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Abstract Book

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ORAL PRESENTATIONS:

1. WHICH INFLUENZA-A SUBTYPES CAN BE FOUND IN FATTENER BLOOD ON HUNGARIAN FARMS WITH HI TESTING? A FIELD STUDY

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Introduction

Swine influenza virus can play a significant role in the Porcine Respiratory Disease Complex. The aim of this study was to gain knowledge of the herd level prevalence, and subtype variance of swine influenza virus in Hungary.

Materials and Methods

We surveyed 25 Hungarian large commercial swine farms between 2019 and 2021. We collected blood samples from 4, 8, 12, 16, 20 and 24 week-old pigs, and fatteners were also sampled. From every age group at least 14 blood samples were taken. We used the IDEXX ELISA for Influenza-A antibody test and HI tests for the identification of subtypes (H1N1, pandemic H1N1, H1N2, H3N2).

Results

We found that 84% of the surveyed farms were Influenza-A positive. 60% of the surveyed farms were positive for H1N1, 65% for pandemic H1N1, 65% for H1N2, and 65% for H3N2.
The influenza-A subtype variance on the surveyed farms:
30% of farms had all 4 subtypes, 20% had none of them. 5% had only pH1N1, 5% had H3N2+pH1N1+H3N2, 10% had pH1N1+H1N2+H3N2, 5% had H1N1+pH1N1+H1N2, 5% had H1N2+H3N2, 5% had H1N1+pH1N1, 5% had H1N1+H1N2+H3N2, 10% had H1N1+H3N2.

Discussion and Conclusions

In order to have an effective swine influenza vaccination protocol, we have to be aware of the virus subtypes being present on the farm. Commercial vaccines are not always effective against all swine influenza subtypes circulating on a farm, hence, in the future there might be a need for farm specific vaccines. After data collection about the subtypes on the farm, we will have more chance to control PRDC instead of using antibiotics, still blood examinations together with PCR tests from organ samples are important tools together.

2. SAME VIRUS, DIFFERENT STORIES: MEASURING SWINE INFLUENZA VIRUS TRANSMISSION IN THREE DUTCH FARMS UNDER ENDEMIC CIRCUMSTANCES

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Introduction and Objectives

Transmission dynamics of swine influenza A viruses (swIAV) during endemic circulation in large pig farms remain poorly understood. Understanding these would enable identification of intervention strategies through in-silico transmission modeling. This study investigated swIAV transmission in three Dutch farms and estimated transmission parameters for within and between pen transmission rates of swIAV.

Materials and Methods

Three farms with a history of swIAV circulation were visited three times per week for three consecutive weeks. First, farms were cross-sectionally screened for swIAV circulation using oral fluid (OF) and udder wipe (UW) samples and subsequent RT-PCR testing. Next, several rooms were selected for both group and individual sampling using OF, UW and nasal swabs (NS). We applied a mathematical infectious-disease model to the collected data to estimate the transmission rate (β). We estimated within-pen transmission from NS data and between-pen transmission from OF and UW.

Results and Discussion

In total, 582/1780 OF, 282/622 NS and 52/478 UW were positive for H1N1 virus clade 1C.2.2. Across all farms viral genome was detected in weaners, but the age of pigs at first positive tests differed per farm at age 4, 5 and 6.5 weeks. In farm A, virus circulation was also found within the finishers and in farm C within the farrowing rooms. The within-pen β was 0.55 day-1 (95% CI: 0.17-1.36) for farm B and 0.96 (0.57-1.63) for farm C. Between pens, β was lower in farms A and C [0.32 day-1 (0.16-0.54) and 0.37 day-1 (0.06-1.15)], whereas farm B had a higher median value of 0.98 day-1 (0.62-1.47).

Conclusion

Despite the H1 viruses on the farms belonged to the same clade, transmission patterns of swIAV varied across the three farms. The transmission parameter estimates enable further development of mathematical models to identify effective intervention measures.

3. ANTIGENIC DRIFT AND NURSERY IMMUNITY GAPS IN FARROW-TO-FINISH HERDS: IMPLICATIONS FOR VACCINATION STRATEGY

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Introduction and Objectives

Swine influenza A virus (swIAV) persists in farrow-to-finish herds despite widespread sow vaccination, yet the mechanisms sustaining endemicity remain poorly quantified. We argue that this failure of control is driven by two interacting forces: antigenic drift, which erodes vaccine–strain match, and stage-specific immunity, particularly maternally derived antibody (MDA) interference in nursery piglets, which limits vaccine effectiveness where transmission is most intense. We aimed to (i) reproduce endemic swIAV dynamics in a calibrated farrow-to-finish setting, (ii) characterize how antigenic drift degrades static versus annually updated autogenous vaccination, and (iii) determine whether reductions in infection burden translate proportionally into reduced reassortment opportunity.

Materials and Methods

We developed a stochastic agent-based model of a closed farrow-to-finish herd with All-In-All-Out batch management and explicit pen/room structure. Pigs carried dynamic antibody profiles shaped by MDA, infection history, and vaccine-derived immunity. A data-driven antigenic drift model generated time-varying introduction of novel variants, creating long-term (15-year) challenge pressure. The model was calibrated to endemic field patterns using approximate Bayesian computation. We evaluated four strategies: no vaccination, static vaccination, and annually updated autogenous vaccination with and without weaner dosing. Outcomes included infection burden, effective reproduction number ($\boldsymbol{R_t}$), antigenic distance, and co-infection-based reassortment opportunity.

Results and Discussion

The model reproduced endemic dynamics driven by early-nursery outbreaks and recurring variant introduction. Under antigenic drift, static vaccination lost strain match and became indistinguishable from no vaccination. Updated autogenous vaccination reduced long-term infection burden. However, nursery peak prevalence did not differ substantially between strategies. MDA interference limited both vaccine effectiveness and the development of robust post-infection immunity in the youngest animals, maintaining the primary transmission reservoir. Consequently, reductions in reassortment opportunity lagged behind reductions in infection burden because multi-strain exposure remained concentrated in these under-protected piglets.

Conclusion

Annual autogenous vaccination is the most effective strategy under antigenic drift but is constrained by MDA-driven immunity gaps in nursery piglets. Addressing this gap through MDA-aware vaccination or targeted monitoring of early cohorts is essential to reduce disease burden and limit reassortment risk. Improving nursery-stage immunity, rather than vaccine updating alone, is critical for effective swIAV control.

4. PRE-PANDEMIC CHARACTERISATION OF SWINE INFLUENZA VIRUSES: A WHO COLLABORATING CENTRE PERSPECTIVE

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Introduction

As a WHO Collaborating Centre, the Worldwide Influenza Centre (WIC) collaborates with public health laboratories globally to characterise human seasonal influenza viruses. Alongside this, the WIC also collaborates with public and animal health institutes by characterising Swine Influenza A virus (SwIAV) infections in humans and performs horizon scanning of SwIAVs with zoonotic potential. This directly feeds into the WHO's Vaccine Composition Meetings to recommend new or updated candidate vaccine viruses (CVVs) for zoonotic influenza viruses.

SwIAVs are enzootic within swine populations but can spillover into humans, with numerous instances of bidirectional transmission between humans and pigs documented. Whilst some SwIAVs currently circulating in pigs originated from humans, over time, diversification has meant limited or no protection afforded by human seasonal influenza vaccines. As such, SwIAVs represent a significant pandemic threat and require routine ongoing surveillance and characterisation.

Materials and Methods

Antigenic characterisation of SwIAVs was performed using ferret antisera raised against existing CVVs, reference viruses and seasonal influenza vaccine viruses using the haemagglutination inhibition (HI) assay. Serological analysis of SwIAVs to assess potential cross-reactivity of contemporary human seasonal influenza vaccines was also performed using pre- and post-vaccination sera from healthy human volunteers using the HI assay. Assessment of antiviral susceptibility of SwIAVs to the neuraminidase inhibitors was performed using the fluorescent MUNANA substrate neuraminidase inhibition assay.

Results and Discussion

Antigenic characterisation of H1 and H3 SwIAVs demonstrated variable recognition by relevant ferret sera, particularly existing CVVs, and this variability was also observed between viruses within the same clade. Serological assessment using a panel of pre-/post-vaccination human sera demonstrated that overall H3 SwIAVs had higher reactivity compared to H1s, but similar variability within clades was also observed. All the SwIAVs tested were susceptible to oseltamivir and zanamivir.

Conclusion

Although in the viruses tested in this study, no reduced susceptibility was observed to current frontline antiviral treatments, there was substantial antigenic and serological variabilities even within clades to existing vaccine strains. These findings highlight the importance of timely and comprehensive phenotypic characterisation of existing and emerging SwIAVs as part of pre-pandemic preparedness activities to ensure existing mitigation strategies remain applicable.

