

SEROLOGICAL SURVEILLANCE OF INFLUENZA A VIRUS AND HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN RUMINANTS IN UKRAINE, 2015–2026

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Summary. This article provides a concise review of the current literature on the circulation of influenza A viruses in cattle and the unprecedented emergence of highly pathogenic avian influenza (HPAI) H5N1 infection in this species. Particular attention is paid to the ecological and biological aspects of influenza A virus cross-species transmission, as well as to the potential implications of these events for veterinary medicine, livestock production, and public health. The study presents the results of the first pilot serological investigation conducted at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', providing evidence of the potential circulation of influenza A virus among ruminants in Ukraine. A total of 510 serum samples collected from cattle and small ruminants in different regions of Ukraine between 2015 and 2026 were tested using an enzyme-linked immunosorbent assay (ELISA). Three (0.59%) seropositive cattle serum samples, collected in 2023 and 2026, were identified, whereas all serum samples obtained from small ruminants tested negative. Overall, the seroprevalence of antibodies against influenza A virus was low, ranging from 1.67% to 5.0%, depending on the year of sampling and the geographical region. Further serological characterization of all influenza A virus-positive sera for antibodies against highly pathogenic avian influenza H5N1 yielded negative results. Thus, the findings do not support the circulation of HPAI H5N1 among the examined ruminant population in Ukraine. Nevertheless, given the remarkable evolutionary plasticity of influenza A viruses, their ability to overcome species barriers, and their potential to establish novel transmission pathways, continued serological, virological, and molecular surveillance of ruminants is warranted. Such monitoring is essential for the early detection of cross-species transmission events, changes in virus ecology, and the emergence of novel biological properties

Keywords: highly pathogenic avian influenza, cattle influenza, serological surveillance, enzyme-linked immunosorbent assay

Introduction. Zoonotic pathogens continue to pose one of the greatest threats to global public health, particularly emerging infectious diseases and those with natural reservoirs in wildlife. Consequently, the One Health concept has become increasingly important, promoting an integrated approach to understanding pathogen circulation among wildlife, domestic animals, and humans, as well as their early detection, risk assessment, and the development of effective response strategies. According to the World Health Organization, influenza pandemics are among the most significant epidemics and pandemics in modern history caused by zoonotic viruses, alongside Marburg virus disease, Ebola virus disease, Nipah virus infection, Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19). Influenza viruses have been responsible for at least four confirmed pandemics within less than a century: the 1918 H1N1 ('Spanish flu'), the 1957 H2N2 ('Asian flu'), the 1968 H3N2 ('Hong Kong flu'), and the 2009 H1N1pdm09 pandemic (Krueger and Gray, 2013; Piret and Boivin, 2020; Scholtissek et al., 1978). Influenza A virus is one of the most extensively studied pathogens affecting both humans and animals. Despite remarkable advances in our understanding of its biology, the development of modern laboratory diagnostic methods, antiviral drugs, and vaccines, influenza remains one of the most dangerous and

unpredictable viral pathogens. Growing concern among public health experts is no longer focused on whether another influenza pandemic will occur, but rather on when it will occur, with influenza A virus once again considered the most likely causative agent. The exceptional pandemic potential of influenza A virus is primarily attributable to its unique biological characteristics. Its segmented genome provides extraordinary evolutionary plasticity, enabling continuous antigenic drift and genetic reassortment, which facilitate the emergence of novel viral variants. Equally important are its continuous circulation among humans, domestic animals, and wildlife, its repeated ability to cross species barriers, adapt to new host species, and expand into new ecological niches (Krammer, Hermann and Rasmussen, 2025; Peacock et al., 2025; Wille and Barr, 2022). The ecology of influenza A viruses is remarkably complex and encompasses a broad range of avian and mammalian host species. Influenza A viruses are currently known to circulate in humans, swine, horses, birds, carnivorous mammals, and bats (Muramoto et al., 2013; Shi et al., 2014; Suarez, 2016; Yamayoshi et al., 2016). However, recent events have demonstrated that this list can no longer be considered exhaustive. Since 2024, cattle have effectively been added as a new host species following the first documented evidence of sustained circulation of highly pathogenic avian influenza (HPAI) H5N1 virus in

