Part 2. Biosafety

UDC 619:616.98-036.22:578.832.1A.083.33:[636.1+599.723/.73](477) DOI 10.36016/JVMBBS-2024-10-3-5

SEROLOGICAL MONITORING OF INFLUENZA A AMONG WILD AND DOMESTIC UNGULATES IN UKRAINE

Rula **O.** M. [1], Muzyka N. M. [1], Drozhzhe Zh. M. [2],
Pishchanskyi **O.** V. [2], Stegniy B. T. [1], Muzyka D. V. [1]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: dmuzyka77@gmail.com
[2] State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine

Summary. The article provides a brief historical background of equine influenza, the spread of this disease worldwide, and the current epizootic situation. The results of serological monitoring by ELISA of wild and domestic ungulates from different farms and regions of Ukraine for the presence of antibodies to influenza A viruses are presented. Blood serum samples from 372 domestic horses and 32 wild ungulates were tested. Samples from animals collected in 2023 and 2024 and archival blood serum samples from 2021 were used and tested according to the manufacturer's instructions using ELISA test systems manufactured by IDEXX, INGEZIM, and IDVet. The data obtained indicate a fairly active circulation of influenza A viruses in populations of unvaccinated domestic horses. The circulation was established not only in recent years (2023–2024, seroprevalence from 10% to 100%), but was observed earlier, as evidenced by the detection of 60.9% of positive samples in samples collected in 2021. In addition, two out of three positive samples were found in wild horses from Kherson Region, which indicates the circulation of influenza A virus among wild animals and requires further investigation. The results correlate with the worsening of the epidemiological situation regarding influenza in animals in Europe. The subsequent phase of the research is serotyping, which involves determining the presence of antibodies to specific virus subtypes by hemagglutinin

Keywords: seroprevalence, equine influenza, enzyme-linked immunosorbent assay

Introduction. Equine influenza (EI) is an acute, highly contagious viral disease of horses, donkeys, mules, and other ungulates that is associated with severe respiratory disorders in animals. Today, the disease is widespread in Europe and North America, and sporadic outbreaks of equine influenza have been reported in Africa, Asia, Australia, and South America (Bryant et al., 2011; Chambers, 2022). From an economic standpoint, equine influenza is regarded as one of the most significant respiratory diseases affecting horses and other ungulates (Olguin-Perglione and Barrandeguy, 2021). At the same time, the disease should be considered a potential threat to human and other animal health. The causative agent of EI, the equine influenza virus (EIV), is an RNA-containing virus belonging to the Orthomyxoviridae family and is a typical representative of the influenza type A virus (Singh, 1994; Bryant et al., 2009; Zhang et al., 2021). Influenza A viruses are currently the most common influenza viruses, primarily infecting humans, horses, birds, and pigs (Cauldwell et al., 2014). They therefore pose a significant threat to both animal and human health. This is particularly important in the context of the worsening epizootic situation with avian influenza, including highly pathogenic influenza, and its ability to cross the interspecies barrier. Cases of infection with avian influenza virus in domestic and wild carnivorous mammals (cats, dogs, foxes, fur-bearing animals) recorded in many European countries (Poland, Finland, etc.) in 2022–2024 (Domańska-Blicharz et al., 2023; WHO, 2023; Lindh et al., 2023; Tammiranta et al., 2023), as well as recent cases in the United States of cows infected with avian influenza virus (highly pathogenic avian influenza virus of subtype H5N1) with subsequent spread in several states and human infection from sick cows (Burki, 2024; Oguzie et al., 2024; Eisfeld et al., 2024), have increased concerns about the next pandemic, which could potentially be caused by a zoonotic influenza virus. It is also necessary to remember that new types of influenza viruses are emerging, the role of which as a dangerous pathogen for human health is still unclear. For example, a new type of influenza virus is the influenza D virus, which has recently been detected in farm animals — cattle, pigs, and horses in different parts of the world (Yu, Li and Wang, 2021; Skelton and Huber, 2022; Nedland et al., 2018).