5. RNA PARTICLE VACCINES ENCODING INFLUENZA A VIRUS HEMAGGLUTININ OR NEURAMINIDASE: SAFETY AND EFFICACY IN PIGS

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Introduction and Objectives

Influenza A virus in swine (IAV-S) remains a major endemic and zoonotic respiratory pathogen that continues to evolve through antigenic drift and shift, challenging current control strategies that rely largely on hemagglutinin (HA)-focused whole inactivated and autogenous vaccines. Here, we evaluated the immunogenicity, efficacy, and safety of next-generation RNA Particle (RNA-P) vaccines targeting either HA or neuraminidase glycoproteins and delivered by either intramuscular (IM) or intradermal (ID) routes.

Materials and Methods

A 1 mL dose of a quadrivalent, adjuvanted NA RNA-P vaccine (Sequivity® IAV-S NA) representing the four major NA clades of contemporary US H1N1, H1N2, and H3N2 viruses was administered twice at three days and three weeks of age and subsequently tested by homologous and heterologous IAV-S challenge in growing pigs. Separately, an adjuvanted HA RNA-P vaccine was assessed as a 1 mL IM regimen (Sequivity® with Microsol Diluvac Forte® [MDF]) and as a low-volume 0.2 mL ID regimen delivered with the IDAL® device (SEQUIVITY® ID), each vaccinated at three and six weeks of age and challenged with homologous H1N1 virus.

Results and Discussion

Across studies, vaccination elicited robust functional antibody responses (including high hemagglutination inhibition or NA inhibition activity), significantly reduced or prevented nasal virus shedding, lowered viral burden in the respiratory tract, and substantially decreased macroscopic lung lesions compared with placebo controls. The NA-based vaccine additionally reduced shedding after heterologous challenge within NA clades, supporting broadened protection. Safety evaluations in large field populations of weaned pigs and pregnant sows demonstrated that both IM and ID HA RNA-P vaccines were well tolerated, with local injection site reactions generally mild and transient and no adverse impact on farrowing outcomes.

Conclusion

Collectively, these results support RNA-P vaccines, delivered IM or ID and targeting HA or NA, as effective tools to mitigate IAV-S respiratory disease and virus spread in swine, with the NA-focused approach offering a complementary strategy to enhance cross-protective immunity in growing pigs.

6. UNDERSTANDING ENDEMIC SWINE INFLUENZA DYNAMICS IN PIG HERDS: INSIGHTS FROM LONGITUDINAL MONITORING AND MACHINE LEARNING

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Introduction and Objectives

Swine influenza A viruses (swIAVs) cause significant respiratory disease in pigs, leading to major animal health issues and economic losses. These viruses, which are also zoonotic, often persist endemically in farrow-to-finish pig herds, making their control particularly challenging. This study aimed to better understand the multi-scale drivers of swIAV infection dynamics by analyzing interactions between host immunity, housing conditions, and animal movement patterns within permanently infected herds.

Materials and Methods

A longitudinal study was conducted on two farrow-to-finish farms on successive pig batches from birth to fattening (33 piglets per batch), combining clinical, virological, serological, and environmental data collected every three weeks through five visits. After swIAV genotyping, hemagglutination inhibition assays were performed to evaluate humoral responses. The analytical workflow combined clustering of piglet serological profiles over time with an integrative machine learning approach to identify key environmental, spatial, and management-driven variables shaping these immune trajectories and underlying infection dynamics.

Results and Discussion

SwIAV infections were most frequently detected at post-weaning (Maternity: 0%, Post-weaning: 22.8%, Fattening: 1.6%). The machine learning models highlighted that features from all data categories jointly contributed to shaping piglet immune profiles. Specifically, post-weaning viral loads, cumulative deviations in room conditions and maternal immunity were predictive of their trajectories. Furthermore, the results demonstrated that animal flows were a critical determinant of within-herd infection patterns. Under Farm#1 structured mixing, a post-weaning room where piglets from different batches were pooled was identified as a transmission “hotspot”. Conversely, Farm#2 relying on continuous mixing across batches showed higher frequencies of successive infections and multi-strain co-circulation. These extensive contact dynamics likely would have promoted viral co-infections and may facilitate reassortment events among co-circulating genotypes.

Conclusion

By integrating longitudinal individual-level data, immunological clustering, and machine learning, this study provides detailed insights into endemic swIAV persistence. The findings reinforce the central role of spatial organization and animal mixing practices in shaping within-herd viral circulation. Ultimately, these results underscore the critical need to incorporate animal movement and batch management into biosecurity strategies to reduce infection pressure, mitigate multi-strain co-circulation, and limit viral reassortment risks in pig production systems.

7. INTEGRATED SURVEILLANCE STRATEGIES OF SWINE INFLUENZA A VIRUSES TO ASSESS ENVIRONMENTAL CONTAMINATION AND OCCUPATIONAL EXPOSURE IN PIG FARMS

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Introduction and Objectives:
Intensive swine production might pose a significant zoonotic risk as workers can be exposed to swine influenza A viruses (swIAV) through contact with infected animals and contaminated farm environments. This study develops an integrated One Health surveillance approach combining animals (AS), environmental (ES) and operator sampling (OS) to assess occupational exposure to swIAV.

Material and Methods:
Environmental samples including bioaerosols, surface gauzes, oral fluids and slurry were collected alongside voluntary self-collected nasal and hand swabs from farm workers during confirmed or suspected swIAV outbreaks. Anonymous questionnaires were administered to collect anamnestic and biosecurity data. Samples from 8 outbreak investigations were screened by RT-qPCR (Nagy et al., 2021; Hoffmann et al., 2006) and dRT-PCR. Subtype characterization was performed through established RT-qPCR assays and Nested-PCR (Graaf et al., 2023; Chiapponi et al., 2021) for H1 and H3 discrimination. For samples characterized by sufficient viral load, whole genome sequencing (WGS) and Sanger sequencing were attempted. To improve ES and OS characterization, the development of novel multiplex RT-qPCRs and RT-PCRs combined with NGS was initiated.

Results and Discussion:
Overall, 98 out of 152 samples tested positive for swIAV RNA by at least one screening assay. Of these, 19 were AS with different clinical manifestations. Positive ES included surfaces, oral fluids, slurry and bioaerosols, demonstrating widespread environmental contamination and the value of multi-matrix surveillance. swIAV RNA was detected in both nasal (9) and hand (12) swabs from workers in five different farms, confirming occupational exposure. Questionnaires highlighted variability in biosecurity measures among farms. During one outbreak, it was possible to compare partial sequences (461 nt) of OS and ES with those of animal-derived samples, and they differed by a single nucleotide substitution. In the remaining investigations, the low viral load in ES and OS prevented further characterization. The development of novel subtyping assays might support enhanced discrimination of H1av, H1pdm, H1hu, and H3 strains.

Conclusions:
Integrated sampling strategies for animals, human and farm environments combined with optimized molecular workflows, proved effective in demonstrating occupational exposure to swIAV. These findings support One Health surveillance strategies and provide a basis for future quantitative assessment of zoonotic risk in intensive swine production systems.

8. REGULAR MONITORING OF SPANISH PIG FARMS IN DIFFERENT REGIONS REVEALS A HIGH DEGREE OF GENETIC DIVERSITY AND DYNAMIC CIRCULATION OF THE INFLUENZA A VIRUS WITHIN THE FARMS

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Introduction and Objectives

Spain provides a relevant setting to study swIAV diversity and evolution because of its large pig population, high-density production areas and intensive systems, where continuous turnover of susceptible animals may favor spread and evolution of the virus. This study characterized swIAV temporal dynamics in Spanish pig farms monitoring respiratory pathogens.

Materials and Methods

Eighty-one farms enrolled in a voluntary 2-year monitoring program. In farms without outbreaks, piglets were randomly selected from farrowing units and nurseries (n=20 each) while in those experiencing respiratory disease samples were collected from sick and healthy age-matched piglets (n=20 each). After testing by RT-qPCR, isolation was attempted (Ct<30) and isolates were sequenced using Illumina technology.

Results and Discussion

Farm prevalence was 70.4% (57/81) and 77 isolates were obtained. H1N2 was predominant (37.6%), followed by H3N2 (33.7%), H1N1 (22.1%) and H3N1 (3.9%). In three cases the isolates contained a mix of HA/NA. The H1N2 set comprised H1avN2s (7/29), H1huN2g (6/29), H1avN2g (6/29), H1pdmN2g (5/29) and H1huN2s (2/29), as well as three cases of H1 coinfections of different lineages. Regarding H1N1, H1avN1av was predominant (11/17), followed by H1pdmN1pdm (4/17), and combinations of H1pdm or H1hu with N1av (two isolates). The HA1 clade distribution (51 isolates) was: 1B.1.2 (12); 1A.3.3.2 (10); 1C.2.2 (9); 1C.2.1 (7); 1C.2.4 (7); and 1C.2.6 (6). As for H3, H3mN2S predominated (16/26), followed by H3mN2G (10/26), all within clade 2000.3.Regarding NA, N2.G was the most common (n = 28), followed by N2.S (n = 26), N1.EA (n = 19), and N1.P (n = 4). A total of 27 genotypes were identified, including a previously unreported H3N2S virus with an avian internal cassette (14.3%, 11/77), which was the most prevalent, followed by genotypes A (12.9%, 10/77) and G (6.5%, 5/77). Of the 16 that have been already examined twice, five had two or more co-circulating subtypes; six cases saw the original subtype replaced by a new one; and 11 cases saw a change in the genotype of the circulating virus over time.