dairy cattle (Shanta et al., 2025). Among the diverse influenza A viruses, highly pathogenic avian influenza (HPAI) is of particular concern, as it currently represents one of the most devastating infectious diseases affecting domestic poultry. The disease is characterized by high mortality, rapid transmission, substantial economic losses to both commercial and backyard poultry production, and significant disruption of international trade in poultry and poultry products. However, the greatest global concern goes beyond its economic impact and primarily centers on the extraordinary pandemic potential of H5 viruses. Consequently, contemporary HPAI H5N1 viruses are now regarded as among the most likely candidates to cause the next human influenza pandemic (EFSA et al., 2026).

Emergence of HPAI H5N1 in cattle. A new stage in the evolution of the global HPAI panzootic, as well as of influenza A virus ecology in general, began with the detection of H5N1 clade 2.3.4.4b (genotype B3.13) infection in dairy cattle in the United States in 2024. This event marked an unprecedented milestone in influenza research, as it provided the first documented evidence not only of cross-species transmission of highly pathogenic avian influenza virus to cattle but also of its subsequent sustained circulation within a new host population. It should be noted that sporadic reports of influenza-like illness in cattle had been documented previously. During the 1940s–1960s and again in the 1990s, serological evidence of antibodies against human influenza A viruses, predominantly H1N1 and H3N2 subtypes, was reported in cattle from Japan, Hungary, the former Soviet Union, the United Kingdom, and several other countries. However, these infections were generally associated with mild or absent clinical signs and were documented primarily through serological studies, without virus isolation or molecular characterization (Shanta et al., 2025; Sreenivasan et al., 2019). In contrast to these earlier observations, the current HPAI H5N1 epizootic in the United States is characterized by confirmed adaptation of the virus to a new host species and its subsequent transmission among cattle. Molecular epidemiological investigations have demonstrated that the outbreak most likely originated from a single cross-species spillover event from a wild bird to dairy cattle that occurred in late 2023. This event subsequently gave rise to widespread transmission among dairy herds across multiple U.S. states, representing the first documented example of sustained large-scale circulation of a highly pathogenic avian influenza virus in cattle. Subsequent investigations confirmed that cross-species transmission from wild birds to cattle was not limited to a single introduction. In 2025, two additional independent introductions of HPAI H5N1 (genotype D1.1) from wild birds into cattle were identified in the United States. These findings further demonstrate that wild birds remain the principal natural reservoir of HPAI viruses and continue to play a pivotal role in generating new spillover events, infecting novel mammalian hosts, and overcoming species barriers (Nguyen et al., 2025). It

is equally important to note that following the virus's adaptation to cattle, several cases of reverse transmission of HPAI H5N1 from infected cattle to other animal species were documented. Infections were confirmed in synanthropic birds, including pigeons and thrushes, as well as in mammals such as domestic cats that had direct contact with infected cattle or contaminated environments. These observations indicate the emergence of novel transmission pathways involving cattle and highlight the increasing ecological complexity of HPAI H5N1 (Nguyen et al., 2025; Peacock et al., 2025). The introduction of HPAI virus into a new host species was accompanied by a distinct change in disease presentation. Although infected cattle exhibited typical influenza-associated clinical signs, including fever, respiratory disease, and serous nasal discharge, the mammary gland became the principal target organ. The disease was characterized by mastitis-like lesions, a marked decline in milk production, and deterioration of milk quality. Importantly, infectious virus was shed in milk at exceptionally high concentrations. The movement of infected cattle, including subclinically infected, between farms, together with the use of shared milking equipment, was identified as one of the principal mechanisms facilitating virus transmission within dairy herds (Caserta et al., 2024; Shanta et al., 2025).