Concerning the peculiarities of equine influenza as an infectious disease, the spread of EI is facilitated by the

uncontrolled movement of animals from their places of residence, stress factors in the keeping of horses at show or sale grounds, and the absence of quarantine measures and preventive vaccination against influenza. Equine influenza is highly seasonal with a peak in the winter and spring (Pusterla et al., 2011, 2015; Vaala et al., 2019). However, it should be remembered that EI can occur in any month of the year (Chappell et al., 2023). At high incidence rates (up to 100%), mortality can be as high as 2% (Bryant et al., 2011; Chambers, 2022; Pusterla et al., 2015; Chappell et al., 2023; Rodriguez et al., 2018; Sack et al., 2019; Oladunni et al., 2021; Paillot et al., 2016), except in donkeys, which can have high mortality rates (up to 30%) at high incidence rates (up to 100%) (Chambers, 2022; Waghmare et al., 2010). The severity of the disease depends on the pathogenicity of the EI virus, the immune status of the animals, and their age — although EI is usually described as a disease of young horses and donkeys, the highest rate of positive PCR results is observed in the age groups 1–4 years and 5–9 years (Chappell et al., 2023; Landolt, 2014; Paillot, 2014). It should also be noted that in adults, death is usually a consequence of the general condition of the body and/or secondary bacterial infection, leading to complications and the development of pleurisy and pneumonia (Sarasola et al., 1992; Liu, 1993; Kästner et al., 1999; Anzai et al., 2000; Muranaka et al., 2012). The animal's sex also plays a role — females are more likely to be infected than males (Chappell et al., 2023).

Historically, the first documented clinical case of EI was reported in the United States in 1872. High incidence (100%) and low mortality (up to 2%) were observed in horses. The second fact is the report that this pathogen could cause influenza infection in poultry in the United States in 1874 (Morens and Taubenberger, 2010). In fact, the first characterized EI virus was isolated in Czechoslovakia in 1956 (A/equine/1/Prague/56, H7N7, equi-1). It did not have highly pathogenic properties, but an outbreak caused by this virus was reported in Italy 13 years later. Subsequently, the virus of this subtype was isolated in India in 1987, in Egypt in 1989, and in Mongolia in 2011 (Singh, 1994; Ismail et al., 1990; Yondon et al., 2013). Another subtype of influenza virus that causes disease in horses is the H3N8, equi-2 virus. This virus was first detected in 1963 during a large epizootic of equine respiratory disease in Miami, Florida (USA), and since then this subtype has circulated continuously in equine populations causing outbreaks of EI worldwide (Waddell, Teigland and Sigel, 1963; Cullinane and Newton, 2013; Daly et al., 2011; Singh et al., 2018; Scholtens et al., 1964).

In the context of the One Health concept and the risks of new zoonotic influenza viruses emerging, it is important to note the detection of viruses in horses that had connections with viruses of other subtypes obtained from different animals, as well as instances of the influenza virus crossing the interspecies barrier. In China, a significant outbreak of H3N8 influenza in horses and donkeys in 1989 was caused by the 'avian-origin' virus (A/equine/Jilin/89) (Webster and Yuanji, 1991; Murcia, Wood and Holmes, 2011). This outbreak led to the infection of 20,000 horses and the death of 400 animals. Similarly, in Mongolia, it was reported that 97 out of 585 tested horses were seropositive for the H3N8 avian influenza virus (Zhu et al., 2019). Additionally, a highly pathogenic avian influenza virus, H5N1, was isolated from clinically ill donkeys in Egypt (Abdel-Moneim, Abdel-Ghany and Shany, 2010).

The authors suggest that avian influenza may be more prevalent among horses and not cause clinical signs, which makes the disease more difficult to detect. However, in the majority of cases, interspecies transmission of this virus typically results in limited further transmission to a new host. Chinese scientists reported that during the surveillance of pigs in 2005 and 2006, they isolated viruses from them. The closest neighbors of these viruses in all eight gene segments were EI viruses from Europe, isolated in 1991–1993 (Tu et al., 2009). Furthermore, H3N8 equine influenza has been isolated from camels, pigs, and infected cats in experimental conditions (Yondon et al., 2014; Su et al., 2014; Tu et al., 2009).