Conclusion

These findings reveal high swIAV diversity and viral turnover in pig farms, suggesting that subtype replacement and reassortment are the main evolutionary mechanism in the examined framework.

9. ONGOING CIRCULATION OF HUMAN-DERIVED H3N2 SWINE INFLUENZA VIRUSES IN NORTHERN ITALY

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Introduction and Objectives

Swine influenza A viruses (IAV-S) represent a major concern for both animal and public health. In Europe, multiple IAV-S genotypes co-circulate in swine populations, contributing to a highly dynamic and genetically diverse viral landscape. Recent evidence has highlighted the emergence of novel H3N2 IAV-S genotypes carrying a hemagglutinin of human seasonal origin, first detected in Italy in 2021. The increased detection of these human-like H3N2 viruses in Northern Italian swine populations highlights the role of pigs as hosts in which human-origin influenza genes may be maintained and further diversified. Here, we provide an update on the detection and genetic characterization of these human-like H3N2 IAV-S in Italy.

Materials and Methods

Respiratory samples collected from pigs during diagnostic activities in Northern Italy were screened for IAV-S by real-time RT-PCR. Positive samples were subjected to virus isolation. Viral RNA was extracted from IAV-S isolates and sequenced with Illumina technology. Consensus sequences were obtained for the eight genomic segments and used for subtype assignment and genetic characterization. Phylogenetic analyses were conducted on a dataset of human and European swine H3 HA and N2 NA sequences obtained from GenBank.

Results and Discussion

Human-like H3N2 IAV-S showed an increasing representation among sequenced Italian IAV-S isolates. After sporadic detection following their first identification in 2021, these viruses accounted for 16.4% of sequenced isolates (21/128) in 2025, while, as of April, 10/49 detections were recorded in the first months of 2026. All detected H3N2 viruses carried an H3 hemagglutinin assigned to the "Other-Human-2010" clade according to BV-BRC classification. Whole-genome analysis identified at least three distinct genomic constellations carrying this human-like H3.

Conclusions

The identification of these new H3N2 genotypes, actively circulating since 2021, highlights the persistent risk of human-to-swine transmission and subsequent reassortment events. The three distinct genotypes identified, carrying the human-derived HA, are characterized by different combinations of neuraminidase (NA) and internal gene cassettes (IGC) of swine and pandemic origin, highlighting the genomic plasticity of IAV-S. These findings emphasize the continuous exchange of genetic material at the human–animal interface and underline the importance of sustained IAV-S surveillance to monitor the evolution, spread, and potential impact of these emerging variants.

10. PERCEPTION OF SWINE INFLUENZA A RISK FOR ANIMAL AND HUMAN HEALTH AND ROLE OF VACCINATION AMONG STAKEHOLDERS IN THE PIG INDUSTRY: A QUALITATIVE STUDY IN BRITTANY, FRANCE

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Introduction and Objectives

Swine influenza A (SwIA) viruses cause infection of pigs that can result in significant economic losses and pose a potential risk of zoonotic transmission. Pigs are susceptible to infection by influenza viruses of various origins, thereby facilitating the emergence of novel reassortant viruses. Although vaccination remains a primary strategy for disease control, its effectiveness in reducing viral transmission remains limited. The objectives of this study were to characterize stakeholder's knowledge in the French pig production industry regarding zoonotic risk and vaccination, and to identify the levers and the obstacles to reduce the zoonotic risk.

Materials and Methods

Qualitative research was carried out in Brittany, the region with the highest pig density in France and a high prevalence of SwIA viruses. In-depth individual interviews, supported by participant observation, were conducted using semi-structured question guides with four veterinarians, three farmers and one swine flu expert. The descriptive thematic analysis consisted in the development of a coding tree based on the themes covered in the various guides, while new codes were added as new themes emerged from the data. A choice experiment was conducted on the selection and comparison of attributes for the implementation of vaccination strategies.

Results and Discussion

Our results highlighted that interviewed farmers care more about the health of their pigs than their own health. Interviewees considered that only a minority of farmers are aware of the zoonotic risk. Only a few farmers are vaccinated against seasonal flu even though the vaccination of professionals in contact with pigs is recommended and reimbursed by the Agricultural Social Mutual Organization. Veterinarians were identified as key informants to spread information concerning the zoonotic risk and seasonal vaccination of professionals. Participants emphasized the importance of the existing surveillance system covering pig farms (RESAVIP), to adapt the pig vaccination strategy to the circulating strains and to develop the surveillance of professionals, to identify potential zoonotic transmission.

Conclusion

Our work identified obstacles and levers to improve protection of swine professionals against the zoonotic risk and provide a list of attributes that can be used to limit this risk in a One Health perspective.

11. UNDERSTANDING SERBIAN PIG FARMERS' PERCEPTIONS FOR EFFECTIVE SWINE INFLUENZA PREVENTION AND CONTROL

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Introduction and Objectives

Farmers' perceptions influence their willingness to invest in animal health prevention and control measures. Understanding these perceptions will help design effective tailored messaging to enhance disease control, zoonotic transmission prevention, and strengthen national pandemic preparedness. The aim of this study was to understand perceptions of Serbian pig farmers related to swine influenza.

Materials and Methods

Data were collected using a specifically designed questionnaire with two sections: 21 questions on farm characteristics, demographics, knowledge, perceptions and awareness of animal-related public health issues with focus on swine influenza virus (SIV), and 9 questions on biosecurity practices. In total, 28 small-scale farms across 6 villages in the Vojvodina region were visited.

Results and Discussion

Among the interviewed farmers, 60.7% knew that diseases can be transmitted from pigs to humans but none identified SIV as zoonotic disease. In total 67.9% were familiar with biosecurity, however, 96.4% had contact with or raised other livestock, most of which were in contact with pigs. African swine fever, diarrhoea, and pneumonia were the main concerns, while none considered SIV a problem on their farm. The potential clinical and economic impact of SIV was the main concern (75%), but most farmers were not concerned about its zoonotic transmission. Motivation to vaccinate against SIV was lacking, mainly due to lack of belief in vaccines, insufficient knowledge of SIV vaccines, and perceived absence of disease. Only 55.6% believed they could prevent zoonotic transmission, while most considered authorities rather than farmers responsible. Farm size emerged as a stronger predictor of knowledge and awareness than years of experience: farmers with larger herds (≥200 pigs) were significantly more likely to recognize zoonotic diseases (83% vs 44%, p=.034) and reported higher familiarity with the One Health concept (p=.003).

Conclusion

Authorities should support farmers' willingness to prevent swine influenza, particularly on smaller farms where knowledge gaps are most pronounced, and increase awareness of its public health risks. Training programmes should be tailored to farm scale, prioritizing smaller operations.

12. BIOSECURITY MEASURES FOR OPTIMIZED MANAGEMENT AND CONTROL OF SWINE INFLUENZA IN PIG HERDS

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Introduction and Objectives

Swine influenza (SwIA) is a respiratory disease of pigs that poses a major threat to the pig sector, affecting animal health and the farm economy. Biosecurity, together with vaccination, is a strategy to prevent and control respiratory diseases at the farm level. This study aimed to identify on-farm prevention and control practices for SwIA with the greatest preventive potential, as determined by expert opinion and scientific literature.

Materials and Methods

Two complementary approaches were used: a bibliographic review of peer-reviewed literature from the Web of Science database, and an expert opinion survey. In total, 96 biosecurity measures grouped in six external and internal biosecurity topics were initially identified. Experts in pig diseases and biosecurity were asked to rate the importance of the topics and the biosecurity measures for SwIA. In the literature review, 73 of the 322 initially identified publications were deemed relevant because they presented and evaluated biosecurity measures against SwIA. The results of the literature review and expert opinion were used to calculate the literature priority index (LPI) and the expertpriority index (EPI) and later integrated into a four-quadrant analysis.

Results and Discussion

'Purchasing/Introduction of breeding pigs and piglets' and 'Animal flow' were the most important topics according to experts (EPI = 1). The strongest evidence in the literature was found for the 'Ventilation/ farm climate' topic, with the effectiveness of several measures being experimentally tested. Regarding biosecurity measures, 93% of them were poorly investigated in literature (LPI < 54). Based on the four-quadrant analysis it was found that only 3 out of 96 measures (Q2) were prioritized by experts and showed a high evidence in literature: 'Density of pigs farm around', 'Type of farm' and 'All-in-all-out system'. Two measures were studied in literature but not prioritized by experts (Q1): 'Animal density' and 'Minimum number of visitors', on the contrary, 31 measures were considered important by experts but not investigated (Q3).

Conclusion

We identified the most important preventing measures for SwIA (measures in Q2) and highlighted research gaps (Q1 and Q3), identifying potential research priorities.

