



Epizootiological aspects of swine influenza in Ukraine for the period 2021–2025

V. S. Garkavenko*, I. O. Sobko**, L. Y. Korniienko***, V. V. Ukhovskiy***, A. V. Pyskun***,
A. V. Kolechko*, I. M. Polupan***, O. D. Polishchuk***, N. V. Shchur***, N. P. Nenysh****

* Vinnytsia National Agrarian University, Vinnytsia, Ukraine

** Centre of Veterinary Diagnostics LTD, Kyiv, Ukraine

*** State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine

**** Chernihiv Regional State Laboratory of the State Service of Ukraine on Food Safety and Consumer Protection, Chernihiv, Ukraine

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Vinnytsia National Agrarian University,
Soniachna st., 3, Vinnytsia, 21008,
Ukraine. Tel.: +38-067-220-07-94.
E-mail: vgarkavenko78@gmail.com

Centre of Veterinary Diagnostics LTD,
Kholodnoyiarska st., 15A, Kyiv, 03022,
Ukraine. Tel.: +38-050-536-23-51.
E-mail: i.sobko@cvd.com.ua

State Scientific and Research Institute
of Laboratory Diagnostics and Veterinary
and Sanitary Expertise, Donetsk st., 30,
Kyiv, 03151, Ukraine. Tel.: +38-066-789-
85-19. E-mail: uhovskiy@ukr.net

Chernihiv Regional State Laboratory
of the State Service of Ukraine on Food
Safety and Consumer Protection,
Hrushevskiy Mykhaylo av., 180, Cher-
nihiv, 14034, Ukraine.
Tel.: +38-073-306-01-86. E-mail:
leonid.kornienko.09@googlegmail.com

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Swine influenza is one of the most common respiratory viral infections in swine farming, causing significant economic losses and posing a potential threat to public health due to the ability of influenza A viruses to undergo inter-species transmission and genetic reassortment. Pigs play an important role in the emergence of new virus variants, as they can be infected by swine, human, and avian strains alike. Given the pandemic and the emergence of new zoonotic variants of influenza viruses, continuous epizootiological surveillance of the swine population is a crucial component of the biosecurity system and early warning system for pandemic threats. The objectives of the study was to assess the prevalence of influenza A virus among Ukraine's pig population in 2021–2025 and to determine the characteristics of its circulation based on the results of molecular and serological studies. The data for the analysis were derived from diagnostic tests conducted by the Centre of Veterinary Diagnostics LTD, a commercial research center (Kyiv city). The Real-Time RT-PCR method was used to detect viral RNA, and enzyme-linked immunosorbent assay (ELISA) was used to determine specific antibodies. Statistical and epizootiological analyses of the obtained data were conducted. During the study period, 132 swine farms and 587 samples of pathological and biological material were tested using Real-Time RT-PCR. Influenza A virus RNA was detected on 33 farms (25.0%) and in 69 samples (11.8%). The proportion of PCR-positive samples gradually decreased from 16.5% in 2021 to 6.1% in 2025. Serological studies covered 173 farms and 7308 blood sera. Antibodies to the influenza A virus were detected on 118 farms (68.2%), and the overall seropositivity rate was 40.5% (2959 positive samples). The highest seropositivity rates were recorded in the Sumy (82.6%), Volyn (79.0%), Ternopil (69.9%), and Lviv (51.2%) oblasts, while the highest rates of viral RNA detection were observed in the Ivano-Frankivsk, Kharkiv, and Kirovohrad oblasts. The results indicate a significantly wider spread of the infection than can be assessed based solely on the results of direct virus detection. It was found that the level of seropositivity exceeded the frequency of detection of viral RNA by more than three times (40.5% versus 11.8%), which indicates long-term circulation of the virus in the pig population and significant spread of infection even in the absence of active virus excretion. The study results confirm the enzootic nature of influenza A virus circulation in the pig population of Ukraine. The combination of high seropositivity with a relatively low frequency of viral RNA detection indicates the long-term persistence of the infection on farms and the development of postinfection immunity. The data obtained justify the need for further comprehensive molecular and serological monitoring of the swine influenza virus as an important element in preventing economic losses in swine farming and minimizing the risks of the emergence of new zoonotic variants of the virus.

Keywords: swine influenza; diagnosis; epizootic monitoring; epizootic situation; commercial swine; serological studies; molecular studies; mapping; One Health.

Introduction

Swine influenza is a highly contagious viral disease of pigs characterized by an acute course, respiratory system involvement, and a significant economic impact on the swine industry (Anderson et al., 2021). In the current context of intensified livestock production, the epizootiological significance of this infection has increased significantly, as it is capable of spreading rapidly within large production complexes (Klivelyeva et al., 2025; Tochetto et al., 2025). According to the CDC, influenza A viruses circulate continuously in pigs, causing regular outbreaks in which the incidence rate can reach 80–100% of the herd, while the mortality rate typically does not exceed 1–4% (Centers for Disease Control and Prevention. 2024. Influenza in swine. www.cdc.gov/flu/swineflu/index.htm). The disease is caused by an influenza A virus belonging to the family Orthomyxoviridae, genus *Alphainfluenzavirus*, which has segmented RNA consisting of eight segments. The most common subtypes in pigs are H1N1, H1N2, and H3N2 (King et al., 2020; Morens et al., 2021). Infections

caused by the influenza A virus can occur in various animal species. Influenza viruses are classified into four types (A, B, C, and D) based on differences in viral nucleoproteins and matrix proteins. The antigenic structure of the virus is determined by two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Thus, influenza A viruses can be further divided into a number of subtypes based on protein structure and genetic characteristics. It is these proteins that determine the antigenic specificity and the body's immune response. The virus evolves through antigenic drift (the gradual accumulation of mutations) and antigenic shift (the exchange of genome segments between different strains) (Hutchinson, 2018). In addition to humans, influenza A virus infection is also quite common among birds and pigs. Both α -2,3-linked sialic acids, or Sias (the avian influenza virus receptor) and influenza viruses H1N2 (NS) and A(H1N1) pdm09 (PB2, PB1, PA, NP, and MP), as well as α -2,6-linked Sias (the receptor for human influenza viruses), circulate in pigs; therefore, pigs are recognized as “genetic mixing vessels” for human and avian influenza viruses, enabling the reassortment of influenza viruses across spe-

cies (Joseph et al., 2017). Thus, pigs are intermediate hosts that can produce new types of latent infectious viruses (Klivleyeva et al., 2025; Tochetto et al., 2025).

Sometimes swine influenza viruses infect humans. Each virus subtype includes various lineages; swine H1N1 viruses are classified by lineage: classical swine H1N1 (CS H1N1), Eurasian avian H1N1 (EAS-H1N1), or the triple reassortant H1N1 (TR-H1N1) (Ma, 2020; Anderson et al., 2021). In 1979, eight segments of the avian-origin H1N1 influenza virus were first isolated from pigs; the virus subsequently spread among pigs in Europe and Asia, leading to its designation as the Eurasian avian-like H1N1 (EAS-H1N1) (Chiapponi et al., 2021; He et al., 2024). In addition to disease burden and production losses, the persistence and evolution of influenza viruses in pigs pose a clear risk to public health. There are reports of the circulation of classical swine H1N1 viruses in pigs for at least 84 years (Lewis & Shope, 1931). It is known that the emergence of new influenza viruses with pandemic potential for the human population is mediated by antigenic drift and antigenic shift (reassortment) in domestic pigs (acting as a “mixing vessel”) (Shorridge et al., 1977). Pigs thus serve as a biological mixing vessel in which viruses of human, avian, and swine origin can circulate simultaneously, creating conditions for genetic reassortment and the emergence of new pandemic variants (Zeller et al., 2024; Ryt-Hansen et al., 2025). It is believed that all three pandemics of the previous century arose as a result of reassortment events (the formation of shift variants) in pigs or humans. The co-circulation and mixing of the triple reassortant H3N2 with a swine lineage led to the emergence of a swine-origin pandemic influenza virus in 2009 (Sun et al., 2020; Abdelwhab & Mettenleiter, 2023). The significance of swine influenza is determined not only by economic losses but also by its significant zoonotic potential (Zeller et al., 2024; Ryt-Hansen et al., 2025).

According to the CDC, the virus can circulate in a pig herd without obvious clinical signs, which significantly complicates its detection and control (Centers for Disease Control and Prevention, 2024. Influenza in swine. www.cdc.gov/flu/swineflu/index.htm). The virus is transmitted primarily through the air. The main mechanisms of transmission are airborne droplets (coughing, sneezing), contact (direct contact between animals), and transmission via contaminated objects (fomites). Experimental studies show that the virus can spread between animals at distances of up to 1.5–2.0 meters, which contributes to rapid spread throughout the entire herd (Abente et al., 2018). Swine influenza is enzootic in nature, with periodic epizootic outbreaks (Khalil et al., 2024). According to the CDC, the duration of an outbreak among pigs is 5–10 days; the spread occurs very rapidly. The epizootic course of swine influenza is wave-like in nature, which is associated with the development of partial immunity and the emergence of new strains. The epizootiology of swine influenza is determined by a complex of biological (high viral mutagenicity, interspecies transmission), technological (high stocking density, intensive production systems), environmental (temperature, ventilation, humidity), and anthropogenic factors (animal movement, personnel as a source of infection transmission). The 2009 pandemic confirmed the virus's ability to spread globally following genetic reassortment among different strains (Morens et al., 2021). Immunity following infection is short-lived and strain-specific. Vaccination reduces disease severity but does not provide complete protection due to antigenic variability. Modern epizootic surveillance and control systems include PCR diagnostics, serological methods, and genomic sequencing (Detmer et al., 2011; Khalil et al., 2024; Ryt-Hansen et al., 2025).

Transmission of the H1N1 pandemic virus in 2009 from humans to pigs (reverse zoonosis) was also reported in many countries (Cardinale et al., 2012; Rajão et al., 2013; Arunorat et al., 2016; Senthikumar et al., 2021). In early April 2009, an outbreak of H1N1 A influenza was detected in Mexico (Senthikumar et al., 2021), which spread to many countries. In June 2009, the World Health Organization (WHO) declared it a pandemic influenza (Fineberg, 2014). During the first year of circulation, the A/H1N1 pdm09 influenza virus caused 151700–575400 deaths worldwide (Juvert et al., 2021). Currently, A/H1N1 pdm09 influenza viruses circulate and cause epidemics in humans as one of the strains of the seasonal influenza virus.