It is also important to note the role of horses in maintaining existing and forming new reservoirs of influenza A viruses, as evidenced by the interspecies transmission of the H3N8 subtype H3N8 virus to dogs in the late 1990s. This resulted in the formation of a separate canine influenza cluster responsible for several outbreaks of acute respiratory infections in dogs. For several years, there was persistent circulation of this virus in dog populations in the United States and sporadic cases of infection among dogs in the United Kingdom and Australia (Parrish, Murcia, and Holmes, 2015; Gibbs and Anderson, 2010; Crawford et al., 2005; Daly et al., 2008; Crispe et al., 2011; Newton et al., 2007).

It is important to consider the potential for human infection with equine influenza. The first documented outbreak of EI in a population of people in contact with horses was reported in 1957 in Kharkiv (Ukraine), and the diagnosis was confirmed serologically (Gaidamaka et al., 1959; Xie et al., 2016), indicating a significant risk of virus exchange between horses and humans.

Regarding the current epizootic situation in the world. Over the past decade (2010–2021), numerous outbreaks of EIV infection have been reported in many countries on different continents. The increase in EIV outbreaks has been reported not only in North America, especially in the United States, where the disease is endemic, but also in Europe, Africa, Asia, and South America (Oladunni et al., 2021). In the United States, outbreaks were reported in 23 states in 2015, 16 states in 2016, 22 states in 2017, and 33 states in 2018–2019 (Sack

et al., 2019; OIE, 2020). It should also be noted that EIV is not a notifiable disease in the United States, so the true picture may be much worse. Europe and the United Kingdom experienced particularly severe outbreaks of EI in 1989, 2003, and 2018–2019. Each outbreak affected approximately 5,000 to 10,000 horses. In Europe in particular, outbreaks have been reported for many years in France, Germany, Ireland, Sweden, and the United Kingdom (Sack et al., 2019). One of the most recent large outbreaks of EI in Europe was reported in 2018–2019, with 228 horses in the United Kingdom, 80 — in Ireland, and 60 — in France (OIE, 2020). These outbreaks occurred in both vaccinated and unvaccinated horses. Until the mid-2000s, EIV outbreaks were rarely reported in Africa, but in 2018–2019 they were reported in many African countries, affecting both horses and donkeys (OIE, 2020; Shittu et al., 2020; Diallo et al., 2021). As a result, more than 66,000 horses and donkeys died in Burkina Faso, Chad, Cameroon, Gambia, Ghana, Mali, Niger, Nigeria, and Senegal. Outbreaks of EIV have also been reported in Asia, South America, and the Middle East (Sack et al., 2019; Oladunni et al., 2021; Motoshima et al., 2011).

The spread of EI among humans is supported by recent serologic studies in Australia, Mongolia, and the United States. Antibodies to H3N8 EIV were detected in 3–36% of human participants in these studies (Burnell et al., 2014; Khurelbaatar et al., 2014; Larson et al., 2015).

As far as the equine influenza epidemic in Ukraine is concerned, the disease is not officially registered today, but there is no data on the surveillance of horses or other animals.

Thus, the above-mentioned facts about the circulation of equine influenza viruses in the world, the concern about the possible emergence of a new pandemic zoonotic virus due to reassortment and crossing of the interspecies barrier, as well as the lack of up-to-date information about the epizootic situation regarding equine influenza in Ukraine, prompted us to start research on the circulation of influenza A viruses among mammals, including horses, in Ukraine.

The first stage of our research aimed to conduct serological monitoring of wild and domestic ungulates from different farms and different regions of Ukraine for the presence of antibodies to influenza A viruses.