13. ANOTHER VIRUS BITES THE DUST: ELECTROSTATIC DUST COLLECTORS AS NON-INVASIVE ALTERNATIVE FOR DETECTION AND SEQUENCING OF INFLUENZA A VIRUS IN PIG HERDS

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Introduction and Objectives

Pigs are reservoirs of former human influenza A virus (IAV) lineages, and act as 'mixing vessels', facilitating opportunities for novel IAVs. IAV surveillance in pig herds is therefore crucial to assess the risk of emerging zoonotic strains. However, effective surveillance of swine influenza is complex due to short-duration shedding and aspecific clinical signs. Moreover, the current golden standard for IAV sampling, nasal swabbing (NS), causes discomfort. Here we tested whether passive air sampling through electrostatic dust collectors (EDCs) could be a non-invasive, practical alternative for the detection and genomic characterization of IAVs circulating in pig herds.

Materials and Methods

During a first pilot in three herds, we collected dust on horizontally placed EDCs, testing three periods of exposure (1, 2, and 7 days), and compared outcomes to well-established IAV detection methods (NS, chewing ropes (CR)). Based on qPCR results, a Bayesian latent class analysis was performed to determine sensitivity and specificity of the matrices. A second pilot was performed in one herd, where EDCs were placed horizontally and vertically, and preserved in DNA/RNA Defend buffer, to reduce suspected RNA degradation. Samples with Ct-values<32 were selected for Oxford Nanopore sequencing.

Results and Discussion

The first pilot showed 90% sensitivity and 91% specificity for 2-day air-exposed EDCs. This was comparable to CR and NS outcomes. Although EDCs had Ct-values<32, the quality of obtained viral sequences was substandard, while WGS were retrieved from other matrices. We hypothesized that either the long exposure of aerosols to captured dust, or the storage of EDCs without stabilizing buffer, may have caused RNA degradation. Preliminary data from the second pilot showed improved quality for sequencing of samples obtained from vertically placed EDCs stored in buffer.

Conclusion

Airborne dust can be sampled with EDCs to detect IAVs by qPCR in pig farms with high sensitivity and specificity and seems promising for IAV lineage classification by sequencing. Ct-values are no reliable predictor of obtaining high-quality WGS from this matrix. Advantages of sampling dust instead of pigs, are the non-invasive nature, lower costs, and opportunities for farmers to do the sampling themselves, which reduces external biosecurity risks.

Ethics statement: In this study, non-invasive animal samples (non-deep nasal swabs, nasal wipes, udder wipes and oral fluids) and environmental samples were collected. Therefore, the study was not considered an animal experiment according to the Animal Welfare Body from Royal GD, Deventer, the Netherlands.

14. DETECTION RATES AND VACCINATION STRATEGIES IN DANISH SOW HERDS 2020-2025

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Introduction and Objectives

Influenza A virus is a common respiratory pathogen in pigs and remains relevant for herd health, diagnostic decision-making, and zoonotic risk assessment. In Denmark, passive surveillance of influenza in pigs is conducted through PCR testing of pooled nasal swabs submitted from herds with clinical signs consistent with influenza. For a subset of submissions, supplementary herd-level information is collected. This report summarizes laboratory findings from 2020 to 2025 in relation to these variables and evaluates patterns in virus detection and vaccine use in Danish pig herds.

Materials and methods

Surveillance data were based on pooled nasal swabs collected by practicing veterinarians from pigs with suspected influenza and analyzed by PCR at Statens Serum Institute. For samples included through collaboration with CEVA Animal Health, additional data on pig age group, pig age at sampling, and herd vaccination program were available. The report describes laboratory findings across the period 2020–2025, with particular emphasis on 2025, and examines associations between influenza detection, animal age, regional distribution, season, and vaccination practices.

Results and Discussion

Influenza was detected predominantly in suckling pigs and weaners, whereas samples from finishers and adult breeding animals were rarely positive. In 2025, 150 herds were examined through 167 submissions comprising 774 pooled samples, and influenza was detected in 125 herds, corresponding to 83% of the investigated herds. Most submissions included samples from suckling pigs or weaners, often from both age groups. Among subtyped isolates, H1pdm09-variants accounted for approximately half of herd findings in 2025, although the proportion varied considerably by region. In some herds, both H1pdm09 and classical swine influenza were detected. The likelihood of influenza detection was highest in older suckling pigs and newly weaned pigs. Among suckling pigs, positivity increased with age, and in 2025 the difference between the youngest and older piglets was statistically significant. Among weaners, there was a tendency toward lower positivity with increasing time after weaning, although this pattern was not statistically significant. Influenza-positive herds were identified throughout the year, with no clear seasonal pattern, indicating that influenza circulation in Danish pigs is not confined to specific seasons. Vaccination practices differed substantially among herds. Most vaccinating herds immunized gilts twice, whereas sow vaccination was implemented either as mass vaccination or around farrowing. However, some herds appeared to vaccinate too infrequently in relation to the year-round occurrence of infection. In addition, vaccine choice was often not aligned with the subtype detected in the herd, and more herds vaccinated only against classical influenza despite the frequent occurrence of H1pdm09.

Conclusion

Influenza remains common in Danish pig herds investigated for respiratory disease, particularly among suckling pigs and weaners. Sampling older suckling pigs and newly weaned pigs appears most suitable when the goal is to detect and subtype influenza virus in positive herds. The absence of seasonality suggests that surveillance and vaccination programs should be maintained year-round. Improved alignment between sampling protocols, laboratory findings and vaccination strategies may strengthen influenza control at herd level.

15. GENOMIC SURVEILLANCE REVEALS DOMINANCE OF EURASIAN AVIAN-LIKE SWINE INFLUENZA A VIRUS AND BIDIRECTIONAL TRANSMISSION IN SWISS PIG HERDS

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Introduction

Influenza A viruses (IAV) pose a persistent public health threat due to their ability to cross species barriers. Pigs act as IAV reservoirs and “mixing vessels”, where viral diversity is frequently amplified by multidirectional spillovers between humans and pigs, driving viral evolution through antigenic drifts and shifts. Switzerland maintains a closed pig production system with limited live-animal importation, potentially reducing external viral introductions and promoting distinct local evolutionary dynamics. We hypothesize that swine IAV (swIAV) circulating in Swiss pig herds exhibits unique genetic characteristics compared to strains circulating elsewhere in Europe.

Methods

We collected nasal swabs from symptomatic and asymptomatic pigs, as well as from symptomatic pig caretakers. Whole genome sequencing (WGS) was performed on qRT-PCR IAV-positive samples using Oxford Nanopore technology. Sequencing data were analyzed using bioinformatic approaches to characterize viral genetic diversity and infer transmission patterns between hosts.

Results and Discussion

Swine IAV was detected in 50% of symptomatic and 31% of asymptomatic herds. Most of the recovered sequences belonged to the swine H1N1 Eurasian avian-like (EA) lineage; clades 1C.2.1 and 1C.2.2, whereas the remaining viruses were reassortants of EA and H1N1 pandemic 2009 (pdm09). Phylogenetic analysis revealed that the EA-pdm09 reassortant viruses likely originated from a spillover of human seasonal pdm09 strains into pigs, followed by reassortment with the dominant EA strains. Furthermore, a case of human infection with swIAV EA was detected in one of the pig herds. Sequence analysis revealed >99% of sequence identity between human and swine IAV sequences, supporting a zoonotic transmission event. The EA viruses detected in Swiss pigs clustered distinctly from contemporary European strains, indicating a unique genetic signature.

Conclusion

Our findings demonstrate that symptomatic and asymptomatic Swiss pigs are predominantly infected by a single swIAV lineage. The unique genetic signature, alongside detection of zoonotic and reverse-zoonotic spillover events, underscores the importance of integrated genomic surveillance at the human–pig interface to better assess transmission risks. These results provide insights into IAV dynamics in Swiss pigs and inform targeted veterinary and public health interventions.

16. ENHANCING INFLUENZA, A SURVEILLANCE IN THE NETHERLANDS: ADDRESSING EPIDEMIOLOGY GAPS OF INFLUENZA A IN A ONE HEALTH CONTEXT

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Background:
The Strong1H project (2023–2026) aimed to enhance the detection and monitoring of influenza A virus (IAV) by conducting targeted, risk-based sampling in populations that are currently underrepresented in existing surveillance systems.

Methods:
Wild boar samples were screened for IAV antibodies using NP-ELISA, with positive samples further characterized via Luminex technology. In addition, samples were collected from domestic outdoor pigs, wild mammals, and environmental sources, and tested for IAV using PCR. Camera traps were used to gain insight into the number and species of scavenging animals with interactions with dead (migratory) birds. Positive IAV-PCR samples were further sequenced, and sequences were uploaded to a national One Health data-sharing platform, facilitating integrated analysis by comparing existing sequencing data from domestic indoor pig surveillance with human, avian, and mammalian reference sequences.