Swine influenza is a globally widespread disease. According to the FAO (Food and Agriculture Organization, 2023. Swine influenza situation update. www.fao.org), the infection is reported in most countries with developed swine industries; the most intense circulation occurs in the United States, the EU, and China. The influenza A virus is endemic in the pig population in the United States; new genetic variants are constantly being identified (United States Department of Agriculture, 2023). The CDC notes regular circulation of the virus throughout the year and periodic outbreaks with high incidence rates. The causative agent of this disease is characterized by interspecies transmission and zoonotic potential. Transmission is possible in both directions, from pigs to humans and from humans to pigs. According to CDC data, human infections are rare but occur regularly; swine farm workers are at the highest risk (Centers for Disease Control and Prevention, 2024. Influenza in swine. www.cdc.gov/flu/swineflu/index.htm).

Pigs are the primary reservoirs of the H1N1 and H3N2 influenza viruses; the latter are endemic in pig populations worldwide and cause one of the most common respiratory diseases in pigs. Pig farming practices that leave pigs susceptible and in regular contact with other species, particularly humans, directly contribute to the circulation of these viruses in pigs and the frequent exchange of viruses between pigs and other species (Lechmann et al., 2025). Pigs are candidates for the role of intermediate hosts for the recombination of influenza A viruses of avian and human origin, as they are the only species of domesticated mammals raised in significant numbers that are susceptible to avian and human influenza viruses and allow efficient viral replication (Scholtissek et al., 1985). This can lead to the emergence of new influenza virus strains, some of which may be transmitted to other species, including humans. This concept is supported by the detection of recombinant viruses in pigs in Europe, as well as by some evidence of subsequent transmission of the virus to the human population (Lechmann et al., 2025). Following interspecies transmission to pigs, some influenza viruses may prove to be genetically highly unstable, leading to the emergence of variants that can facilitate repeated crossing of the species barrier. Ultimately, a stable lineage derived from the dominant variant may become established in pigs. Genetic drift occurs, in particular, in genes encoding surface glycoproteins, but typically does not result in the same level of antigenic variability observed in the predominant strains circulating in the human population (Markin et al., 2023; Li et al., 2024). The adaptation of a transmitted influenza virus to pigs can take many years. Both the human H3N2 and avian H1N1 strains were detected in pigs many years before they acquired the ability to spread rapidly and became associated with outbreaks of influenza A in pigs (Petro-Tumquist et al., 2024). Infections caused by influenza A viruses can occur in various animal species. Eurasian avian-like swine influenza A (H1N1) (EAS-H1N1) viruses are prevalent among pigs and occasionally infect humans. For instance, A Eurasian avian-like influenza A virus (H1N1) was isolated from a child with a fever; this strain was designated A/Shandong-binzhou/01/2021 (H1N1) (He et al., 2024). Whole-genome sequencing and phylogenetic analysis revealed that A/Shandong-binzhou/01/2021 (H1N1) is a novel triple reassortant of the EAS-H1N1 lineage, containing gene segments from EAS-H1N1 (HA and NA), a triple reassortant of the swine influenza virus H1N2 (NS), and the A(H1N1) pdm09 (PB2, PB1, PA, NP and MP). Serological evidence of swine infection with the H1N1 influenza virus has been reported in many countries (Chastagner et al., 2019). Subsequently, Danish swine herds experienced the spread of a triple-reassortant H3N2 virus, comprising an HA of human origin from the 2004–2005 seasonal virus, N2 from endemic swine viruses, and internal genes from the 2009 pH1N1 virus (Krog et al., 2017). A reassortment between the 2009 pH1N1 virus and contemporary swine viruses in European swine populations has increased the number of genotypes of circulating swine influenza viruses, posing a significant challenge for disease control and swine production (Pippig et al., 2016; Lechmann et al., 2025).

Serological and molecular studies in commercial swine farming are an important component of epizootiological monitoring of the spread of the swine influenza virus. Such studies provide the basis for

assessing the potential effectiveness of the vaccines used. In this study, we aimed to conduct an epizootiological analysis of influenza virus circulation in the pig population in Ukraine during the period 2021–2025.

Material and methods

Blood serum samples, swabs, and nasal secretions were collected from unvaccinated pigs on farms of various ownership types across different regions of Ukraine during 2021–2025. The pigs from which the samples were collected were of various ages and production types (sows, boars, finishing piglets, etc.). Nasal swabs were collected using moistened cotton swabs in sterile PBS, stored at 4 °C, and transported in storage containers. Whole blood was collected by puncturing the anterior *vena cava* or the ear vein, followed by serum separation, and stored at –20 °C. Data from the Centre of Veterinary Diagnostics LTD, a commercial research center (Kyiv city), were used to study the prevalence of the influenza virus in Ukraine (<https://cvd.com.ua/pro nas>).

Commercial ELISA kits were used to detect antibodies to swine influenza (HA H1 SIV) in accordance with the manufacturer’s instructions (HerdChek Swine Influenza H1N1 Antibody Kit) (IDEXX Laboratories, Inc., USA). The HerdChek Swine Influenza H1N1 Antibody Test kit (manufactured by IDEXX) is an enzyme-linked immunosorbent assay (ELISA) used to detect serum antibodies to the swine influenza virus (SIV) subtype H1N1 in swine herds. It provides veterinary laboratories with a reliable tool for herd screening and disease diagnosis. All serum samples were inactivated at 56 °C for 30 minutes and adsorbed onto chicken erythrocytes for 60 minutes at room temperature prior to testing.

Table 1
Results of studies of swine farms for the presence of the influenza virus and antibodies to it in Ukraine during the period 2021–2025

Year	Target sequence – influenza A virus RNA (Real-Time RT-PCR)					Specific antibodies to influenza A virus in pig serum (ELISA)				
	farms tested	positive farms	number of samples tested	number of positive samples	proportion of positive results, %	farms tested	positive farms	number of samples tested	number of positive samples	proportion of positive results, %
2021	17	7	103	17	16.50	20	12	659	239	36.27
2022	23	6	63	10	15.87	37	26	1313	488	37.17
2023	32	7	168	23	13.69	62	44	2833	1185	41.83
2024	20	4	88	9	10.23	22	15	1476	554	37.53
2025	40	9	165	10	6.06	32	21	1027	493	48.00
Total	132	33	587	69	11.75	173	118	7308	2959	40.49

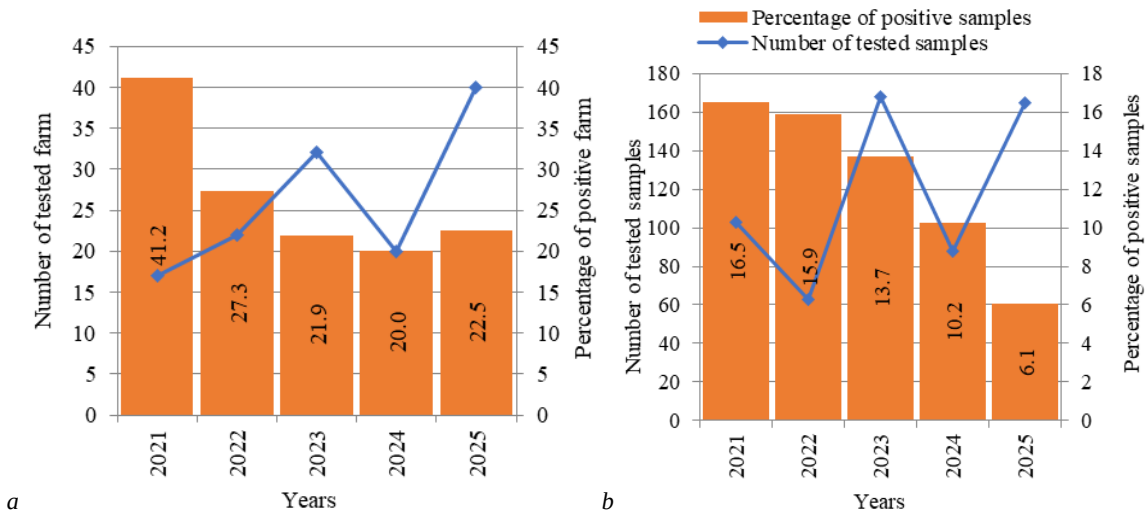


Fig. 1. Dynamics of PCR-based swine influenza research (2021–2025 years): *a* – farm; *b* – samples

The data on the volume and results of molecular diagnostics of swine for the detection of influenza A virus RNA on farms are broken down by region of Ukraine (figures 2–5). During the study period, material from 132 farms was tested for the presence of influenza A virus RNA using RT PCR (Fig. 2); in this case, positive results were obtained from 33 farms (Fig. 3), accounting for 25.0%. A total of 587

The Kylt® Influenza A Real-Time RT-PCR kit (Kylt®, SAN Gro-up Biotech Germany GmbH) was used to detect the antigen. The product is intended for the detection of influenza A virus in blood samples from birds and pigs. The Real-Time PCR detection kit contains all the necessary reagents and controls for detecting the influenza A virus in biological samples.

Results

We conducted the study to detect swine influenza virus RNA using Real-Time RT-PCR and to detect antibodies to the swine influenza virus using the ELISA serological method during the period 2021–2025. The results broken down by region of Ukraine are presented in Table 1.

The analysis of the data in Table 1 shows that between 2021 and 2025, samples from 132 farms of various ownership types were tested for the presence of swine influenza virus RNA using Real-Time RT-PCR, and samples from 173 farms were tested for the presence of antibodies using the ELISA serological method. According to the Real-Time RT-PCR results, viral RNA was detected on 33 farms, accounting for 25.0%, while antibodies to the swine influenza virus were detected on 118 farms, or 68.2%.