Materials and methods. The study was conducted in the Department of Poultry Diseases and Molecular Diagnostics of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

Sampling and preparation of blood sera from animals were performed according to classical methods. Blood serum samples from 372 domestic horses and 32 wild ungulates were tested. The study used samples from animals collected in 2023 and 2024, as well as archival blood serum samples from 2021. The list of samples used in the study is shown in Table 1.

Table 1 — List of selected blood serum samples from ungulates

Year of sampling	Animal species	Region	Number of surveyed farms	Total number of samples collected
2021 (archive samples)	Horse (*Equus ferus caballus*)	Poltava	1	23
	Sika deer (*Cervus nippon*)	Kherson	1	7
	Przewalski's horse (*Equus ferus przewalskii*)		1	3
	Saiga (*Saiga tatarica*)		1	2
2023	Horse	Kharkiv	6	56
		Vinnytsia	1	20
		Dnipropetrovsk	1	10
		Zaporizhzhia	1	20
		Sumy	1	20
		Lviv	2	30
		Volyn	1	20
		Ternopil	1	20
		Ivano-Frankivsk	1	13
2024	Horse	Cherkasy	1	10
		Chernihiv	1	10
		Chernivtsi	1	10
		Zhytomyr	1	10
		Odesa	1	10
		Dnipropetrovsk	1	10
		Rivne	1	10
		Zakarpattia	1	10

Table 1 — continuation

Year of sampling	Animal species	Region	Number of surveyed farms	Total number of samples collected
2024	Horse	Khmelnytskyi	1	10
		Kharkiv	1	10
		Kherson	1	10
		Kirovohrad	1	10
		Kyiv	1	10
		Poltava	1	10
	Mouflon (*Ovis gmelini*)	Lviv	1	20

Blood sera from ungulates were tested for the presence of antibodies to Influenza A virus by ELISA using the following test systems: IDEXX Influenza A Ab Test ELISA (USA), INGEZIM Influenza A, manufactured by Ingenasa (Spain), and IDVet ID Screen Influenza A Antibody Competition Multi-species-FLUACA (France). The ELISA was performed, the reaction was recorded, and the results were interpreted according to the instructions of the test system manufacturers. All studies were performed following good laboratory practice and in compliance with all biosafety and biosecurity requirements.

Results. The results of serologic studies in domestic horses are shown in Tables 2 and 3.

Table 2 — Results of the ELISA test for the presence of antibodies to influenza A in horses from farms in different regions of Ukraine in 2021 and 2023

No. of the farm	Number of samples			Seropre-valence, %
	total	positive	negative	
Archive (2021), Poltava Region				
1	23	14	9	60.9
2023, Kharkiv Region				
2	10	6	4	60.0
3	10	4	6	40.0
4	10	0	10	0.0
5	10	0	10	0.0
6	10	7	3	70.0
7	6	4	2	66.6
2023, Vinnytsia Region				
8	20	2	18	10.0
2023, Dnipripetrovsk Region				
9	10	10	0	100.0
2023, Zaporizhzhia Region				
10	20	0	20	0.0
2023, Sumy Region				
11	20	0	20	0.0
2023, Lviv Region				
12	20	0	20	0.0
13	10	10	0	100.0
2023, Volyn Region				
14	20	4	16	20.0
2023, Ternopil Region				
15	20	4	16	20.0
2023, Ivano-Frankivsk Region				
16	13	2	11	15.4
Total	232	67	165	2.9

Table 3 — Results of the ELISA test for the presence of antibodies to influenza A in horses from farms in different regions of Ukraine in 2024

No. of the farm	Number of samples			Seropre-valence, %
	total	positive	negative	
Cherkasy Region				
1	10	1	9	10.0
Chernihiv Region				
2	10	10	0	100.0
Chernivtsi Region				
3	10	0	10	0.0
Zhytomyr Region				
4	10	0	10	0.0
Odesa Region				
5	10	2	8	20.0
Dnipropetrovsk Region				
6	10	2	8	20.0
Rivne Region				
7	10	1	9	10.0
Zakarpattia Region				
8	10	5	5	50.0
Khmelnytskyi Region				
9	10	2	8	20.0
Kharkiv Region				
10	10	8	2	80.0
Kherson Region				
11	10	1	9	10.0
Kirovohrad Region				
12	10	0	10	0.0
Kyiv Region				
13	10	3	7	30.0
Poltava Region				
14	10	4	6	40.0
Total	140	39	101	27.9