Results:
With NP-ELISA, antibodies against IAV were detected in 18.5% of the 2585 wild boar samples collected between 2017 and 2025. Further serotyping with Luminex identified antibodies against swine Influenza, as well as H5N8 and H5N1 subtypes. In total, 306 non-invasive oral fluid samples from 27 outdoor pig farms were collected and all tested PCR negative for IAV. In 2025, two red foxes were positive for H5N1 (DI.2 genotype). Camera trap results confirmed predation on bird carcasses by mammalian carnivores and rodents. Surface water samples from the winters of 2024–25 and 2025–26 frequently yielded pan-influenza PCR positive results, though only once an H5-specific positive sample was detected.

Conclusions:
This study offers fresh insights into the epidemiology of IAV in animals not currently included in monitoring programs, as well as in the environment. By combining expertise and data from veterinary, environmental, and human health sectors, and concentrating on under-sampled wildlife and environmental reservoirs, our project enhances IAV One Health surveillance. It also lays the basis for developing a sustainable IAV One Health surveillance roadmap in the Netherlands.

17. EVOLUTION OF SURFACE PROTEINS IN SWINE INFLUENZA A VIRUSES CIRCULATING IN BELGIUM (2023–2026)

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Introduction and objectives

Swine influenza A viruses (swIAV) evolve rapidly through mutations and reassortment, enabling the emergence of zoonotic variants. Continuous surveillance is essential for detecting variants with zoonotic potential and vaccine selection. This study characterizes swIAV subtypes circulating in Belgian swine and their genetic and antigenic.

Materials and Methods

A total of 158 samples from pigs with respiratory symptoms (2023-2025) yielded 67 isolates for sequencing and phylogenetic analysis. Antigenic properties were assessed using cross-hemagglutination inhibition (HI) and cross-neuraminidase inhibition (NI) assays with reference strains, including seasonal human and swine vaccine strains. Additionally, HI assays were performed with sera from pigs vaccinated with the 2 commercial swIAV vaccines available in Belgium (Respiporc® FLU3 and FLUpan).

Results and Discussion

The dominant swIAV subtype was EUH1avN1av (55.2%), and H3N2 was absent. Out of the 11 distinct genotypes identified, most carried the 2009 pandemic M gene, including 5 newly detected ones in Belgium. Clade 1C.2.2 emerged as the dominant clade. Cross-HI analysis showed marked antigenic drift in several 1A.3.3.2 isolates, whereas clades 1B.1.2.1 and 1C.2.2 remained broadly reactive with recent European reference strains. Notably, none of the isolates reacted with 2018 or 2022 human vaccine strains, indicating clear antigenic separation. NI analysis showed distinct evolutionary patterns among 3 neuraminidase (NA) lineages identified. N2Gent-84 (from European human-like H3N2) showed pronounced antigenic drift, separating into 3 antigenically distinct clusters with cluster-specific serum reactivity. In contrast, N1av (from European avian-like H1N1) and N2Scot-94 (from European human-like H1N2) remained antigenically stable. Together, these findings suggest that the 3 NA lineages undergo differential evolutionary pressures. Serologic cross-reactivity showed clear gaps: sera from pigs vaccinated with the 2009 pandemic strain (Respiporc® FLUpan) failed to react with all 1A.3.3.2 isolates. Also, sera from pigs vaccinated with the avian-like strain (Respiporc® FLU3) failed to react with 35% of 1C.2.2 isolates, indicating insufficient coverage of circulating field strains.

Conclusions

The lack of cross-reactivity between contemporary field isolates and human seasonal vaccine strains, with gaps in swine vaccine coverage, underscores clade-specific pandemic risks that immunization strategies cannot address. These findings show that continuous surveillance and vaccine strain evolution are essential for effective preparedness.

18. H3N2 INFLUENZA A VIRUS DETECTED IN PIGS IN GREAT BRITAIN FOR THE FIRST TIME IN THREE DECADES

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Globally, influenza A(H3N2) viruses have circulated widely and evolved for decades in both human and swine populations. In Great Britain, however, H3N2 viruses have not been detected in pigs since 1997, until recent surveillance identified an H3N2 influenza A virus in an English pig herd. Phylogenetic analysis revealed that the haemagglutinin belonged to the human seasonal G.2 subclade, previously classified within 3C.2a1b.2a.2b (2b) last detected in humans around 2023. The neuraminidase gene segment was closely related to human seasonal N2 viruses of the A.2.2.2 clade, which was associated with G.2 viruses. All characterised viruses possessed the remaining six internal gene segments of A(H1N1)pdm09 origin, forming a genomic constellation comprising human-seasonal HA and NA segments on an A(H1N1)pdm09-derived internal gene backbone. In addition, a H1N2 swine influenza A virus belonging to the 1B.1.1.1 HA clade, possessing internal gene segments of A(H1N1)pdm09 origin, was also detected within the same herd.
Antigenic characterisation by haemagglutination inhibition testing showed strong recognition of the swine H3N2 viruses by ferret antisera to representative a human seasonal G.2 clade virus, but poor recognition by ferret antisera to recent seasonal vaccine strains. Cross-reactivity was further assessed using paired pre- and post-vaccination sera from volunteers receiving the 2025–26 northern hemisphere influenza vaccine, which showed limited vaccine-induced reactivity against both the vaccine strains and the swine H3N2 viruses. Taken together, these data suggest that current human seasonal vaccine strains provide limited antigenic coverage against these contemporary swine H3N2 viruses.
This finding follows the detection of a subclade J.2 H3N2 virus in pigs in Northern Ireland, suggesting renewed incursions of contemporary H3N2 viruses into UK swine populations. These events are particularly significant in the context of the 2025–26 northern hemisphere influenza season, during which the human A(H3N2) K subclade expanded rapidly, highlighting the continued capacity of H3N2 viruses for rapid diversification and the potential for reverse-zoonotic spillover into pigs.

POSTER PRESENTATIONS:

19. Pandemic Risk of European Swine Influenza Viruses Replicative fitness in a human model

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Introduction and Objectives
Influenza A viruses have a significant impact on human and animal health, and the economy worldwide. Swine Influenza A viruses (swIAVs) have been known to spillover into the human population and cause zoonotic infections. Such detections are called 'Influenza variant viruses of swine origin' or, 'variants' and cause varying disease severities, and were the origin of the 2009 influenza pandemic. There is currently little research into understanding the phenotypic characteristics as to why certain swIAVs spillover into humans, and whether currently circulating swIAVs also possess this potential.

Materials and Methods
Human lung epithelial (A549) and newborn pig tracheal (NPTr) cell lines were infected with a selection of variant viruses and swIAVs and samples were taken for quantification of viral growth to assess viral fitness in a human cell line.

Results and Discussion
Three variant viruses and one swIAV grew to significantly increased titres in A549s compared to NPTrs, and another two variants and swIAVs grew to high titres in both cell lines. These viruses either exceeded, or grew to the equivalent titres of that of the human seasonal H1N1 control in A549s.

Conclusion
These results suggest an increased replicative fitness in human cells of variant and contemporary swIAVs circulating in Europe in recent years, this might reflect an increased zoonotic potential and therefore has consequences for pandemic preparedness efforts.

20. GENETIC CHARACTERIZATION OF SWINE INFLUENZA VIRUSES, INCLUDING A REASSORTANT STRAIN, IN FINLAND, 2014–2025

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Introduction and Objectives

Swine influenza A viruses (swIAV) are genetically diverse pathogens capable of reassortment, enabling the emergence of novel variants with zoonotic potential. Finland represents a geographically and epidemiologically semi-closed pig production system with minimal live-animal movement, providing a unique environment to study long-term viral evolution. This study aimed to characterize the genetic diversity, temporal dynamics and reassortment events of swIAV circulating in Finland between 2014 and 2025, and to assess the emergence and spread of newly detected genotypes

Materials and Methods

Diagnostic samples (n=955) were submitted from 235 pig holdings for influenza A virus detection. Samples consisted of nasal swabs (19.5%) and pathological tissues (80.5%). Influenza A–positive samples were identified using real-time RT-PCR. Whole-genome sequencing was performed on Illumina MiSeq using Uni12/Inf primer sets for full-segment amplification. Libraries were prepared with Nextera XT or Illumina DNA Prep kits. Sequence reads were processed and assembled using CLC Genomics Workbench v25.0 with default parameters. Genotypes were assigned based on established HA/NA/inner-gene lineage nomenclature, and phylogenetic analyses were conducted for all eight gene segments. Statistical calculations were performed using Epitools.

Results and Discussion

Of the 955 samples, 149 (16%) tested positive for influenza A virus. Whole-genome sequencing identified three circulating genotypes: avian-like H1C.2.1/N1EA/EEEEEE (n=28), avian-like H1C.2.4/N1EA/EEEEEE (n=6), and pandemic H1A.3.3.2/N1P/PPPPPP (n=4). Phylogenetic analysis revealed a previously undescribed reassortant strain combining an HA gene from genotype H1C.2.4 with the remaining seven segments from H1C.2.1, demonstrating active co-circulation and genetic exchange between endemic lineages. A marked increase in detections in 2019 coincided with the emergence of genotype H1C.2.1, suggesting altered transmissibility or clinical impact. Despite a 61% reduction in pig holdings during the study period, sample submissions remained relatively stable, reflecting heightened awareness following new strain introductions.