The proportion of farms with positive Real-Time RT-PCR results was 41.2% in 2021, 27.3% in 2022, 21.9% in 2023, 20.0% in 2024, and 22.5% in 2025 (Fig. 1a). In total, 587 samples were tested using the Real-Time RT-PCR method, of which 69 were positive, accounting for 11.8%. The proportion of positive samples by year was as follows: 16.5% in 2021, 15.9% in 2022, 13.7% in 2023, 10.2% in 2024, and 6.1% in 2025 (Fig. 1b). The average number of samples tested using the RT-PCR method was 4.45 per farm.

samples of pathological and biological material were tested (Fig. 4), of which 69 were positive (11.8%, Fig. 5).

In the eastern region of Ukraine, Real-Time RT-PCR testing was not conducted in the Donetsk, Zaporizhzhia, and Kherson oblasts; data were also unavailable for Luhansk Oblast. In Kharkiv Oblast, 2 farms and 2 samples were tested, of which one was positive, accounting for 50.0%. In Dnipropetrovsk Oblast, 3 farms and 3 samples were

for 16.67% in Volyn Oblast; 11.1% in Ternopil Oblast; and 10.7% in Khmelnytskyi Oblast. No positive results were detected in Chernivtsi Oblast. Real-Time RT-PCR testing was not conducted in the Rivne and Zakarpattia oblasts. In general, the analysis of the results presented in Fig. 2–5 indicates an uneven distribution of the swine influenza A virus across Ukraine and significant regional differences in the detection rate of viral RNA.

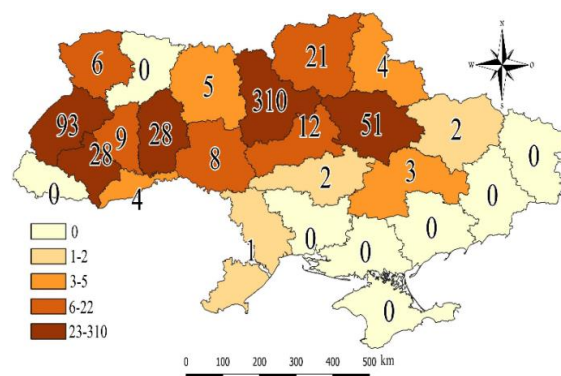


Fig. 4. Quantity of samples tested for swine influenza virus by PCR (2021–2025 years)

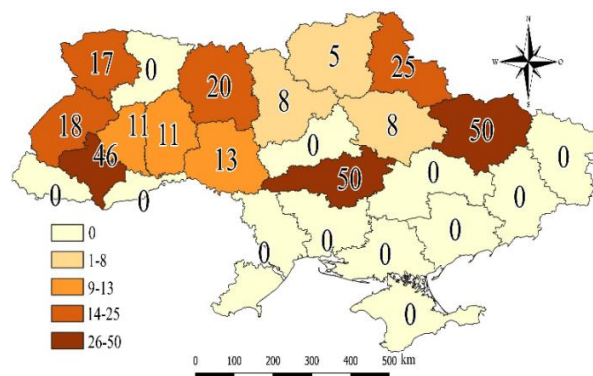


Fig. 5. Percentage of samples positive for swine influenza virus by PCR (2021–2025 years)

The serological testing using the ELISA method was conducted on a larger scale. A total of 173 farms were tested, and antibodies to the influenza A virus were detected on 118 farms (68.2%). The proportion of farms with positive ELISA results was 60.0% in 2021, 70.3% in 2022, 71.0% in 2023, 68.2% in 2024, and 65.6% in 2025 (Fig. 6a). In total, 7308 blood sera were tested using the ELISA method, of which 2959 tested positive, accounting for 40.5%. The seropositivity rate was 36.3% (239) in 2021, 37.2% (488) in 2022, 41.8% (1185) in 2023, 37.5% (554) in 2024, and 48.0% (493) in 2025 (Fig. 6b). The average number of samples tested by ELISA was 42.2 per farm.

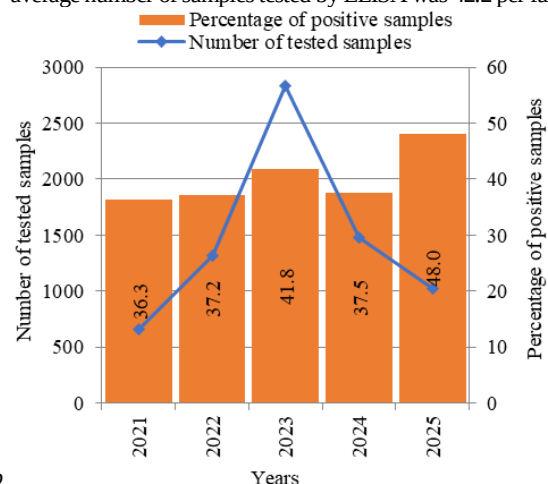


Fig. 6. Dynamics of ELISA-based swine influenza research (2021–2025 years): *a* – farm; *b* – samples

The data on the volume and results of serological testing of pigs for the detection of antibodies to the influenza A virus on farms, broken down by region of Ukraine, are presented in figures 7–10.

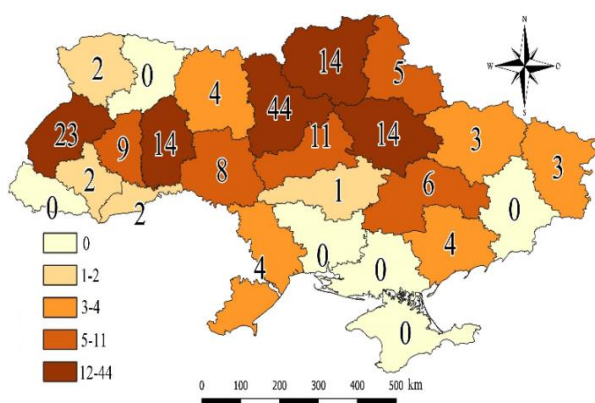


Fig. 7. Quantity of farms tested for swine influenza based on ELISA testing (2021–2025 years)

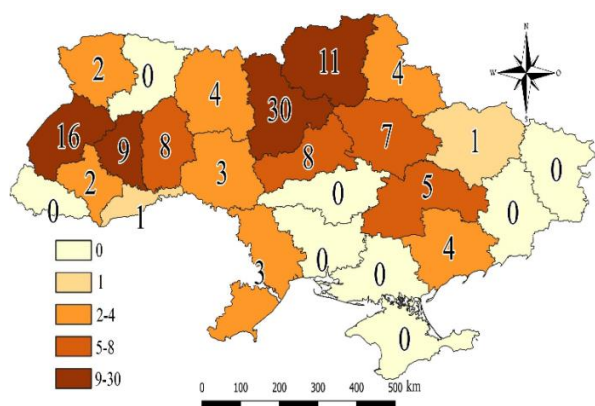


Fig. 8. Quantity of pig farms with swine influenza virus detected by ELISA (2021–2025 years)

Thus, in the eastern oblasts, the seropositivity rates were as follows: 24.6% in Kharkiv, 40.4% in Zaporizhzhia, and 37.0% in Dnipropetrovsk. In Luhansk Oblast, all results were negative. No testing was conducted in the Donetsk and Kherson oblasts. Among the central regions, the largest number of serum samples was tested in Kyiv – 4865, of which 1913 (39.3%) were positive. The seropositivity rate was 31.3% in Poltava, 26.8% in Cherkasy; 23.1% in Vinnytsia; and 38.1% in Zhytomyr. No positive results were detected in Kirovohrad Oblast. In the northern regions, the highest seropositivity rate was recorded in Sumy Oblast, measuring 82.6%, while in Chernihiv Oblast, this figure stood at 31.6%. Among the western regions, high seropositivity rates were recorded in Volyn (79.0%), Ternopil (69.9%), Lviv (51.2%), Ivano-Frankivsk (46.0%), Chernivtsi (33.3%), and Khmelnytskyi oblasts (31.5%). No studies were conducted in the Zakarpattia and Rivne oblasts.

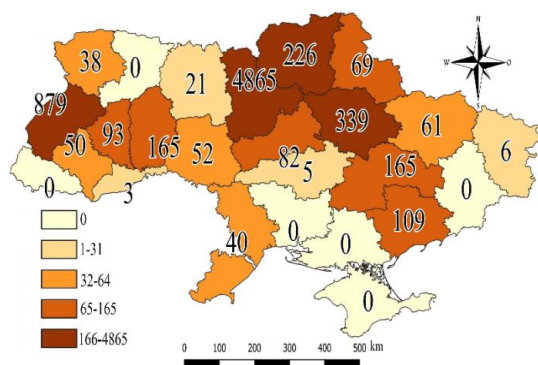


Fig. 9. Quantity of samples tested for swine influenza virus by ELISA (2021–2025 years)

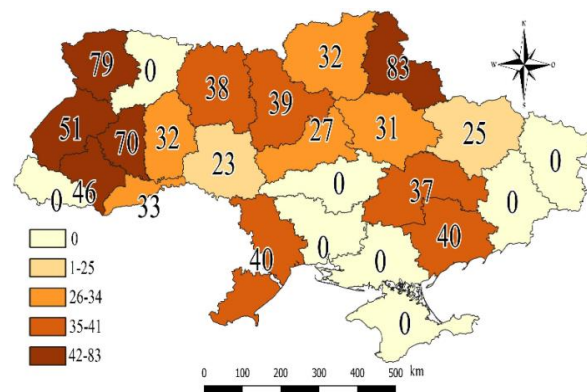


Fig. 10. Percentage of positive samples for swine influenza virus by ELISA (2021–2025 years)

Thus, the study results indicate a widespread prevalence of the influenza A virus among Ukraine's pig population. The significantly higher seropositivity rate compared with the frequency of viral RNA detection suggests active circulation of the virus in the pig population during previous periods and the development of postinfectious immunity in the animals. According to the results of serological studies, the Sumy, Volyn, Ternopil, and Lviv oblasts were found to be the most epizootically affected (Fig. 8 and 10), whereas, according to the results of Real-Time RT-PCR studies, the highest detection rates of viral RNA were observed in the Ivano-Frankivsk, Kharkiv, and Kirovohrad oblasts (Fig. 3 and 5).

Discussion

The results of the studies confirmed that the swine influenza A virus continues to circulate actively in pig farms in Ukraine, forming a complex enzootic process with a high rate of seroconversion among animals. The detection of antibodies to the virus in 68.2% of farms and the presence of PCR-positive cases in 25.0% of the farms indicate a significant spread of the infection among the pig population and the potential for the virus to persist in the population for an extended period. Of particular note is the significant difference between the seropositivity rate and the frequency of viral RNA detection, which may be a consequence of the subclinical course of the infection, postinfection persistence of antibodies, and the continuous enzootic circulation of swIAV on farms.