Studies on the presence of antibodies to the influenza A virus in horses were conducted in 16 farms in different regions of Ukraine, all samples were taken from animals older than one year of working productivity. Table 2 illustrates that the percentage of positive samples in horses from different farms ranged from 10–20% (4 farms) to 40–100% (7 farms) in 2021 and 2023. Antibodies were not detected in horses from 5 of the 16 farms studied.

In 2024 antibodies to influenza A were not detected only in animals from three farms in Zhytomyr, Kirovohrad and Chernivtsi regions, in all other farms the percentage of positive samples ranged from 10 to 100%.

Blood sera from wild ungulates of the following species were also tested: sika deer, Przewalski's horse, saiga, and mouflon. All animals were over one year old. The results are shown in Table 4.

Table 4 — Results of the ELISA test for the presence of antibodies to influenza A in wild ungulates

Animal species	Number of samples			Seropre-valence, %
	total	positive	negative	
2021, Kherson Region				
Sika deer	7	0	7	0.0
Przewalski's horse	3	2	1	66.6
Saiga	2	0	2	0.0
2024, Lviv Region				
Mouflon	20	0	20	0.0

As illustrated in Table 4, the analysis revealed the presence of antibodies to the influenza A virus in two out of three samples from Przewalski's horses (Kherson Region).

Discussion. Previously, the issue of equine influenza was regarded as an economic concern. For many years, two subtypes of the influenza A virus (H7N7 and H3N8) have been closely associated with respiratory diseases in horses, resulting in significant economic losses due to the treatment and prevention of the disease (Singh, 1994; Waddell, Teigland and Sigel, 1963). Given the recent instances of interspecies transmission, monitoring the circulation of influenza viruses among mammalian hosts, including horses, has become a priority for the early detection of new reassortant viruses.

Monitoring studies, particularly serological studies, are an effective tool for controlling the epizootic status of equine influenza in a specific animal population. The results of serological studies can provide up-to-date information on the potential circulation of the virus in a herd of animals or establish the strength of group immunity following preventive vaccinations. In light of the ongoing uncertainty regarding the equine influenza situation in Ukraine, our initial research phase entailed conducting serological monitoring of domestic, wild horses, and other ungulates to ascertain the presence of specific antibodies to influenza A viruses.

Serological surveillance in Ukraine confirmed the circulation of influenza A viruses among unvaccinated domestic horses. According to the ELISA results, the percentage of positive horses ranged from 10% to 100% in different farms. Such a high seroprevalence is not surprising, although it may vary in different countries and regions. Numerous studies have been conducted worldwide on the seroprevalence of influenza in horses and donkeys, and this figure is very variable (Baydar et al., 2023). For example, the seropositivity of horses to influenza is 38% in Mexico (Blitvich et al., 2010), 11% in Pakistan (Sajid et al., 2013), and 44.7% in Brazil (Daly et al., 2021).

On the other hand, the seroprevalence in five different regions of Turkey in a study of more than 600 horses was 31% (Ataseven and Daly, 2007), and according to the results of other researchers from the same country, it was 3.03%.

Only New Zealand and Iceland, with their large horse populations, have remained EIV-free. Some countries, including Australia and South Africa, have eradicated EIV after past outbreaks. However, EIV is generally considered an enzootic in Europe, the Americas, and Asia (Lim et al., 2023). With its almost worldwide distribution, this highly contagious infection can lead to infection through direct and indirect contact and sometimes have a subclinical course.