Conclusion

This 12-year surveillance study provides comprehensive genomic insight into swIAV evolution in a geographically isolated production system. The identification of a novel reassortant strain underscores the ongoing evolutionary activity of swIAV in Finland and highlights the necessity of continuous genomic monitoring to detect emerging variants with potential implications for animal and public health.

21. OPTIMIZING SWINE INFLUENZA A VIRUS SAMPLING FOR HERD-LEVEL SURVEILLANCE AND STRAIN CHARACTERIZATION

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Introduction and Objectives

Swine influenza A virus (swIAV) monitoring is challenged by transient viral shedding, enzootic circulation, low within-herd prevalence, and co-circulation of multiple strains. This review summarizes recent evidence to guide practical farm and slaughterhouse sampling strategies for sensitive herd-level detection of swIAV circulation and epidemiological relevant information on circulating strains.

Materials and Methods

Sampling approaches evaluated across production stages and at slaughter included nasal swabs, tracheobronchial and bronchial swabs, lung tissue, serum, and group-based samples such as oral fluids, contact wipes, dust wipes, and udder skin wipes. Detection was performed by Matrix-gene RT-qPCR, followed by subtype-specific multiplex RT-qPCR when viral loads allowed. Serological monitoring was performed using NP-based ELISAs and hemagglutination inhibition assays with antigen panels of varying breadth. The effect of stabilizing media and cooled storage on oral-fluid sample quality was also assessed.

Results and Discussion

Population-based samples, particularly oral fluids, are valuable tools for herd-level surveillance because they are welfare-friendly, time-efficient, and representative of large animal groups. They provide high detection rates and opportunities for subtype identification. Environmental samples such as dust wipes generally contain lower viral loads and are less suitable for subtype characterization. Nasal swabs remain the preferred sample type for detailed strain characterization. In breeding and nursery systems, sampling suckling piglets and multiple nursery age groups is essential, as different subtypes may circulate simultaneously. In fattening herds, repeated oral-fluid sampling can detect circulation but may miss infections because shedding is short. At slaughter, bronchial swabs and lung tissue outperform nasal swabs and oral fluids for RT-qPCR detection and subtyping, with bronchial swabs providing the highest diagnostic value. Serology substantially improves herd-level detection, while expanded HI panels provide additional subtype information. Stabilizers and cooled storage improve oral-fluid RT-qPCR performance.

Conclusion

Effective swIAV surveillance requires a combination of complementary sampling approaches adapted to the production stage and diagnostic objective. The most informative strategy combines repeated oral-fluid sampling for herd-level surveillance, targeted nasal swabs for strain characterization, serology for assessing herd exposure, and bronchial swabs or lung tissue at slaughter as complementary tools for detecting ongoing circulating strains.

22. SWINE INFLUENZA A VIRUS SURVEILLANCE IN SLOVENIAN PIG FARMS USING NON-INVASIVE ENVIRONMENTAL AND AIR SAMPLING APPROACHES

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Introduction and Objectives

Swine influenza A virus (swIAV) is an important respiratory pathogen in pigs and a potential zoonotic threat due to the ability of influenza A viruses to cross species barriers. Conventional sampling often requires handling of animals, whereas environmental and air sampling may offer a practical, non-invasive alternative. This study aimed to evaluate the presence of swIAV on Slovenian pig farms with respiratory clinical signs and to assess environmental samples as non-invasive diagnostic specimens.

Materials and Methods

The study was conducted in 2025-2026 within the IAVSur-BME project. Targeted surveillance was performed on 14 Slovenian pig farms with observed respiratory clinical signs. Nasal swabs were collected from clinically affected pigs, followed by environmental sampling in the same pens, including swabs from pen walls or bars, areas around feeders, and air samples. In total, 202 nasal swabs, 55 environmental swabs, and 18 air samples were tested by real-time RT-PCR targeting the influenza A matrix (M) gene according to WOAH-recommended protocols. Influenza A virus-positive samples were subsequently subtyped using a real-time RT-PCR assay as described by Hassan et al. (2022; doi: 10.3390/v14020415).

Results and Discussion

Two of the 14 farms tested positive for swIAV. Positive results were detected in nasal swabs, environmental swabs, and air samples, and the viruses were identified as subtype H1N1. These findings support the use of environmental and air sampling as complementary, non-invasive tools for swIAV surveillance, particularly on farms with respiratory clinical signs. Previous Slovenian studies have shown substantial herd-level exposure to swIAV, with predominance of H1N1 and evidence of genetically diverse strains, including H1avN2 detected in 2023. Together with these data, our findings indicate the ongoing presence and diversity of swIAV in Slovenian pig herds.

Conclusion

The results confirm swIAV circulation in Slovenian pig farms and support environmental and air samples as useful complementary, non-invasive tools for targeted surveillance. Continuous monitoring and timely communication of results to farmers and veterinarians are important to improve awareness, strengthen biosecurity, and support early detection of viruses with animal and public health relevance.

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23. TRANSMISSION EVENTS OF SWINE- AND HUMAN-ORIGIN INFLUENZA A VIRUS IN TURKEYS (*Meleagris gallopavo*)

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Introduction

While transmission of influenza A viruses (IAVs) from birds to humans and swine is frequently reported, infections of poultry with human- or swine-adapted IAVs remain poorly documented. Here, we describe three independent interspecies transmission events detected in commercial turkey farms in Northern Italy, a region characterized by intensive poultry and swine production.

Materials and Methods

Tracheal swabs and/or cloacal swabs were collected in 2019, 2020 and 2024 from three turkey farms showing clinical signs compatible with low pathogenic avian influenza infection (mild respiratory signs and reduced egg production). IAVs were detected by rRT-PCR, followed by subtyping, whole-genome sequencing (Illumina-MiSeq/Nanopore-MinION) and phylogenetic analysis. Serological tests (ELISA and HI) and epidemiological investigations were also performed for each outbreak.

Results

In 2019, an H1N1pdm09 virus closely related to human seasonal strains was detected in a breeder turkey farm. Evidence suggests a human-to-bird transmission, which likely occurred during artificial insemination. Serological, virological and genetic data indicated subsequent spread of the virus within the farm and signs of virus adaptation to birds, as suggested by the emergence of the HA-Q223R substitution, which is associated with an increased affinity to avian-type receptors.
The following year, a swine-origin H1N1 virus (clade 1C.2.1) caused an outbreak in a meat turkey farm. Genomic similarity with local swine viruses supported swine-to-turkey transmission, with shared personnel and feed transport identified as most likely routes of transmission.
The third outbreak occurred in 2024 in a breeder turkey farm and was caused by a swine-origin H1N1 virus (clade 1C.2.4) closely related to local swine strains. Shared personnel between swine and turkey farms were the most likely transmission route. Serological analyses revealed that all the collected serum samples (N=10) were positive for H1 influenza A, indicating a seroconversion in the flock. A novel HA-N168S substitution in the receptor-binding domain was identified.

Conclusions

These findings highlight the occasional transmission of IAV from mammals to poultry and suggest that swine play a dual role as both mixing vessels and active spillover sources. Such events may promote reassortment between mammalian- and avian-origin influenza viruses, increasing the risk of emergence of novel zoonotic strains. Strengthening One Health surveillance across swine, poultry and exposed workers is essential for the early detection and the prompt implementation of preventive and control measures.

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24. MULTISECTORAL SURVEILLANCE REVEALS GENETIC DIVERSITY AND POTENTIAL REVERSE ZOONOTIC TRANSMISSION OF SWINE INFLUENZA A VIRUSES IN AUSTRIA

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Introduction and Objectives

Swine influenza A viruses (swIAV) cause respiratory disease in pigs, leading to economic losses and posing zoonotic risks. Until 2023, Austria lacked systematic swIAV-surveillance. To address this gap, a pilot study launched in 2023 established a multisectoral network integrating diagnostic laboratories, academia, industry stakeholders, and animal health services. The objectives of this study were to (i) evaluate the performance of this multisectoral surveillance approach and (ii) characterize the genetic diversity and epidemiology of swIAV circulating in Austrian pig herds.

Materials and Methods

Respiratory samples collected from 386 pig farms between 2018 and 2026 were screened using RT qPCR targeting the matrix gene. Samples with sufficient viral load were selected for whole-genome sequencing. Genetic characterization was performed through subtyping, phylogenetic analyses, and genotyping using the OctoFLU classification system.

Results and Discussion

SwIAV was detected in 11% of samples, corresponding to a farm-level positivity rate of 18.6%. Most successfully characterized viruses belonged to the avian-like lineage 1C (88%), while lineages 1A (7.2%) and 1B (4.8%) were detected less frequently. No H3N2 viruses were identified during the study period. Within lineage 1C, clade 1C.2.2 predominated. Additionally, six detections of H1N1pdm09 viruses were identified on five farms between 2024 and 2026. Phylogenetic analysis showed that Austrian swine-derived H1N1pdm09 viruses clustered closely with contemporaneous seasonal human influenza A viruses from Austria, suggesting potential human-to-swine transmission events.