A wide range of pandemic viruses, including the influenza A/H1N1 pdm09, is causing concern among national and international public health organizations, prompting them to closely monitor and control the transmission of zoonotic diseases from animals to humans, from humans to humans, as well as bidirectional transmission from humans to animals. Pigs can serve as a permanent reservoir for these viral infections following mild or asymptomatic infections, leading to potential new genetic reassortments, recombinations, and evolutionary events, as well as drug-resistant and immunodeficient variants (Khalil et al., 2024). From 1959 to 2014, only 396 confirmed swIAV infections in humans were reported worldwide (Freidl et al., 2014). From 2010 to 2021, fewer than 700 confirmed cases were reported worldwide, with the majority occurring in young people or immunocompromised patients. However, several studies have shown subclinical infections in farm workers and slaughterhouse workers ranging from 15% to 40% (Hennig et al., 2022). Zoonotic transmission of seasonal and pandemic hIAV to pigs has led to the establishment of a long-term reservoir in pigs for zoonotic IAVs (Kessler et al., 2021; Glud et al., 2021). It should be noted that several cases of AIV have been reported in pigs, including H5, H7, and H9 viruses (Hennig et al., 2022; Abdelwhab & Mettenleiter, 2023).

Thus, swine influenza typically causes high morbidity (up to 100%) and low mortality in infected pigs; however, it can result in 10–15% mortality in unvaccinated pigs. Swine influenza causes significant economic losses to swine producers, primarily due to weight loss and reduced weight gain in infected pigs; however, some cases can be much more severe when coinfecting with other pathogens. The

swine influenza virus, together with other swine pathogens such as porcine reproductive and respiratory syndrome virus, porcine circovirus 2, *Mycoplasma hyopneumoniae*, *Bordetella bronchiseptica*, and *Actinobacillus pleuropneumoniae*, can cause a complex of respiratory diseases in pigs, leading to a significant annual increase in mortality and economic losses in the swine industry (Schultz-Cherry et al., 2013; Khalil et al., 2024). Although swine influenza was previously considered a seasonal disease, similar to seasonal influenza in humans, which is predominantly observed in winter and spring, this is no longer the case due to the adoption of high-density housing systems in the swine industry (Petro-Turnquist et al., 2024).

Sequencing of influenza virus genomes reveals a number of gene mutations that encode amino acid substitutions associated with increased virulence and the likelihood of transmission to other mammals and humans. Therefore, continuing and strengthening surveillance of influenza viruses in pigs is extremely important, as pigs are a host species capable of producing new viruses that could lead to another pandemic among humans (He et al., 2024). These processes ensure the constant emergence of new variants, which complicates infection control and reduces the effectiveness of vaccination. The primary source of infection is sick or infected pigs that shed the virus in their respiratory secretions. Viral shedding begins as early as 24 hours after infection and lasts up to 5–7 days. A particularly important epizootiological role is played by latently infected animals, subclinical carriers (serving as additional reservoirs of infection), humans (reverse zoonosis), wild boars, and birds (Khalil et al., 2024).

Senthilkumar with coauthors conducted a serological study to assess the prevalence of H1N1 infection in pigs across various states of India between 2009 and 2016. Based on hemagglutination inhibition (HI) assays, the seroprevalence of the H1N1 virus ranged from 5.2% (2009) to 36.3% (2011) (Senthilkumar et al., 2021). Our results indicate an even higher prevalence of the influenza A virus among pigs in Ukraine. Specifically, during 2021–2025, antibodies to the swine influenza virus were detected by ELISA in 68.2% of the farms studied, and the overall serum seropositivity rate was 40.5%. In some regions, the seropositivity rate exceeded 70–80%, particularly in the Sumy and Volyn oblasts. This may indicate prolonged enzootic circulation of the virus in the pig population and constant exposure of animals to various variants of the influenza A virus.

Pigs have receptors for both avian and human influenza; therefore, this disease poses a serious threat to public health due to the emergence of new reassortant strains and the transmission of the virus to humans (Kong et al., 2016). The increase in the number of influenza cases in humans has been accompanied by a rise in antibody prevalence in pigs, indicating that influenza manifests as a reverse zoonosis (transmission from humans to pigs), a phenomenon that has also been reported previously in other countries (Senthilkumar et al., 2021).

Swine influenza cannot be effectively controlled, despite the significant number of vaccines used, just like seasonal human influenza. It is very important to note that in pigs, a new virus could emerge that has the ability to infect humans and the potential to adapt to humans, thereby causing an epidemic or pandemic in the future, as was the case with the pH1N1 virus in 2009. The pH1N1 virus of 2009 originated in North America, likely in Mexico (Mena et al., 2016), rather than in a “wet market” in Southeast Asia, which is considered the epicenter of pandemic influenza viruses (Shortridge, 1992). This indicates that a potential pandemic virus can emerge anywhere and underscores the importance of monitoring pigs in other regions. Furthermore, other existing swine viruses, such as the North American triple reassortant virus H3N2 in the United States and the Eurasian avian virus H1N1 in China, have acquired increased infectivity for humans through reassortment with other viruses, leading to the emergence of the variant virus H3N2 and the Eurasian reassortant virus H1N1 in their respective regions, which have caused human infections (Nelson et al., 2016; Sun et al., 2020). Therefore, enhanced surveillance of these viruses is necessary to better understand the ecology of invasive viruses in pigs worldwide (Ma, 2020).

The seroprevalence rate in pigs and the trend of influenza infections in humans indicate possible human-to-pig transmission of influenza. Consequently, in addition to serological surveillance, continu-

ous and systematic molecular surveillance should be implemented in the swine population to mitigate and quantify the risk of a pandemic influenza outbreak (Senthilkumar et al., 2021). The proximity of swine and poultry farms, the production and trade of farm animals and animal-derived products, as well as people working on swine farms, all contribute to the potential for genetic exchange and interspecies transmission of influenza A viruses. Therefore, to prevent severe infections and reduce the burden on the healthcare system, it is necessary to restrict contact between humans and pigs and also to carry out continuous surveillance of both pigs and humans, especially those at increased risk of complications from infection due to contact at the animal-human interface (Mollett et al., 2023; Klivleyeva et al., 2025).

It has been shown that the 2009 pandemic virus pH1N1 infects and causes disease in pigs without prior adaptation (Ma, 2020). It has been detected in pig herds worldwide, even in countries previously considered free of swine influenza, such as Australia and Norway (Holyoake et al., 2011). It has been documented worldwide in pigs, often co-circulating with locally endemic viruses (Nelson et al., 2015). The most well-known cases involved the swine influenza virus variants H3N2 and H1N2, which were reassortants consisting of seven genes from modern North American triple-reassortant viruses and the M gene of the pH1N1 virus in 2009, which led to more than 300 human infections and several deaths in the United States (Bowman et al., 2014; Nelson et al., 2016). Importantly, a similar variant of the H3N2 swine influenza virus was detected in pigs in Korea (Kim et al., 2013); although no human infections were reported in that country, the virus most likely entered Korea through the importation of pigs from the United States.

Numerous swine influenza viruses, characterized by significant genetic diversity, exist and circulate in pig populations worldwide (Lewis et al., 2016; Anderson et al., 2021), and new genotypes and subtypes of the virus can emerge in pigs at any time as a result of infection with avian and/or human influenza viruses. In addition, live pigs are transported around the world due to the growth of international trade, which has accelerated the evolution of the virus and complicated the swine flu situation globally. This has led to the introduction of new swine influenza viruses into nonimmune pig herds, such as the North American triple reassortant and the Eurasian swine influenza virus H1N1, which are similar to avian influenza and were introduced into China from North American and European countries (Zhu et al., 2013).

Annual seasonal influenza epidemics of varying severity, caused by influenza A and B viruses, impose a significant burden of disease worldwide. The circulation of seasonal influenza viruses declined significantly in 2020–2021 following the emergence of SARS-CoV-2, but increased in 2021–2022. Sporadic zoonotic infections with novel influenza A viruses of avian or swine origin continue to pose a pandemic threat (Uyeki et al., 2022). Although some animal influenza viruses pose a high zoonotic risk (e.g., avian and swine influenza viruses), others have low or negligible zoonotic potential (e.g., influenza viruses of horses, dogs, bats, and cattle). Transmission can occur directly from animals (particularly poultry and swine) to humans, or via reassortant viruses within host organisms, which act as “mixing vessels.” Currently, there are fewer than 3,000 confirmed cases of human infection with avian-origin viruses and fewer than 7,000 subclinical infections. Nevertheless, there are a number of hosts that carry both types of receptors and can act as potential “mixing vessels.” High vigilance is required to prevent the next pandemic caused by animal influenza viruses in humans (Abdelwhab & Mettenleiter, 2023; Chaves et al., 2024).

PCR testing is the most sensitive and objective method for diagnosing influenza in pigs (Ma et al., 2024; Moraes et al., 2025; Shi et al., 2025). Of particular note in our study is the gradual decline in the proportion of PCR-positive samples from 2021 to 2025. Such changes may be associated not only with the cyclical nature of the virus’s circulation but also with changes in pig husbandry practices, enhanced biosecurity measures, a reduction in the frequency of animal movements between farms, and the partial development of herd immunity. At the same time, the consistently high level of seropositivity indicates that the virus continues to circulate enzootically even

in the absence of widespread clinical manifestations. Abnormal microRNA expression plays a crucial role in regulating interspecies infection by influenza viruses, laying a solid foundation for further research into the molecular regulatory mechanism of microRNAs in interspecies infection and potential targets against this virus (Wang et al., 2024). The influenza A H1N1 virus (A/swine/Guangdong/1/2011) can spread rapidly among animals via various routes of transmission, and new data indicate that the ocular surface is an important entry point (Li et al., 2024). Further confirming the zoonotic risk, the researchers presented phylogenetic evidence of 17 cases of pdm09 transmission from pigs to humans between 2010 and 2021, 11 of which had not previously been classified as variants, and each of the zoonotic cases was associated with sustained circulation of pdm09 in pigs. These data demonstrate that reverse zoonoses and the evolution of pdm09 in pigs lead to the emergence of viruses capable of zoonotic transmission and pose a potential pandemic threat (Markin et al., 2023).