The HPAI H5N1 outbreak in dairy cattle was also accompanied by human infections among individuals with direct occupational exposure to infected animals or contaminated biological materials. In most cases, the disease was mild and manifested primarily as conjunctivitis or mild respiratory illness. Nevertheless, each new replication cycle of the virus in humans or other mammals provides additional opportunities for the accumulation of adaptive mutations, alterations in viral biological properties, and the generation of reassortants with seasonal human influenza viruses. Although sustained human-to-human transmission of contemporary H5N1 clade 2.3.4.4b viruses had not been documented as of mid-2026, continued circulation of the virus in cattle and other mammals substantially increases the risk of further adaptation to humans and, consequently, the likelihood of the emergence of a new influenza pandemic (Krammer, Hermann and Rasmussen, 2025; Lombard et al., 2025; Nguyen et al., 2025; Pekar et al., 2026).

The situation in Europe and the rationale for investigating ruminants in Ukraine. In contrast to the United States, where HPAI H5N1 has established sustained circulation in dairy cattle, no confirmed cases of HPAI H5N1 infection in cattle had been reported in Europe as of 2026. The only available evidence comes from a serological survey conducted in the Netherlands in 2025, in which antibodies against H5N1 virus were detected in cattle on a single dairy farm. However, none of the seropositive animals showed clinical signs of disease, and viral RNA was not detected in any of the collected clinical samples. These findings therefore indicate previous exposure of individual animals to the virus rather than active circulation of HPAI H5N1 in

cattle in Europe (EFSA et al., 2026). In addition, a single case of HPAI H5N1 infection in a sheep was confirmed in the United Kingdom in 2025. Epidemiological investigation demonstrated that this infection resulted from a cross-species transmission event originating from infected poultry and was not followed by onward transmission within the sheep flock. Current evidence suggests that such cases remain sporadic and, at present, provide no indication that ruminants have become a novel reservoir for HPAI H5N1 (EFSA et al., 2025). Despite the absence of evidence for sustained circulation of HPAI H5N1 among ruminants in Europe, these events demonstrate the capacity of highly pathogenic avian influenza viruses to overcome species barriers and infect new mammalian hosts. Given the ongoing global HPAI H5N1 panzootic, the expanding range of natural and incidental hosts, and the emergence of novel transmission pathways, surveillance of ruminants has become an increasingly important component of early warning systems for the detection of emerging epizootic and zoonotic threats.

In Ukraine, information regarding the possible circulation of influenza A viruses, including HPAI H5N1, in cattle and small ruminants remains virtually unavailable. Although various influenza A virus subtypes have circulated in wild and domestic birds for many years (Muzyka et al., 2012, 2016a, 2016b, 2019), as well as in swine and horses (Kovalenko et al., 2017; Rula et al., 2024; Stegnyy et al., 2023), no studies investigating influenza A viruses in ruminants have previously been

conducted in Ukraine. Consequently, information on the occurrence and distribution of influenza A viruses in cattle and small ruminants in the country remains extremely limited.

In response to the current global epizootic situation and the lack of relevant national data, the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' initiated surveillance for influenza A virus and highly pathogenic avian influenza H5N1 in ruminants in Ukraine. The first phase of the study consisted of a retrospective serological analysis of archived serum samples collected from cattle and small ruminants in different regions of Ukraine between 2015 and 2026. The results provided the first evidence for assessing previous exposure of ruminants to influenza A viruses in Ukraine and established a scientific basis for further virological and molecular investigations.

Materials and methods. The study was conducted at the Department of Avian Diseases and Molecular Diagnostics, National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Sampling. Blood samples were collected from cattle and small ruminants using standard veterinary sampling procedures. A total of 510 cattle serum samples were included in the study. These comprised serum samples collected between 2023 and 2026, together with archived sera collected during 2015 and 2020–2022, originating from different regions of Ukraine (Table 1). In addition, 92 serum samples from small ruminants, collected in 2023, were examined (Table 2).