Conclusions. Our data in Ukraine indicate that influenza A viruses are currently circulating among unvaccinated domestic horses. This circulation has been observed not only in recent years (2023–2024), but also earlier, as evidenced by the detection of 60.9% of positive samples in samples collected in 2021. Furthermore, two out of three tested samples from wild horses in Kherson Region were positive, indicating the potential circulation of the influenza A virus among wild animals. Further investigation is required to confirm this. The data obtained correlate with the worsening of the epizootic situation regarding influenza in Europe among animals. The detection of specific antibodies to the influenza virus in wild ungulates is a notable finding that requires further investigation. Additionally, the subsequent phase of research is serotyping, which involves determining the presence of antibodies to specific virus subtypes by hemagglutinin.

Acknowledgements. The authors would like to express their gratitude to the National Research Foundation of Ukraine for providing financial support for the research project No. 2021.01/0006 'Study of the circulation of zoonotic influenza A viruses in a natural reservoir, assessment of their epidemic risks and danger to human health in Ukraine', conducted under the competition 'Science for Security and Sustainable Development of Ukraine'.

References

Abdel-Moneim, A. S., Abdel-Ghany, A. E. and Shany, S. A. (2010) 'Isolation and characterization of highly pathogenic avian influenza virus subtype H5N1 from donkeys', *Journal of Biomedical Science*, 17(1), p. 25. doi: 10.1186/1423-0127-17-25.

Anzai, T., Walker, J. A., Blair, M. B., Chambers, T. M. and Timoney, J. F. (2000) 'Comparison of the phenotypes of *Streptococcus zooepidemicus* isolated from tonsils of healthy horses and specimens obtained from foals and donkeys with pneumonia', *American Journal of Veterinary Research*, 61(2), pp. 162–166. doi: 10.2460/ajvr.2000.61.162.

Ataseven, V. S. and Daly, J. M. (2007) 'Seroepidemiology of equine influenza virus infection in Turkey', *Turkish Journal of Veterinary & Animal Sciences*, 31(3), 198–202. Available at: https://journals.tubitak.gov.tr/veterinary/vol31/iss3/9.

Baydar, E., Aydogdu, U., Utuk, A. E., Kaya, F., TiMurkan, O., Erol, U. and Babur, C. (2023) 'Occurrence of the selected parasites and viral infections in horses and donkeys in Turkey', *Medycyna Weterynaryjna*, 79(2), pp. 6743–2023. doi: 10.21521/mw.6743.

Blitvich, B. J., Ibarra-Juarez, L. A., Cortes-Guzman, A. J., Root, J. J., Franklin, A. B., Sullivan, H. J. and Fernandez-Salas, I. (2010) 'Seroprevalence of equine influenza virus in northeast and southern Mexico', *Veterinary Record*, 166(18), pp. 565–567. doi: 10.1136/vr.b4845.

Bryant, N. A., Rash, A. S., Russell, C. A., Ross, J., Cooke, A., Bowman, S., MacRae, S., Lewis, N. S., Paillot, R., Zanoni, R., Meier, H., Griffiths, L. A., Daly, J. M., Tiwari, A., Chambers, T. M., Newton, J. R. and Elton, D. M. (2009) 'Antigenic and genetic variations in European and North American equine influenza virus strains (H3N8) isolated from 2006 to 2007', *Veterinary Microbiology*, 138(1–2), pp. 41–52. doi: 10.1016/j.vetmic.2009.03.004.

Bryant, N. A., Rash, A. S., Woodward, A. L., Medcalf, E., Helwegen, M., Wohlfender, F., Cruz, F., Herrmann, C., Borchers, K., Tiwari, A., Chambers, T. M., Newton, J. R., Mumford, J. A. and Elton, D. M. (2011) 'Isolation and characterisation of equine influenza viruses (H3N8) from Europe and North America from 2008 to 2009', *Veterinary Microbiology*, 147(1–2), pp. 19–27. doi: 10.1016/j.vetmic.2010.05.040.

Burki, T. (2024) 'Avian influenza in cattle in the USA', *The Lancet Infectious Diseases*, 24(7), pp. e424–e425. doi: 10.1016/S1473-3099(24)00369-4.

