokine-mediated pathways by the virus. Additionally, the activation of TLR signaling pathways was suggested by the overexpression of Toll-like receptors (TLRs), namely TLR2 and TLR3. These results emphasize the intricate interactions between pro-inflammatory cytokines, chemokines, and TLR pathways during infection and offer important new insights into the host immune response to ASFv.

Overall, this knowledge furthers our understanding of ASFv pathogenesis and may be the foundation for further studies aimed at developing effective immunological interventions to manage and prevent ASF.

Keywords: ASFv, immune response, mRNA expression, Philippines

Infection with Moderately Virulent African Swine Fever Virus Conveys Protection Against Highly Virulent Challenge for at Least Six Months

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African swine fever virus (ASFV) is a significant threat to both pork production and wild boar populations worldwide. This research aimed to examine the duration of immunity following inoculation with a moderately virulent strain of ASFV, while also evaluating immune robustness towards a highly virulent variant. Pigs inoculated with the moderately virulent ASFV 'Estonia14' predominantly exhibited mild clinical manifestations and transient viremia. Six months post-inoculation, all pigs were subjected to challenge infection with the highly virulent ASFV 'Armenia08'. Only one pig displayed mild clinical symptoms. All control animals manifested typical signs of acute ASF. Furthermore, some of the previously recovered pigs were moderately positive for viral genome after challenge. Virus isolation further validated these observations, revealing minimal levels of infectious particles in the organs of previously inoculated pigs (28 days post-challenge). In addition, the assessment of IgM and IgG kinetics in all animals allowed a detailed overview of humoral immune responses, with IgG concentrations remaining elevated after inoculation and showing moderate increases post-challenge. Finally, plasma analysis indicated higher levels of complement factor C3a following both inoculation and challenge in recovered pigs, which correlated with the presence of the challenge virus. In contrast, C3a and C5a levels were significantly higher in control animals. These results indicate that the duration and robustness of ASFV-specific immunity lasts at least six months following recovery from a moderately virulent ASFV infection.

Vaccines

An Immortalized Porcine Alveolar Macrophage Cell Line with Potential for Live ASFV Vaccine Production

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In addition to the development of suitable vaccine strains, the commercialization of live virus ASF vaccines requires cell lines capable of stably producing them. However, the development of a virus-sensitive cell line is impeded by the fact that *in vivo* ASFV replication is largely restricted to terminally differentiated macrophages.

Porcine alveolar macrophages (PAM) express the cellular receptor CD163, which is essential for PRRSV entry and may be involved in ASFV infection. Primary PAM isolated from pigs were immortalized with the SV40 T antigen and characterized. A single clone (designated iPAM) was selected from the generated cell pool based on CD163 expression data. The iPAM cell line fully supports PRRSV replication and has been tested for ASFV propagation. To date, all genotype I and genotype II viruses tested have shown growth characteristics similar to viruses cultured in primary PAM. In most cases, higher virus titers could be achieved in iPAM than in PAM.

The genome stability of four different genotype II vaccine candidate strains (three Lv17/WB/Rie1 and one Georgia 2007/1 derivatives) were studied by NGS (Illumina platform) sequencing after a minimum of 5-rounds of cell culture passage. Three of the investigated viruses did not show significant genetic changes after five passages. Genetic instability could be detected only in one of the Lv17/WB/Rie1 vaccine candidates in the form of minor genome arrangements and single nucleotide polymorphisms.

Our results demonstrate that iPAM has the potential to replace PAM in many areas of research, and following appropriate regulatory approval, can be suitable for vaccine production.

Comparison of oral, nasal and anal swabs for detection of African swine fever virus by qPCR in vaccine efficacy studies

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African Swine Fever Virus (ASFV) studies often assess virus shedding using oral (OS), nasal (NS), and anal (AS) swabs. To improve animal welfare, reducing the number of swabs may be considered if research objectives allow. We compared ASF viral DNA detection via qPCR in OS, NS, and AS samples during a multicenter vaccine efficacy study conducted at Friedrich Loeffler Institute (FLI), Germany, and at Wageningen Bioveterinary Research (WBVR), Netherlands, within an EU collaboration.