To identify the mechanisms influencing reassortment and evolution, American researchers conducted a phylogenetic analysis of N1 genes and their paired hemagglutinin genes of the swine influenza virus in North America between 1930 and 2020. The researchers analyzed and described 14 N1 clades within the Eurasian avian N1 lineage (including the pandemic N1 clade), the classical swine N1 lineage, and the seasonal human N1 lineage. Seven genetic clades of N1 showed evidence of current circulation (Hufnagel et al., 2023).

A serological study of samples from pig production systems in the United States provided evidence of infection caused by the seasonal human H3N2 virus. One farm in the United States and four farms in Mexico had concurrent virological evidence. The H3N2 3C.2a1b.2a.2b strain isolated from pigs was antigenically distinct from the endemic H3N2 swine strains 1990.4.A, 2010.1, and 2010.2, but retained antigenic similarity to a recent seasonal human H3N2 virus (A/Darwin/6/2021) (Zeller et al., 2024). American researchers also noted the relative frequency of detection of the phylogenetic clade H3 1990.4.a (commonly known as C-IVA) in pigs in the United States: It decreased to 7% in 2017 but increased to 32% in 2019 (Neveau et al., 2022; Thomas et al., 2024).

Sequencing conducted by Danish researchers identified two genotypes in the swine flu H1pdm09N1av: Genotype 1 contained the entire internal gene cassette of H1N1pdm09 origin, while genotype 2 differed in that it carried an NS gene segment of H1N1av. Both H1pdm09N1av genotypes were transmitted between ferrets via direct contact, but only genotype 1 was capable of effective aerosol transmission. The rapid spread of the H1pdm09N1av viruses in Danish pig herds raises concerns regarding the health of pigs and humans (Ryt-Hansen et al., 2025).

Dutch researchers collected and tested respiratory samples from pigs (2019–2023) for swine influenza virus and detected the pathogen in 342 (42%) of 824 respiratory samples from 90 farms. Whole-genome sequencing identified 73 H1N1 viruses, 51 H1N2 viruses, and one H3N2 virus. The study also highlighted sporadic transmission of the virus between humans and pigs (van der Vries et al., 2025). Analysis of viral sequences showed clustering with swIAV from pigs in a recently implemented surveillance system on pig farms. These human cases indicate that swIAV viruses with zoonotic potential are enzootic in the Netherlands. The coincidence of these findings suggests that human infections may occur more frequently than is currently recognized (Eggink et al., 2025).

Between 2017 and 2018, Chinese researchers collected 1,421 samples from the provinces of Guangdong, Fujian, Henan, Yunnan, and Jiangxi and isolated 29 viruses. Among them were the 21 H1N1, 5 H1N2, and 3 H3N2 strains. Genome analysis showed that the domestic epidemic genotypes of H1N1 were predominantly G4 and G5 reassortants of swine H1N1 (Cai et al., 2024). From 2020 to 2021, other Chinese researchers monitored swine influenza viruses H1N2 (swH1N2) on pig farms located in Guangdong, Yunnan, and Guizhou provinces in southern China, as well as in Henan and Shandong provinces in northern China. The authors systematically analyzed the evolution and pathogenicity of swH1N2 isolates and characterized their ability to replicate and transmit. The isolated viruses were found

to be quadruple reassortant H1N2 viruses; these modern swH1N2 viruses have zoonotic potential, underscoring the need for enhanced surveillance of swine influenza viruses (Cui et al., 2024). Chinese researchers who studied some of the H1 viruses noted a high mortality rate of the isolated viruses in mice. Overall, the authors conclude that the H1subtypes that have recently begun circulating in China pose a potential threat to human health and emphasize the importance of continuing close monitoring of their evolution and spread (Yan et al., 2025). Studies provide evidence of pigs' involvement in the genetic evolution of the influenza A virus and highlight the need for increased attention to new reassortant viruses in pigs (Lan et al., 2025).

Swiss researchers attribute the absence of the H1N2 and H3N2 subtypes, which circulate in the rest of Europe, to the relatively low diversity and distinct clustering of Swiss H1N1 strains, as well as the characteristics of the Swiss swine industry, which is inherently isolated and subject to low levels of animal imports (Lechmann et al., 2025). In Switzerland, the influenza virus was detected in pigs on 375 of the 674 farms studied. H1N1 has been the only subtype detected in local pigs to date. The seasonal human influenza A (H1N1) pdm09 strain (hemagglutinin (HA) clade 1A) was detected in seven of the 375 farm samples. No evidence of sustained human-to-human transmission was found. Continuous surveillance of influenza viruses at the pig-human interface is of great importance for the early detection of new subtypes of influenza viruses and changes in the epidemiological situation (Lechmann et al., 2025).

Serological studies in Zambia showed that 49% and 32% of sera collected in 2011 were positive in the hemagglutination inhibition test and the neutralization test for the A (H1N1) pdm09 virus, respectively (Harima et al., 2022). Brazilian researchers compiled 14 years of influenza virus surveillance data and described the subtypes and major lineages of H1 and H3 viruses co-circulating in Brazilian pigs. Using multiplex RT-qPCR and sequencing, they identified H1N1pdm in 41.3% of the subtyped samples (165/399), followed by H1huN2 (108/399), H3N2 (77/399), and H1N1hu (9/399). The high diversity observed indicates that invasive-associated viruses from different lineages are widespread throughout the country (Tochetto et al., 2025).

The high seropositivity rate observed in our study indicates active and prolonged circulation of the influenza A virus in the pig population of Ukraine. In this case, the significant difference between the antibody detection rate and the frequency of viral RNA detection may indicate a predominance of subclinical or latent forms of infection, in which animals no longer actively shed the virus but remain seropositive following past infection. Similar patterns have also been described in a number of European and Asian studies, where high seroconversion rates were associated with relatively low levels of viral shedding.

Researchers argue that human-animal interface surveillance is a more effective and less expensive approach to identifying future pre-pandemic threats (El Zowalaty et al., 2022). Undoubtedly, national and international monitoring programs in animals and humans, coupled with internationally coordinated early-warning systems, are a crucial component of influenza virus surveillance worldwide. In the future, all stakeholders (including farmers, veterinarians, meat producers, and veterinary authorities) must be involved, and communities should take steps to raise awareness of the disease (Lechmann et al., 2025).

Our findings in Ukraine may also indicate the existence of regional variations in the epizootic process. In regions with high seropositivity but a low detection rate of viral RNA, chronic enzootic circulation of the virus likely predominates, with periodic outbreaks among susceptible young animals. By contrast, regions with high PCR-positivity rates may be characterized by more active circulation of specific viral variants at the time of the studies.

Vaccination is currently the primary tool for controlling swine influenza, although its effectiveness is reduced against heterologous strains or in the presence of maternal antibodies (Mancera Gracia et al., 2020). Vaccines against current strains of the H1N1 virus are quite effective and help control swine influenza (Nuwarda et al., 2021). Vaccination against swine influenza can protect pigs from

infection and is typically used in sows, as piglets are believed to be protected by maternal immunity against homologous strains of the influenza A virus (Chamba Pardo et al., 2019; Li & Robertson, 2021). In our upcoming studies, we plan to investigate this specific aspect – the potential effect of vaccinating sows on the epizootiological indicators of swine influenza.

Swine flu can result in significant economic losses for the swine industry and could potentially lead to a pandemic among humans. The 2009 H1N1 pandemic had significant economic consequences due to costs associated with prevention, treatment, and hospitalization. The 2009 H1N1 pandemic, caused by a new strain of the H1N1 virus, spread rapidly to more than 200 countries, causing an estimated 284,400 deaths worldwide, according to the data from the World Health Organization (Meles et al., 2025). Since 2010, at least 21 cases of human transmission of the virus have been identified, three of which occurred between July and December 2023. At the same time, the data from some researchers indicate that the H1-1C influenza virus poses a relatively high pandemic risk (Le Sage et al., 2025). The co-circulation of different strains of swine influenza viruses on pig farms may facilitate gene reassortment between strains, leading to the emergence of new circulating strains in pigs and strains with pandemic potential. Close contact between pigs and swine farm workers creates opportunities for zoonotic transmission of the swine influenza virus. To control the potential for swine influenza pandemics developing in humans, monitoring of swine influenza on pig farms is necessary (Li & Robertson, 2021). Chinese researchers have demonstrated that H1 SIV subtype viruses, which have recently circulated in China, pose a potential threat to human health, and emphasize the importance of continuing to closely monitor their evolution and spread (Yan et al., 2025).

Influenza A viruses of the H1 and H3 subtypes circulating in European pigs differ markedly from those circulating in other global pig populations. These viruses exhibit significant genetic diversity, which is further amplified by periodic interspecies transmission of the influenza virus from humans to pigs, followed by sustained circulation (Coggon et al., 2025). Swine influenza control is not a priority for the swine industry in many countries, and there is concern that some strains may become more pathogenic and/or contagious as they circulate in the wild. Routine surveillance has shown that EA H1N1 viruses acquire different internal genes from various swine influenza viruses and form various new genotypes (Meng et al., 2022). The passive surveillance system for swine influenza virus in the United States (U.S.) provides aggregated metrics for quantifying spatial and temporal changes in genetic diversity (Thomas et al., 2025).

The prevalence and pandemic status of the H1N1 virus among pigs can be better assessed through seroepidemiological studies, which provide valuable information on infection and the risk associated with individuals who care for pigs. Pigs can also serve as “sentinels” for this infection, providing useful information about the influenza virus circulating in the human population. In addition to serological surveillance of the influenza virus, there is an urgent need for an improved and continuous molecular surveillance program in the pig population to study evolutionary changes in genetic components, whose reassortant subtypes may pose a major threat to public health (Senthilkumar et al., 2021). The results of our PCR studies confirm the feasibility of this approach. Between 2021 and 2025, swine influenza virus RNA was detected in 25.0% of the farms tested, and the overall proportion of positive samples was 11.8%. In this case, a gradual decrease in the proportion of positive PCR samples was observed: from 16.5% in 2021 to 6.1% in 2025, which may indicate both the cyclical nature of the virus’s circulation and the impact of improved biosecurity and specific preventive measures on farms.