Table 1 — Distribution of cattle serum samples collected in different years

Region	Number of samples							
	2015	2020	2021	2022	2023	2024	2025	2026
Chernihiv	—	6	8	—	—	—	—	10
Khmelnitskyi	10	—	—	—	30	—	—	—
Kirovohrad	—	—	—	—	10	—	—	10
Poltava	—	8	—	—	20	45	—	—
Sumy	—	—	—	—	34	10	10	10
Vinnitsia	—	—	—	—	10	—	—	—
Zhytomyr	—	—	—	—	60	—	—	—
Dnipropetrovsk	—	—	—	—	6	—	—	—
Kharkiv	—	22	5	10	10	10	20	40
Kherson	—	10	—	—	—	—	—	—
Odesa	—	—	—	—	46	10	20	10
Total in years	10	46	13	10	226	75	50	80
Total	510							

Table 2 — Distribution of small ruminants serum samples collected in 2023

Region	Number of samples
Cherkasy	8
Volyn	30
Dnipropetrovsk	29
Kharkiv	25
Total	92

Serological analysis. Serological testing for antibodies against influenza A viruses was performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits, ID Screen® Influenza A Antibody Competition Multi-species and ID Screen® Influenza H5 Antibody Competition 3.0 Multi-species (IDvet, France). As a confirmatory assay, the microneutralization (MN) test was performed at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, Italy) by Dr. F. Bonfante.

All serological analyses were conducted according to the manufacturers' instructions and established laboratory protocols, in compliance with applicable biosafety and biosecurity requirements.

Results and discussion. The study was designed as a series of consecutive analytical stages. In the first stage, all serum samples were screened for antibodies against the influenza A virus nucleoprotein (NP), a highly conserved antigen present in all influenza A viruses. Screening was performed using a competitive ELISA designed to detect antibodies against influenza A virus irrespective of subtype. No antibodies against influenza A virus were detected in serum samples collected from small ruminants originating from four regions of Ukraine, namely Cherkasy, Volyn, Dnipropetrovsk, and Kharkiv (Table 3).

Among the 510 cattle serum samples examined, three (0.59%) were positive for antibodies against influenza A

virus. Seropositive samples were detected in two sampling years. In 2023, one seropositive sample was identified in cattle from Zhytomyr Region, corresponding to a seroprevalence of 1.67%. In 2026, two seropositive samples were detected in cattle from Kharkiv Region, corresponding to a seroprevalence of 5.0%. Importantly, all seropositive animals were clinically healthy at the time of sample collection (Table 4).

Table 3 — ELISA results for detection of antibodies against influenza A virus in small ruminants, 2023

Region	Samples			Seroprevalence, %
	Total	Negative	Positive	
Cherkasy	8	8	0	0
Volyn	30	30	0	0
Dnipropetrovsk	29	29	0	0
Kharkiv	25	25	0	0

Table 4 — ELISA results for detection of antibodies against influenza A virus in cattle serum samples

Region	Positive samples/total (seroprevalence, %)							
	2015	2020	2021	2022	2023	2024	2025	2026
Chernigiv	—	0/6 (0)	0/8	—	—	—	—	0/10 (0)
Khmelnyskyi	0/10 (0)	—	—	—	0/30 (0)	—	—	—
Kirovograd	—	—	—	—	0/10 (0)	—	—	0/10 (0)
Poltava	—	0/8 (0)	—	—	—	0/45 (0)	—	—
Sumy	—	—	—	—	0/20 (0)	0/10 (0)	0/10 (0)	—
Vinnitsia	—	—	—	—	0/34 (0)	—	—	0/10 (0)
Zhytomyr	—	—	—	—	1/60 (1,67)	—	—	—
Dnipropetrovsk	—	—	—	—	0/6 (0)	—	—	—
Kharkiv	—	0/22 (0)	0/5 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/20 (0)	2/40 (5)
Kherson	—	0/10 (0)	—	—	—	—	—	—
Odesa	—	—	—	—	0/46 (0)	0/10 (0)	0/20 (0)	0/10 (0)
Total	0/10 (0)	0/46 (0)	0/13 (0)	0/10 (0)	1/226 (0,44)	0/75 (0)	0/50 (0)	2/80 (2,5)

The second stage of the study involved testing all influenza A virus-seropositive serum samples for antibodies against HPAI H5 virus. Based on the ELISA results, one of the three influenza A virus-seropositive cattle serum samples, collected in Zhytomyr Region in 2023, tested positive for H5-specific antibodies. To confirm the ELISA result, the H5-seropositive serum sample was further analyzed using a microneutralization (MN) assay with an HPAI H5N1 clade 2.3.4.4b virus antigen at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, Italy). No HPAI H5N1-specific neutralizing antibodies were detected, indicating that the confirmatory assay did not confirm the initial ELISA-positive result.