Three live attenuated vaccine candidates (n=15, FLI; n=10, WBVR) were tested via oral administration. One of the candidates was tested intramuscularly (IM, n=8, WBVR) as an efficacy control, and unvaccinated controls (n=5 at both institutes) were included. All animals were challenged oronasally with the Armenia'08 strain. Swabs were collected during the vaccination and challenge phases, and qPCR was used to detect viral DNA.

At WBVR, ASFV DNA was detected in 7/152 OS (oral vaccines) and 10/32 OS (IM vaccine) samples during the vaccination phase. Only 1/32 NS and no AS samples tested positive. During the challenge phase, 34/43 OS samples were positive at 2 days post-infection (dpi), compared to 6 NS and 0 AS. NS and AS positivity increased at later time points, but overall, the numbers of positive OS was higher. At FLI, one OS and one NS (out of 180) samples were positive during the vaccination phase. After challenge, 23/50 OS, 18/45 NS, and 11/50 AS samples were positive at 4 dpi. Positive OS and NS numbers equalized later, while AS remained consistently lower.

OS were the most sensitive samples for detecting virus shedding during the vaccination phase and early post-challenge and were superior or comparable to NS and AS at later stages. These results suggest OS sampling alone is sufficient for assessing ASFV shedding in vaccine studies, improving animal welfare by minimizing invasive procedures.

Establishment of a Replication-Restricted Mutant of African Swine Fever Virus

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African swine fever (ASF) is a fatal febrile infectious disease affecting pigs and wild boars, caused by the ASF virus (ASFV). Since 2007, genotype II ASFV has continued to spread across countries in the Caucasus, Europe, the Asia-Pacific region, and the Caribbean. Although several live attenuated strains have been developed as vaccine candidates, concerns over their safety have emerged due to prolonged persistence in inoculated pigs, raising the risk of virulence reversion. To develop safer vaccine candidates, we aimed to create a replication-restricted mutant of ASFV by deleting the S273R gene, which is crucial for core-shell formation. Using genetically modified immortalized porcine kidney macrophages (IPKMs) which stably express the S273R gene as host cells, we successfully generated a replication-restricted mutant (AQSΔS273R) derived from the parental virulent strain, AQS-C-1-22. This mutant virus produced non-infectious, immature progeny *in vitro* and *in vivo*, suggesting a lack of mature particle formation. Pigs inoculated with AQSΔS273R did not develop lethal ASF symptoms, although they were not fully protected from a challenge with the parental virus.

In conclusion, we successfully generated a replication-restricted ASFV mutant using modified IPKMs expressing a key gene. This innovative approach provides a new tool for basic and applied ASF research.

Vaccine strain ASFV-G-ΔI177L reverts quickly to virulence upon serial passaging in pigs and negatively impacts reproductive performance of sows

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ASFV-G-ΔI177L is a gene-deleted modified-live ASFV vaccine strain that is incorporated in a commercially available vaccine. Its safety in pregnant sows and its genetic stability in a reversion-to-virulence experiment were evaluated.

Two sows in their third trimester of pregnancy were inoculated with ASFV-G-ΔI177L, resulting in the development of moderate ASF-related clinical signs in one of the two pregnant animals. The offspring of both inoculated sows became viremic and developed ASF-specific clinical signs. Reproductive performance was severely compromised with 43% of piglets born dead and only 17% of the remaining live-born piglets stayed alive until study end.

ASFV-G-ΔI177L reverted to virulence in an *in vivo* passaging experiment performed according to international regulatory guidelines (EMA, VICH). Severe ASF-specific clinical signs were already observed in passage 3 and 4, associated with increased virus titres in blood. Full genome sequence analysis pinpointed to the C257L protein as the possible cause of the increased replication fitness and virulence. The data demonstrated that ASFV-G-ΔI177L is not suited for use in an ASF vaccine, as it appeared not stably attenuated and unsafe for pregnant sows.