Adaptive mutations acquired by influenza viruses do indeed help them overcome species barriers to infect humans in a phenomenon known as zoonosis. In addition, these adaptive mutations help variant strains spread horizontally to other animal species with which they come into contact, including domestic and wild animals (zoonanthroponosis) (Khalil et al., 2024). Studies show that even such exotic influenza virus lineages as H1-1C from pigs pose a relatively high pandemic risk to humans (Le Sage et al., 2025). Numerous public

health organizations have published comprehensive guidelines containing advice for the general public and participants in agricultural fairs. These recommendations generally emphasize handwashing, avoiding close contact with pigs, and taking preventive measures to avoid contamination of mucous membranes, such as prohibiting eating and drinking in pig pens. In addition, they urge young children and anyone at risk of more severe illness from seasonal human influenza viruses to avoid pigs and pig pens at fairs (Meles et al., 2025).

Research by epizootiologists and epidemiologists underscores the importance of the “One Health” approach in disease control, particularly in ecosystems where humans are the primary source of invasive-associated viruses for pig populations, and emphasizes the need for continuous surveillance and enhanced biosecurity measures. The co-circulation of several subtypes in regions with high densities of pig populations facilitates viral exchange, underscoring the importance of monitoring viral evolution to inform vaccine selection and public health policies at the local and global levels (Ciuderis et al., 2024). Our results also demonstrate marked regional variability in the spread of the swine influenza virus in Ukraine. The highest seropositivity rates were found in the Sumy, Volyn, Ternopil, and Lviv oblasts, while the highest rates of viral RNA detection by PCR were observed in the Ivano-Frankivsk, Kharkiv, and Kirovohrad oblasts. These differences may be related to variations in livestock density, the intensity of animal movement, biosecurity levels, and the circulation of different genetic variants of the virus in specific regions. The “One Health” approach and intersectoral collaboration employed by the CDC, FAO, and USDA ensure integrated infection control. Overall, the results of our studies confirm global trends regarding the widespread circulation of the influenza A virus in the swine population and the high prevalence of latent or subclinical infection. A significantly higher seropositivity rate compared with the frequency of viral RNA detection indicates that the epizootic process is continuously active even in the absence of active clinical disease. This creates conditions for the long-term persistence of the virus in the swine population, the emergence of new reassortant variants, and potential interspecies transmission. Swine influenza is a complex, multifactorial epizootiological system characterized by high variability, global distribution, and significant zoonotic potential. Effective control is possible only through a combination of biosecurity measures, vaccination, and continuous epizootiological monitoring.

Continuous assessment of the presence of antibodies to swine influenza in pigs is necessary due to the virus’s susceptibility to avian and mammalian influenza viruses and its role in interspecies transmission (Khalil et al., 2024). It is important to note that in our studies, the average number of sera tested per farm was nearly 10 times higher than the amount of material tested by PCR. This is due to the nature of serological monitoring, which allows for an assessment of the population’s exposure to the virus over a longer period of time. By contrast, PCR-based diagnostics reflect only the active phase of infection and depend to a large extent on the timing of sample collection, the intensity of viral shedding, and the clinical status of the animals. This is precisely why the combination of molecular-genetic and serological methods is the most informative approach for assessing the epizootic situation regarding swine influenza.

Thus, our results confirm that swine influenza in Ukraine exhibits characteristics of an enzootic infection with persistent viral circulation in the population. High seropositivity, combined with periodic detection of viral RNA, creates conditions for the long-term persistence and evolution of the virus on swine farms. Under conditions of intensive swine farming, this may contribute to the emergence of new reassortant variants of the virus that have the potential for interspecies transmission and pose a risk to both animal husbandry and public health.

Conclusions

Studies conducted in 2021–2025 indicate that influenza A virus is widespread among the pig population in Ukraine and confirm its constant circulation on pig farms of various forms of ownership. According to the results of the studies, influenza virus RNA was

detected in 25.0% of the farms, while antibodies to the influenza virus were detected in 68.2% of the farms. In general, during 2021–2025, of all the samples tested, the percentage of viral RNA detection was within 6.06–16.5%, and the seroprevalence ranged from 36.3% to 48.0%.

The obtained results demonstrate the feasibility of the comprehensive use of molecular-genetic and serological diagnostic methods when monitoring swine influenza. RT-PCR allows confirming infection at a specific point in time, while ELISA provides a generalized assessment of the prevalence of the influenza virus in the pig population, and also makes it possible to analyze the epizootic situation and plan a set of anti-epizootic measures.