Conclusion

This study provides new insights into the circulation and genetic diversity of swIAV in Austria and demonstrates the value of multisectoral surveillance. The detection of potential reverse zoonotic transmission events highlights the importance of the human–animal interface in swIAV epidemiology.Building on this pilot, further expansion of the surveillance network and harmonization of
sampling strategies will be essential to improve coverage, operational efficiency, and the quality of epidemiological metadata.

25. AN END-TO-END PIPELINE FOR ASSEMBLY, GENOTYPING AND SURVEILLANCE OF SWINE INFLUENZA

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Introduction and Objectives

Swine Influenza A Virus (swIAV) poses a significant risk to both animal and human health due to its capacity for genomic reassortment and zoonotic transmission. With Denmark's intensive pig farming industry—producing over 30 million pigs annually—there is significant human-animal contact daily, amplifying the potential for interspecies transmission and the emergence of novel influenza strains. Reassortment events can result in viruses with altered antigenicity and virulence, underscoring the need for robust genomic surveillance. To facilitate this, we have developed a diagnostic pipeline that automates referenced-based assembly and genotyping of swIAV genomes.

Materials and Methods

The pipeline is implemented as a reproducible snakemake workflow, integrating read quality control, reference selection, annotation and phylogenetic characterization within a single workflow. Various programs with differing parameters were tested and evaluated based on speed and resource usage. Using a standardized dataset, the workflow was benchmarked against other workflows.

Results and Discussion

The resulting pipeline works as follows: The quality of input data from either Illumina or Nanopore sequencing platforms is assessed with fastp. Selection of the optimal reference genome segments is done with KMA by employing a k-mer-based mapping of the reads to a deduplicated influenza database. Consensus sequences are then generated through an iterative assembly procedure that repeatedly maps and refines the assembly until convergence is achieved. Coverage statistics, ambiguity rates and segment completeness metrics are reported for each segment. The resulting genome is annotated, and the quality is assessed using custom Julia packages that verify segment structure and coding sequence integrity. The finalized genome is genotyped by constructing segment-level phylogenies with sequences from a curated database. The phylogenetic placement of each segment is evaluated with emergent genotypes and reassortment events flagged for manual inspection. The workflow can run on a laptop and assemble genomes in a minute time frame. It is faster and less resource heavy than other known workflows.

Conclusion

We have developed a scalable and reproducible swine influenza pipeline that transforms raw sequencing data to annotated, genotyped genomes.

26. VIROLOGICAL BACKGROUND OF SWINE IAV-POSITIVE PIGS IN SWITZERLAND: PRELIMINARY EVIDENCE OF PPIV1, SOV AND PHEV

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Introduction and Objectives

Swine influenza A virus (swIAV) is a major respiratory pathogen in pigs; however, under field conditions, swIAV may be detected in different clinical and epidemiological contexts, ranging from respiratory disease outbreaks to asymptomatic pig herds, and alongside other viral agents. In Switzerland, the virological background in which swIAV circulates remains incompletely characterized. Porcine parainfluenza virus 1 (PPIV1) and swine orthopneumovirus (SOV) have been reported in several European countries, but, to our knowledge, have not yet been documented in Swiss pigs. Although porcine hemagglutinating encephalomyelitis virus (PHEV) has been reported in Switzerland in the 1970s, its current circulation has not been systematically investigated. As an initial step within a broader project, this work focused on swIAV-positive samples to assess selected viral co-detections and support multiplex RT-qPCR development.

Material and Methods

We performed targeted molecular screening of archived individual nasal swab samples from Swiss pigs previously identified as swIAV-positive. Samples were tested by RT-qPCR for PPIV1, SOV, and PHEV using published primer/probe sets, with minor adaptation of the PHEV probe.

Results and Discussion

Screening of 96 swIAV-positive samples for PHEV and PPIV1 detected PHEV in 41/96 samples (42.7%) and PPIV1 in 27/96 samples (28.1%). Among these, 16/96 samples (16.7%) were positive for both viruses. SOV testing was performed on 78 of the 96 samples, with SOV detected in 6/78 (7.7%). Among the 78 swIAV-positive samples tested for PPIV1, SOV, and PHEV, viral co-detections were observed, including one PPIV1/SOV-positive sample, two PHEV/SOV-positive samples, and one sample with co-detection of PHEV/PPIV1/SOV. Since all samples were swIAV-positive, these findings indicate viral co-detections in swIAV-positive nasal swab samples. To our knowledge, these data provide the first molecular evidence of PPIV1 and SOV in Swiss pigs and suggest that PHEV remains detectable decades after its first description in the country.

Conclusions

These preliminary findings expand current knowledge of swine respiratory viral co-detections in Switzerland. They support further investigation of their association with available clinical and epidemiological metadata, and the continued development of multiplex diagnostic tools for surveillance and biological risk profiling.

27. EVOLUTIONARY DYNAMICS OF SWIAV QUASISPECIES DURING A SCHEDULED MONITORING OF REPIRATORY PATHOGENS IN SPANISH PIG FARMS

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Introduction and Objectives

Swine influenza A virus (swIAV) is a major respiratory pathogen of pigs and a zoonotic threat. Spain is a relevant setting for studying swIAV and evolution because of its large industrial pig population concentrated in high-density areas where continuous turnover of susceptible animals may facilitate viral persistence, co-circulation and reassortment. This study aimed to describe and characterize swIAV quasispecies, genetic evolution and evolutionary dynamics in six Spanish pig farms belonging to the same company between 2024 and 2025.

Materials and Methods

Nasal swabs collected periodically for monitoring purposes during a two-year period were tested by RT-qPCR. Positive samples (Ct values <30) were isolated in MDCK cells and sequenced using Illumina technology. Viral quasispecies structure was inferred from the nucleotide proportions at each position across viral segments, and entropy and mean coverage per position were calculated. Each isolate was genotyped from the consensus sequence. Bayesian phylogenetic trees were built using MrBayes, including reference strains for comparison. Circulating lineages of the HA, NA and PB2 segments were compared between consecutive sampling times. When the same lineage was detected, mutations between consensus sequences were classified as pre-existing if already present in the previous quasispecies, or as *ex novo* if not previously detected.

Results and Discussion

At least two genotypes and subtypes were detected in every farm, with some farms showing up to five genotypes over time and evidence of co-infections. Comparisons between consecutive isolates within farms showed that many mutations detected in consensus sequences were already present as minor variants in the previous quasispecies. Overall, 262 mutations were identified in HA, NA and PB2, of which 64.9% (170/262) were pre-existing and 35.1% (92/262) were *ex novo,* suggesting that swIAV evolution between sampling times was driven more by selection and expansion of low-frequency variants than by new mutations. To note, the proportion of changes in the consensus for each viral protein differed between farms.

Conclusion

SwIAV evolution within the studied farms involved both expansion of pre-existing quasispecies variants and emergence of *ex novo* mutations. These results highlight the value of scheduled longitudinal sampling and quasispecies-level analysis, since consensus sequences alone may underestimate viral diversity before specific variants become predominant.

28. UNUSUAL RISE OF H1N1pdm09 SWINE INFLUENZA VIRUS IN FRANCE: INSIGHTS FROM THE CEVA VIROLOGICAL SURVEILLANCE NETWORK (2019–2026)

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Introduction and Objectives

Swine influenza A virus (swIAV) causes respiratory and reproductive disorders in pig herds and represents a significant zoonotic risk. The distribution of circulating subtypes is closely monitored through clinical event-based virological surveillance, notably by the Ceva surveillance network. This study compares swIAV subtype distributions during the first semester of each year from 2019 to 2026, with a particular focus on the H1N1pdm09 subtype.

Materials and Methods

Ceva clinical event-based virological surveillance is based on the collection of samples (nasal swabs, broncho-tracheal mucus, lungs or oral fluids) by veterinary practitioners in farms facing flu evocative clinical signs. The presence of swIAV is assessed by RT-qPCR targeting the M gene on pooled samples (up to 5 samples per pool). Pools with a Cq below 30 undergo subtyping by RT-qPCR or, since 2023, by whole genome sequencing

Results and Discussion

SwIAV subtype distributions were analyzed over the first six months of each year from 2019 to 2026. The number of subtyped viruses ranged from 16 (2019) to 55 (2021). Between 2019 and 2025, H1N1pdm09 detection frequency ranged from 0% (2019) to a maximum of 22% (2022). Strikingly, in the first semester of 2026, 9 of 26 subtyped swIAV (35%) belonged to the H1N1pdm09 subtype, the highest proportion ever recorded in French swine surveillance.