Conclusions. Overall, the findings of this first serological screening study of influenza A virus infection in cattle and small ruminants suggest the possible circulation of influenza A viruses in selected cattle populations in Ukraine. The overall seroprevalence in cattle was low (0.59%). Antibodies against influenza A virus were detected only in cattle from Zhytomyr and Kharkiv regions in samples collected in 2023 and 2026,

respectively. In contrast, no seropositive samples were identified among the archived sera or among sera collected from small ruminants. Furthermore, all influenza A virus-seropositive sera tested negative for antibodies against HPAI H5 virus in subsequent confirmatory testing. Therefore, the present study provides no serological evidence of HPAI H5 virus circulation in cattle in Ukraine or previous HPAI H5 infection in the examined animals.

The detection of antibodies against influenza A virus in cattle should not be regarded as an entirely novel finding. Serological evidence of exposure to influenza A viruses, predominantly viruses of human origin, was reported in cattle as early as the 1940s–1960s and later during the 1990s in several European and Asian countries (Ballmann et al., 2026). More recently, a serological survey conducted in the Netherlands in 2026 demonstrated that approximately 1% of tested cattle were positive for influenza A virus antibodies by ELISA while remaining seronegative for H5 virus. Depending on the region, seroprevalence ranged from 0.51% to 1.42% (Ballmann et al., 2026). Thus, the results obtained

in the present study in Ukraine are generally consistent with observations reported from other European countries.

In our opinion, the detection of influenza A virus antibodies in cattle should be interpreted within a broader ecological and epizootiological framework. At present, the specific influenza A virus subtypes responsible for the detected antibody responses in Ukrainian cattle remain unknown. This question is of particular importance because influenza A viruses have previously been documented in swine (Rula et al., 2024), horses (Stegniy et al., 2023), and wild boar (Kovalenko et al., 2017) in Ukraine. In addition, extensive circulation of multiple influenza A virus subtypes, including highly pathogenic avian influenza viruses, has long been documented in wild waterfowl (Muzyka et al., 2016a, 2019; Sapachova et al., 2021), while recent studies have also demonstrated influenza A virus infection in passerine birds, representing previously underappreciated hosts (Mironenko et al., 2026; Muzyka et al., 2025). Consequently, future cross-species transmission of influenza viruses from wild or domestic birds to cattle in Ukraine cannot be excluded, particularly in light of the ongoing global changes in influenza virus ecology and the continued evolution of HPAI H5 viruses.

The ongoing HPAI H5N1 epizootic in dairy cattle in the United States further emphasizes the importance of these findings. Despite extensive investigations, the precise circumstances under which the virus first crossed the species barrier from wild birds to cattle remain unclear. As additional data become available, new questions continue to emerge, while fundamental aspects of virus emergence, host adaptation, transmission dynamics, and long-term maintenance in cattle populations remain incompletely understood (Nguyen et al., 2025). Consequently, investigations of novel susceptible hosts and potential reservoirs of influenza A viruses, particularly highly pathogenic avian influenza viruses, are essential for the early detection of emerging

epizootic and zoonotic threats, improved risk assessment, and strengthened pandemic preparedness.

Future research should focus on expanding the ongoing serological surveillance of ruminants in Ukraine by increasing both the geographical coverage and sample size. Surveillance should include animals from large commercial dairy operations as well as smallholder and backyard farms. In addition to serological testing, future studies should incorporate virological and molecular approaches to enable direct detection, isolation, and genetic characterization of influenza viruses. The integration of serological, virological, and molecular data will provide a more comprehensive assessment of the occurrence of influenza A viruses in ruminants in Ukraine, facilitate identification of potential sources of infection, and improve our understanding of the epizootiological and zoonotic significance of these viruses.

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Declaration of competing interest. The authors declare that they have no conflict of interest.

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