References

- Abdelwhab, E. M., & Mettenleiter, T. C. (2023). Zoonotic animal influenza virus and potential mixing vessel hosts. *Viruses*, 15(4), 980.
- Abente, E. J., Rajao, D. S., Santos, J., Kaplan, B. S., Nicholson, T. L., Brockmeier, S. L., Gauger, P. C., Perez, D. R., & Vincent, A. L. (2018). Comparison of adjuvanted-whole inactivated virus and live-attenuated virus vaccines against challenge with contemporary, antigenically distinct H3N2 influenza A viruses. *Journal of Virology*, 92(22), e01323-18.
- Anderson, T. K., Chang, J., Arendsee, Z. W., Venkatesh, D., Souza, C. K., Kimble, J. B., Lewis, N. S., Davis, C. T., & Vincent, A. L. (2021). Swine influenza A viruses and the tangled relationship with humans. *Cold Spring Harbor Perspectives in Medicine*, 11(3), a038737.
- Arunorat, J., Charoenvisal, N., Woonwong, Y., Kedkovid, R., & Thanawongnuwech, R. (2016). Determination of current reference viruses for serological study of swine influenza viruses after the introduction of pandemic 2009 H1N1 (pdmH1N1) in Thailand. *Journal of Virological Methods*, 236, 5–9.
- Bowman, A. S., Nelson, S. W., Page, S. L., Nolting, J. M., Killian, M. L., Sreevatsan, S., & Slemons, R. D. (2014). Swine-to-human transmission of influenza A (H3N2) virus at agricultural fairs, Ohio, USA, 2012. *Emerging Infectious Diseases*, 20(9), 1472–1480.
- Cai, M., Wei, Z., Hu, X., Ji, Y., Li, S., Huang, J., Jin, R., Liang, Q., Zhang, G., Zheng, Z., Gong, L., & Li, M. (2024). The evolution, complexity, and diversity of swine influenza viruses in China: A hidden public health threat. *Virology*, 598, 110167.
- Cardinale, E., Pascalis, H., Temmam, S., Hervé, S., Saulnier, A., Turpin, M., Barbier, N., Hoarau, J., Quéguiner, S., Gorin, S., Foray, C., Roger, M., Porphyre, V., André, P., Thomas, T., de Lamballerie, X., Dellagi, K., & Simon, G. (2012). Influenza A (H1N1) pdm09 virus in pigs, Réunion Island. *Emerging Infectious Diseases*, 18(10), 1665–1668.
- Chamba Pardo, F. O., Wayne, S., Culhane, M. R., Perez, A., Allerson, M., & Torremorell, M. (2019). Effect of strain-specific maternally-derived antibodies on influenza A virus infection dynamics in nursery pigs. *PLoS One*, 14(1), e0210700.
- Chastagner, A., Enouf, V., Peroz, D., Hervé, S., Lucas, P., Quéguiner, S., Gorin, S., Beven, V., Behillil, S., Leneveu, P., Garin, E., Blanchard, Y., van der Werf, S., & Simon, G. (2019). Bidirectional human-swine transmission of seasonal influenza A (H1N1) pdm09 virus in pig herd, France, 2018. *Emerging Infectious Diseases*, 25(10), 1940–1943.
- Chaves, L. C. S., Orr-Burks, N., Vanover, D., Mosur, V. V., Hosking, S. R., Kumar E K P., Jeong, H., Jung, Y., Assumpção, J. A. F., Peck, H. E., Nelson, S. L., Burke, K. N., Garrison, M. A., Arthur, R. A., Claussen, H., Heaton, N. S., Lafontaine, E. R., Hogan, R. J., Zurla, C., & Santangelo, P. J. (2024). mRNA-encoded Cas13 treatment of influenza via site-specific degradation of genomic RNA. *PLoS Pathogens*, 20(7), e1012345.
- Chiapponi, C., Prosperi, A., Moreno, A., Baioni, L., Faccini, S., Manfredi, R., Zanni, I., Gabbi, V., Calanchi, I., Fusaro, A., Beato, M. S., Cavicchio, L., Torreggiani, C., Alborali, G. L., & Luppi, A. (2021). Genetic variability among swine influenza viruses in Italy: Data analysis of the period 2017–2020. *Viruses*, 14(1), 47.
- Ciuoderis, K., Usuga, J., Pérez-Restrepo, L. S., Gonzalez-Ramirez, M., Carvajal, L., Cardona, A., Moreno, I., Diaz, A., Peña, M., Hernández-Ortiz, J. P., & Osorio, J. E. (2024). Genomic diversity and evolutionary dynamics of Influenza A viruses in Colombian swine: Implications for one health surveillance and control. *Emerging Microbes and Infections*, 13(1), 2368202.
- Coggon, A., Lopes, S., Simon, G., Arendsee, Z., Chen, K.-F., Chiapponi, C., Essen, S., Everett, H., Hervé, S., Hufnagel, D. E., Mollett, B., Moreno, A., Pekosz, A., Richard, G., Rothman, R. E., Shaw-Saliba, K., Van Reeth, K., Venkatesh, D., Brown, I. H., Anderson, T. K., ... Lewis, N. S. (2025). Quantifying the zoonotic risk profile of European influenza A viruses in swine from 2010 to 2020 inclusive. *Journal of Virology*, 99(7), e0030625.
- Cui, X., Ma, J., Pang, Z., Chi, L., Mai, C., Liu, H., Liao, M., & Sun, H. (2024). The evolution, pathogenicity and transmissibility of quadruple reassortant H1N2 swine influenza virus in China: A potential threat to public health. *Virologica Sinica*, 39(2), 205–217.
- Detmer, S. E., Patnayak, D. P., Jiang, Y., Gramer, M. R., & Goyal, S. M. (2011). Detection of influenza A virus in porcine oral fluid samples. *Journal of Veterinary Diagnostic Investigation*, 23(2), 241–247.
- Eggink, D., Kroneman, A., Dingemans, J., Goderski, G., van den Brink, S., Bagheri, M., Lexmond, P., Pronk, M., van der Vries, E., Gemmeraad, E., Brandwagt, D., Houben, M., Hooiveld, M., van der Giessen, J., van Gageldonk-Lafeber, R., Fouchier, R., & Meijer, A. (2025). Human infections with Eurasian avian-like swine influenza virus detected by coincidence via routine respiratory surveillance systems, the Netherlands, 2020 to 2023. *Euro Surveillance: Bulletin Européen sur les Maladies Transmissibles*, 30(19), 2400662.
- El Zowalaty, M. E., Abdelgadir, A., Borkenhagen, L. K., Ducatez, M. F., Bailey, E. S., & Gray, G. C. (2022). Influenza A viruses are likely highly prevalent in South African swine farms. *Transboundary and Emerging Diseases*, 69(4), 2373–2383.
- Fineberg H. V. (2014). Pandemic preparedness and response—lessons from the H1N1 influenza of 2009. *The New England Journal of Medicine*, 370(14), 1335–1342.
- Freidl, G. S., Meijer, A., de Bruin, E., de Nardi, M., Munoz, O., Capua, I., Breed, A. C., Harris, K., Hill, A., Kosmider, R., Banks, J., von Dobschuetz, S., Stark, K., Wieland, B., Stevens, K., van der Werf, S., Enouf, V., van der Meulen, K., Van Reeth, K., Dauphin, G., & Koopmans, M. (2014). Flurisk consortium. Influenza at the animal-human interface: A review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1). *Euro Surveillance: Bulletin Européen sur les Maladies Transmissibles*, 19(18), 20793.
- Glud, H. A., George, S., Skovgaard, K., & Larsen, L. E. (2021). Zoonotic and reverse zoonotic transmission of viruses between humans and pigs. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, 129(12), 675–693.
- Harima, H., Okuya, K., Kajihara, M., Ogawa, H., Simulundu, E., Bwalya, E., Qiu, Y., Mori-Kajihara, A., Munyeme, M., Sakoda, Y., Saito, T., Hang'ombe, B. M., Sawa, H., Mweene, A. S., & Takada, A. (2022). Serological and molecular epidemiological study on swine influenza in Zambia. *Transboundary and Emerging Diseases*, 69(4), e931–e943.
- He, Y., Song, S., Wu, J., Wu, J., Zhang, L., Sun, L., Li, Z., Wang, X., Kou, Z., & Liu, T. (2024). Emergence of Eurasian avian-like swine influenza A (H1N1) virus in a child in Shandong Province, China. *BMC Infectious Diseases*, 24(1), 550.
- Hennig, C., Graaf, A., Petric, P. P., Graf, L., Schwemmler, M., Beer, M., & Harder, T. (2022). Are pigs overestimated as a source of zoonotic influenza viruses? *Porcine Health Management*, 8(1), 30.
- Holyoake, P. K., Kirkland, P. D., Davis, R. J., Arzey, K. E., Watson, J., Lunt, R. A., Wang, J., Wong, F., Moloney, B. J., & Dunn, S. E. (2011). The first identified case of pandemic H1N1 influenza in pigs in Australia. *Australian Veterinary Journal*, 89(11), 427–431.
- Hufnagel, D. E., Young, K. M., Arendsee, Z. W., Gay, L. C., Caceres, C. J., Rajão, D. S., Perez, D. R., Vincent Baker, A. L., & Anderson, T. K. (2023). Characterizing a century of genetic diversity and contemporary antigenic diversity of N1 neuraminidase in influenza A virus from North American swine. *Virus Evolution*, 9(1), vead015.
- Hutchinson, E. C. (2018). Influenza virus. *Trends in Microbiology*, 26(9), 809–810.
- Joseph, U., Su, Y. C., Vijaykrishna, D., & Smith, G. J. (2017). The ecology and adaptive evolution of influenza A interspecies transmission. *Influenza and Other Respiratory Viruses*, 11(1), 74–84.
- Juvet, L. K., Robertson, A. H., Laake, I., Mjaaland, S., & Trogstad, L. (2021). Safety of influenza A H1N1pdm09 vaccines: An overview of systematic reviews. *Frontiers in Immunology*, 12, 740048.
- Kessler, S., Harder, T. C., Schwemmler, M., & Cimini, K. (2021). Influenza A viruses and zoonotic events – are we creating our own reservoirs? *Viruses*, 13(11), 2250.
- Khalil, A. M., Martinez-Sobrido, L., & Mostafa, A. (2024). Zoonosis and zoonanthroposis of emerging respiratory viruses. *Frontiers in Cellular and Infection Microbiology*, 13, 1232772.
- Kim, S. H., Kim, H. J., Jin, Y. H., Yeoul, J. J., Lee, K. K., Oem, J. K., Lee, M. H., & Park, C. K. (2013). Isolation of influenza A(H3N2) virus from pigs and characterization of its biological properties in pigs and mice. *Archives of Virology*, 158(11), 2351–2357.
- King, J., Harder, T., Beer, M., & Pohlmann, A. (2020). Rapid multiplex MinION nanopore sequencing workflow for influenza A viruses. *BMC Infectious Diseases*, 20(1), 648.
- Klivelyeva, N., Glebova, T., Saktaganov, N., & Webby, R. (2025). Cases of interspecies transmission of influenza A virus from swine to humans. *Veterinary Sciences*, 12(9), 873.
- Kong, W., Liu, Q., Sun, Y., Wang, Y., Gao, H., Liu, L., Qin, Z., He, Q., Sun, H., Pu, J., Wang, D., Guo, X., Yang, H., Chang, K. C., Shu, Y., & Liu, J.