Conclusion

An unprecedented proportion of H1N1pdm09 viruses was detected in French swine herds during the first semester of 2026. Second-semester data will be critical to determine whether this shift reflects a lasting epidemiological change. If confirmed, this trend would carry three major implications: (i) current vaccination strategies in swine farms may require adaptation; (ii) the zoonotic risk would increase, as H1N1pdm09 is regularly involved in bidirectional human-swine transmission; and (iii) co-circulation with others enzootic swIAV subtypes could facilitate reassortment events, potentially generating novel viral variants. Continued surveillance remains essential to monitor this evolving situation.

29. BEYOND THE MATRIX; DIAGNOSTIC GENOMICS FOR INFLUENZA A VIRUS DETECTION IN PIGS

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Introduction and Objectives

Most diagnostic RT-qPCR assays target the conserved matrix gene and provide a rapid, sensitive and cost-effective method for detecting influenza A virus (IAV) RNA in respiratory samples. However, these assays generally provide only a binary positive/negative result and do not yield information on other gene segments, information that is critical for monitoring and risk assessment of IAV in pigs. Third generation sequence technologies like Oxford Nanopore Technology (ONT) can overcome this. However, widespread implementation of ONT-based IAV diagnostics remains hampered by technical and bioinformatical challenges. Here, we describe a user-friendly and easily scalable workflow for whole-genome sequencing of IAV, including a transparent in-house developed bioinformatics pipeline.

Materials and Methods

Viral RNA was isolated and all eight IAV segments were amplified by RT-PCR with universal influenza primers and genetically characterized using an optimized protocol for the Native Barcoding Kit. In addition to genome detective for assembly, a genome analysis and characterization toolkit has been developed that leverages open-source tools and provides efficient coupling with the genome detective platform. The toolkit implements comprehensive quality control statistics on raw reads and assembled genomes, as well as clade/lineage assignment (octoflu), phylogenetic analysis (mafft, iqtree) and reassortment detection (treesort).

Results and Discussion

Our toolkit enabled comprehensive QC allowing stringent selection of high-quality genomes for further characterization, including a novel statistic: minimum depth of coverage. The further characterization resulted in classification of HA/NA segments into subtypes and their phylogenetic clades and phylogenetic inference with a representative set of references. Using this workflow, we identified three distinct influenza strains circulating in a single farm with high confidence.

Conclusion

Our in-house developed diagnostic workflow enabled comprehensive analysis of IAV in both pigs and environmental samples collected from pig farms. The workflow can enhance monitoring of circulating IAVs in pig farms and support the early detection of new variants with zoonotic and pandemic potential.

30. FIELD EVALUATION OF PIGLET PERFORMANCE FOLLOWING VACCINATION OF THE BREEDING HERD AGAINST PANDEMIC SWIAV

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Introduction and Objectives

Swine influenza A virus (swIAV) is a major health challenge in pig production. In addition to respiratory disease, swIAV infection has been associated with reproductive disorders in breeding herds resulting in considerable economic losses. This study describes the impact of introducing a swIAV vaccination programme against H1pdmN1 in a breeding herd experiencing respiratory and reproductive problems.

Materials and Methods

A 3,400-sow farrow-to-finish farm was positive for porcine reproductive and respiratory syndrome virus (PRRSV), swIAV and *Mycoplasma hyopneumoniae*. The existing vaccination programme included PRRSv mass vaccination of sows, PRRSv piglet vaccination, and mass sow vaccination with Respiporc Flu3 against swIAV. Despite this, the herd experienced increased fever, coughing, sneezing and reproductive failures, while wild-type PRRSv continued to circulate in nursery pigs after 18 months of vaccination, confirmed by PCR and sequencing. Hemagglutination inhibition testing in September 2025 demonstrated seropositivity to H1pdmN1 in both breeding and growing pigs. In December 2025, H1pdmN1 infection was confirmed by RT-qPCR on nasal swabs collected from five-week-old nursery pigs. Following the initial diagnosis, the breeding herd received a two-dose mass vaccination with Respiporc FLUpan, administered three weeks apart in October and November 2025.

Results and Discussion

Following implementation of the new vaccination strategy, suckling piglet mortality compared from the first semester of 2025 to the same period in 2026 decreased from 20.03% to 11.80%. In parallel, the number of weaned piglets per sow increased from 12.66 to 13.31. These improvements coincided with the introduction of Respiporc FLUpan mass vaccination and the vets reported improvement in herd health, especially in relation to respiratory clinical signs and reproductive performance. These findings suggest that improved control of circulating swIAV strains contributed to enhanced production outcomes.

Conclusions

This case highlights the impact of pandemic-lineage swIAV on both respiratory and reproductive performance in breeding herds. Broader antigenic coverage through mass vaccination with Respiporc FLU3 and Respiporc FLUpan may improve control of circulating influenza viruses and contribute to improved piglet survival and productivity under field conditions.

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31. TOWARDS A HARMONIZED ASSESSMENT OF THE ECONOMIC IMPACT OF SWINE INFLUENZA A VIRUS (SWIAV) IN EUROPE

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Introduction and Objectives

Swine Influenza A Virus (SwIAV) remains one of the most important endemic respiratory pathogens affecting pig production worldwide. Although its impact on animal health is well recognized, the overall economic burden of SwIAV is difficult to quantify due to variability in production systems, surveillance strategies, co-infections, and methods used to estimate production losses. Furthermore, the One Health relevance of SwIAV, including its zoonotic potential and role in viral reassortment, emphasizes the need for a broader assessment beyond direct production losses. This Short-Term Scientific Mission (STSM), conducted within COST Action ESFLU Working Group 3.3, aims to contribute to the harmonization of approaches used to assess the economic impact of SwIAV in Europe by combining evidence from the scientific literature with expert opinions collected through an international survey.

Materials and Methods

A rapid literature review is being performed to identify studies reporting production losses, economic indicators, and methodological approaches related to SwIAV. Information is extracted into a standardized database including production stage, disease characteristics, reported economic outcomes, presence of co-infections, surveillance strategies, and One Health aspects. In parallel, an anonymous questionnaire has been developed and distributed to veterinarians, researchers, consultants, and professionals involved in swine production across Europe. The survey investigates expert perceptions regarding production impacts, economic consequences, data availability, and priorities for improving economic assessments of SwIAV.

Expected outcomes

The project is expected to provide a structured overview of existing evidence, identify current knowledge gaps, and compare published data with expert perceptions across European countries. Integrating both approaches will support the development of harmonized methodologies for future economic assessments of SwIAV and contribute to identifying research priorities within ESFLU Working Group 3.3.

Conclusions

The combined use of systematic evidence synthesis and expert elicitation represents a practical approach for improving understanding of the economic consequences of SwIAV. The outputs of this STSM are expected to support future collaborative research, facilitate standardized economic assessments, and strengthen the One Health perspective within the ESFLU network.

32. SEROLOGICAL SCREENING AND PRELIMINARY SUBTYPING OF SWINE INFLUENZA VIRUS IN CROATIAN WILD BOARS (2024–2025)

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Introduction and Objectives

Swine influenza A viruses (swIAV) are endemic in domestic pigs worldwide and pose a continuous risk of livestock–wildlife spillover and zoonotic transmission. In Croatia, extensive and backyard husbandry bordering wild boar habitats increases the potential for strain exchange, yet national surveillance data for influenza A viruses (IAV) in wildlife remain scarce. This study evaluated IAV exposure in wild boar populations across eight Croatian counties using serological surveillance.

Materials and Methods

Five hundred wild boar serum samples collected during the 2024 and 2025 hunting seasons were screened for antibodies against the IAV nucleoprotein (NP) by ELISA. Positive samples from 2025 were tested for H5-specific antibodies by competitive ELISA. Seropositive samples with sufficient remaining serum were subtyped by haemagglutination inhibition (HI) using reference swIAV H1N1, H1N2 and H3N2 antigens and an H5 antigen from a Croatian HPAI H5N1 isolate.

Results and Discussion

Overall IAV seroprevalence was 5.0%, with considerable regional variation: Šibenik-Knin County 16.7%, Karlovac County 6.7% and Sisak-Moslavina County 5.5%. No antibodies were detected in Brod-Posavina, Lika-Senj, Osijek-Baranja, Split-Dalmatia or Vukovar-Srijem Counties. H5-specific antibodies were found in two animals (1/3 tested in Karlovac; 1/12 in Sisak-Moslavina), suggesting possible exposure at the interface with wild birds, although HI provided no evidence of past HPAI infection. H1avN1 was the dominant subtype, detected in 38% of ELISA-positive samples, and possible co-infection with H1N2 and H3N2 was recorded in one animal from Šibenik-Knin County. Contrary to expectations, no seropositive animals were found in the counties with the most intensive pig production, possibly reflecting high farm biosecurity and intensified culling of wild boars following the African swine fever outbreak.

Conclusion

Seroprevalence of swIAV in Croatian wild boars is low, with Eurasian avian-like H1N1 predominating, as reported in other European countries. Even at low prevalence, wild boars represent an as yet unquantified infection risk to domestic pigs. Repurposing the national ASF surveillance infrastructure has proved a robust, cost-effective One Health mechanism for monitoring secondary viral pathogens.