- (2016). Transmission and pathogenicity of novel reassortants derived from Eurasian avian-like and 2009 pandemic H1N1 influenza viruses in mice and guinea pigs. *Scientific Reports*, 6, 27067.
- Krog, J. S., Hjulsgaard, C. K., Larsen, M. A., & Larsen, L. E. (2017). Triple-reassortant influenza A virus with H3 of human seasonal origin, NA of swine origin, and internal A(H1N1) pandemic 2009 genes is established in Danish pigs. *Influenza and Other Respiratory Viruses*, 11(3), 298–303.
- Lan, R., Yang, J., Li, J., Li, H., Cao, X., Tao, M., Chang, H., Yu, H., Tong, Q., Lu, L., Liu, J., & Sun, H. (2025). Continuous evolution of Eurasian avian-like H1N1 swine influenza viruses with pdm/09-derived internal genes enhances pathogenicity in mice. *Journal of Virology*, 99(10), e0043025.
- Le Sage, V., Souza, C. K., Rockey, N. C., Shephard, M., Zanella, G. C., Arruda, B., Wang, S., Drapeau, E. M., Doyle, J. D., Xu, L., Barbeau, D. J., Paulson, J. C., McElroy, A. K., Hensley, S. E., Anderson, T. K., Vincent Baker, A. L., & Lakdawala, S. S. (2025). Eurasian 1C swine influenza A virus exhibits high pandemic risk traits. *Emerging Microbes and Infections*, 14(1), 2492210.
- Lechmann, J., Szelecsenyi, A., Bruhn, S., Harisberger, M., Wyler, M., Bachofen, C., Hadorn, D., Suter-Boquete, P., Tobler, K., Krauer, F., Fraefel, C., & Gonçalves Cabecinhas, A. R. (2025). Das nationale programm zur –überwachung von influenza A viren bei schweinen und menschen in der schweiz: genetische variabilität und zoonotische übertragungen von 2010 bis 2022 [The Swiss national program for the -surveillance of influenza A viruses in pigs and humans: genetic variability and zoonotic transmissions from 2010–2022]. *Schweizer Archiv für Tierheilkunde*, 167(11), 600–616 (in German).
- Lewis, N. S., Russell, C. A., Langat, P., Anderson, T. K., Berger, K., Bielejec, F., Burke, D. F., Dudas, G., Fonville, J. M., Fouchier, R. A., Kellam, P., Koel, B. F., Lemey, P., Nguyen, T., Nuansrichy, B., Peiris, J. M., Saito, T., Simon, G., Skepner, E., Takemae, N., Webby, R. J., Van Reeth, K., Brookes, S. M., Larsen, L., Watson, S. J., Brown, I. H., & Vincent, A. L. (2016). The global antigenic diversity of swine influenza A viruses. *eLife*, 5, e12217.
- Lewis, P. A., & Shope, R. E. (1931). Swine influenza: II. A hemophilic bacillus from the respiratory tract of infected swine. *The Journal of Experimental Medicine*, 54(3), 361–371.
- Li, S., Peng, X., Wang, M., Wang, W., Liu, Y., & Yang, Q. (2024). Influenza A virus utilizes the nasolacrimal system to establish respiratory infection after ocular exposure in the swine model. *Transboundary and Emerging Diseases*, 8192499.
- Li, Y., & Robertson, I. (2021). The epidemiology of swine influenza. *Animal Diseases*, 1(1), 21.
- Ma, W. (2020). Swine influenza virus: Current status and challenge. *Virus Research*, 288, 198118.
- Ma, W., Vincent, A. L., Gramer, M. R., Brockwell, C. B., Lager, K. M., Janke, B. H., Gauger, P. C., Patnayak, D. P., Webby, R. J., & Richt, J. A. (2007). Identification of H2N3 influenza A viruses from swine in the United States. *Proceedings of the National Academy of Sciences of the United States of America*, 104(52), 20949–20954.
- Ma, Y., Shi, K., Chen, Z., Shi, Y., Zhou, Q., Mo, S., Wei, H., Hu, L., & Mo, M. (2024). Simultaneous detection of porcine respiratory coronavirus, porcine reproductive and respiratory syndrome virus, swine influenza virus, and pseudorabies virus via quadruplex one-step RT-qPCR. *Pathogens*, 13(4), 341.
- Mancera Gracia, J. C., Pearce, D. S., Masic, A., & Balasch, M. (2020). Influenza A virus in swine: Epidemiology, challenges and vaccination strategies. *Frontiers in Veterinary Science*, 7, 647.
- Markin, A., Ciacchi Zanella, G., Arendsee, Z. W., Zhang, J., Krueger, K. M., Gauger, P. C., Vincent Baker, A. L., & Anderson, T. K. (2023). Reverse-zoonoses of 2009 H1N1 pandemic influenza A viruses and evolution in United States swine results in viruses with zoonotic potential. *PLoS Pathogens*, 19(7), e1011476.
- Meles, D. K., Khairullah, A. R., Rimayanti, R., Mustofa, I., Wurlina, W., Utama, S., Lestari, T. D., Mulyati, S., Ahmad, R. Z., Moses, I. B., Wibowo, S., Kusala, M. K. J., Wardhani, B. W. K., Fauziah, I., Kumiasih, D. A. A., Anggraini, L., Ekawasti, F., & Akintunde, A. O. (2025). The global burden of swine influenza and its mitigation. *Open Veterinary Journal*, 15(5), 1866–1879.
- Mena, I., Nelson, M. I., Quezada-Monroy, F., Dutta, J., Cortes-Fernández, R., Lara-Puente, J. H., Castro-Peralta, F., Cunha, L. F., Trovão, N. S., Lozano-Dubernard, B., Rambaut, A., van Bakel, H., & García-Sastre, A. (2016). Origins of the 2009 H1N1 influenza pandemic in swine in Mexico. *eLife*, 5, e16777.
- Meng, F., Yang, H., Qu, Z., Chen, Y., Zhang, Y., Zhang, Y., Liu, L., Zeng, X., Li, C., Kawaoka, Y., & Chen, H. (2022). A Eurasian avian-like H1N1 swine influenza reassortant virus became pathogenic and highly transmissible due to mutations in its PA gene. *Proceedings of the National Academy of Sciences of the United States of America*, 119(34), e2203919119.
- Mollett, B. C., Everett, H. E., van Diemen, P. M., Byrne, A. M. P., Ramsay, A., James, J., Reid, S. M., Hansen, R. D. E., Lewis, N. S., Brown, I. H., & Banyard, A. C. (2023). JMM profile: swine influenza A virus: A neglected virus with pandemic potential. *Journal of Medical Microbiology*, 72, 1623.
- Moraes, D. C. A., Cezar, G. A., Magalhães, E. S., Nicolino, R. R., Rupasinghe, K., Chandra, S., Silva, G. S., Almeida, M. N., Crim, B., Burrough, E. R., Gauger, P. C., Madson, D., Thomas, J., Zeller, M. A., Main, R., Thurn, M., Lages, P., Corzo, C. A., Sturos, M., Naikare, H., & Trevisan, G. (2025). Macroeconomic trends of influenza A virus detection through reverse transcription real-time polymerase chain reaction (RT-rtPCR) in porcine samples in the United States over the last 20 years. *Frontiers in Veterinary Science*, 12, 1572237.
- Morens, D. M., Taubenberger, J. K., & Fauci, A. S. (2021). A century tale of two pandemics: the 1918 influenza pandemic and COVID-19, part II. *American Journal of Public Health*, 111(7), 1267–1272.
- Nelson, M. I., Stratton, J., Killian, M. L., Janas-Martindale, A., & Vincent, A. L. (2015). Continual reintroduction of human pandemic H1N1 influenza A viruses into swine in the United States, 2009 to 2014. *Journal of Virology*, 89(12), 6218–6226.
- Nelson, M. I., Stucker, K. M., Schobel, S. A., Trovão, N. S., Das, S. R., Dugan, V. G., Nelson, S. W., Sreevatsan, S., Killian, M. L., Noltling, J. M., Wentworth, D. E., & Bowman, A. S. (2016). Introduction, evolution, and dissemination of influenza A viruses in exhibition swine in the United States during 2009 to 2013. *Journal of Virology*, 90(23), 10963–10971.
- Neveau, M. N., Zeller, M. A., Kaplan, B. S., Souza, C. K., Gauger, P. C., Vincent, A. L., & Anderson, T. K. (2022). Genetic and antigenic characterization of an expanding H3 influenza A virus clade in U.S. swine visualized by nextstrain. *MSphere*, 7(3), e0099421.
- Nuwarda, R. F., Alharbi, A. A., & Kayser, V. (2021). An overview of influenza viruses and vaccines. *Vaccines*, 9(9), 1032.
- Petro-Tumquist, E., Pekarek, M. J., & Weaver, E. A. (2024). Swine influenza A virus: Challenges and novel vaccine strategies. *Frontiers in Cellular and Infection Microbiology*, 14, 1336013.
- Pippig, J., Ritzmann, M., Büttner, M., & Neubauer-Juric, A. (2016). Influenza A viruses detected in swine in southern Germany after the H1N1 pandemic in 2009. *Zoonoses and Public Health*, 63(7), 555–568.
- Rajão, D. S., Costa, A. T., Brasil, B. S., Del Puerto, H. L., Oliveira, F. G., Alves, F., Braz, G. F., Reis, J. K., Guedes, R. M., Lobato, Z. I., & Leite, R. C. (2013). Genetic characterization of influenza virus circulating in Brazilian pigs during 2009 and 2010 reveals a high prevalence of the pandemic H1N1 subtype. *Influenza and Other Respiratory Viruses*, 7(5), 783–790.
- Ryt-Hansen, P., George, S., Hjulsgaard, C. K., Trebbien, R., Krog, J. S., Ciucani, M. M., Langerhuus, S. N., DeBeauchamp, J., Crumpton, J. C., Hibler, T., Webby, R. J., & Larsen, L. E. (2025). Rapid surge of reassortant A(H1N1) influenza viruses in Danish swine and their zoonotic potential. *Emerging Microbes and Infections*, 14, 2466686.
- Scholtissek, C., Bürger, H., Kistner, O., & Shortridge, K. F. (1985). The nucleoprotein as a possible major factor in determining host specificity of influenza H3N2 viruses. *Virology*, 147(2), 287–294.
- Schultz-Cherry, S., Olsen, C. W., & Easterday, B. C. (2013). History of swine influenza. *Current Topics in Microbiology and Immunology*, 370, 21–28.
- Senthilkumar, D., Kulkarni, D. D., Venkatesh, G., Gupta, V., Patel, P., Dixit, M., Singh, B., Bhatia, S., Tosh, C., Dubey, S. C., & Singh, V. P. (2021). Widespread prevalence of antibodies against swine influenza A (pdm H1N1/09) virus in pigs of Eastern Uttar Pradesh, India. *Current Microbiology*, 78(7), 2753–2761.
- Shi, Y., Shi, K., Ma, Y., Yin, Y., Long, F., Feng, S., Mo, M., He, J., & Wei, Z. (2025). Development of a triplex crystal digital PCR for the detection of PRCov, PRRSV, and SIV. *Frontiers in Veterinary Science*, 12, 1562444.
- Shortridge, K. F. (1992). Pandemic influenza: A zoonosis? *Seminars in Respiratory Infections*, 7(1), 11–25.
- Shortridge, K. F., Webster, R. G., Butterfield, W. K., & Campbell, C. H. (1977). Persistence of Hong Kong influenza virus variants in pigs. *Science*, 196(4297), 1454–1455.
- Sun, H., Xiao, Y., Liu, J., Wang, D., Li, F., Wang, C., Li, C., Zhu, J., Song, J., Sun, H., Jiang, Z., Liu, L., Zhang, X., Wei, K., Hou, D., Pu, J., Sun, Y., Tong, Q., Bi, Y., Chang, K. C., Liu, S., Gao, G. F., & Liu, J. (2020). Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection. *Proceedings of the National Academy of Sciences of the United States of America*, 117(29), 17204–17210.
- Thomas, M. N., Zanella, G. C., Cowan, B., Cáceres, C. J., Rajão, D. S., Perez, D. R., Gauger, P. C., Vincent Baker, A. L., & Anderson, T. K. (2024). Nucleoprotein reassortment enhanced transmissibility of H3 1990.4a clade influenza A virus in swine. *Journal of Virology*, 98(3), e0170323.
- Tochetto, C., Gava, D., Haach, V., & Schaefer, R. (2025). Passive surveillance for Influenza A virus among swine, Brazil, 2009–2023. *Brazilian Journal of Microbiology*, 56(4), 2865–2872.
- Uyeki, T. M., Hui, D. S., Zambon, M., Wentworth, D. E., & Monto, A. S. (2022). Influenza. *Lancet*, 400(10353), 693–706.

- van der Vries, E., Germeraad, E. A., Kroneman, A., Dieste-Pérez, L., Eggink, D., Willems, E., Byrne, A. M., Lewis, N., Houben, M., Fouchier, R., Meijer, A., & van der Giessen, J. (2025). Swine influenza virus surveillance programme pilot to assess the risk for animal and public health, the Netherlands, 2022 to 2023. *Euro Surveillance: Bulletin Européen sur les Maladies Transmissibles*, 30(22), 2400664.
- Wang, Q., Liu, Z., Zeng, X., Zheng, Y., Lan, L., Wang, X., Lai, Z., Hou, X., Gao, L., Liang, L., Tang, S., Zhang, Z., Leng, J., & Fan, X. (2024). Integrated analysis of miRNA-mRNA expression of newly emerging swine H3N2 influenza virus cross-species infection with tree shrews. *Virology Journal*, 21, 4.
- Yan, M., Ma, T., Shi, X., Chen, Q., Li, L., Xu, B., Pan, X., Teng, Q., Yuan, C., Yan, D., Zhang, Z., Liu, Q., & Li, Z. (2025). Isolation and characterization of H1 subtype swine influenza viruses recently circulating in China. *Viruses*, 17(2), 185.
- Zeller, M. A., Camevale de Almeida Moraes, D., Ciacci Zanella, G., Souza, C. K., Anderson, T. K., Baker, A. L., & Gauger, P. C. (2024). Reverse zoonosis of the 2022–2023 human seasonal H3N2 detected in swine. *NPJ Viruses*, 2, 27.
- Zhu, H., Webby, R., Lam, T. T., Smith, D. K., Peiris, J. S., & Guan, Y. (2013). History of Swine influenza viruses in Asia. *Current Topics in Microbiology and Immunology*, 370, 57–68.