Burnell, F. J., Holmes, M. A., Roiko, A. H., Lowe, J. B., Heil, G. L., White, S. K. and Gray, G. C. (2014) 'Little evidence of human infection with equine influenza during the 2007 epizootic, Queensland, Australia', *Journal of Clinical Virology*, 59(2), pp. 100–103. doi: 10.1016/j.jcv.2013.11.011.

Cauldwell, A. V., Long, J. S., Moncorgé, O. and Barclay, W. S. (2014) 'Viral determinants of influenza A virus host range', *Journal of General Virology*, 95(6), pp. 1193–1210. doi: 10.1099/vir.0.062836-0.

Chambers, T. M. (2022) 'Equine influenza', *Cold Spring Harbor Perspectives in Medicine*, 12(1), p. a038331. doi: 10.1101/cshperspect.a038331

Chappell, D. E., Barnett, D. C., James, K., Craig, B., Bain, F., Gaughan, E., Schneider, C., Vaala, W., Barnum, S. M. and Pusterla, N. (2023) 'Voluntary Surveillance Program for Equine Influenza Virus in the United States during 2008–2021', *Pathogens*, 12(2), p. 192. doi: 10.3390/pathogens12020192.

Crawford, P. C., Dubovi, E. J., Castleman, W. L., Stephenson, I., Gibbs, E. P. J., Chen, L., Smith, C., Hill, R. C., Ferro, P., Pompey, J., Bright, R. A., Medina, M.-J., Influenza Genomics Group, Johnson, C. M., Olsen, C. W., Cox, N. J., Klimov, A. I., Katz, J. M. and Donis, R. O. (2005) 'Transmission of equine influenza virus to dogs', *Science*, 310(5747), pp. 482–485. doi: 10.1126/science.1117950.

Crispe, E., Finlaison, D., Hurt, A. and Kirkland, P. (2011) 'Infection of dogs with equine influenza virus: Evidence for transmission from horses during the Australian outbreak', *Australian Veterinary Journal*, 89(s1), pp. 27–28. doi: 10.1111/j.1751-0813.2011.00734.x.

Cullinane, A. and Newton, J. R. (2013) 'Equine influenza — A global perspective', *Veterinary Microbiology*, 167(1–2), pp. 205–214. doi: 10.1016/j.vetmic.2013.03.029.

Daly, J. M., Blunden, A. S., MacRae, S., Miller, J., Bowman, S. J., Kolodziejek, J., Nowotny, N. and Smith, K. C. (2008) 'Transmission of equine influenza virus to English foxhounds', *Emerging Infectious Diseases*, 14(3), pp. 461–464. doi: 10.3201/eid1403.070643.

Daly, J. M., MacRae, S., Newton, J. R., Wattrang, E. and Elton, D. M. (2011) 'Equine influenza: A review of an unpredictable virus', *The Veterinary Journal*, 189(1), pp. 7–14. doi: 10.1016/j.tvjl.2010.06.026.

Daly, J. M., Megid, J., Langoni, H., De Nardi Júnior, G. and Ribeiro, M. G. (2021) 'Retrospective serological survey for influenza in horses from Brazil', *Brazilian Journal of Microbiology*, 52(1), pp. 461–466. doi: 10.1007/s42770-020-00398-8.

Diallo, A. A., Souley, M. M., Issa Ibrahim, A., Alassane, A., Issa, R., Gagara, H., Yaou, B., Issiakou, A., Diop, M., Ba Diouf, R. O., Lo, F. T., Lo, M. M., Bakhoum, T., Sylla, M., Seck, M. T., Meseko, C., Shittu, I., Cullinane, A., Settypalli, T. B. K., Lamien, C. E., Dundon, W. G. and Cattoli, G. (2021) 'Transboundary spread of equine influenza viruses (H3N8) in West and Central Africa: Molecular characterization of identified viruses during outbreaks in Niger and Senegal, in 2019', *Transboundary and Emerging Diseases*, 68(3), pp. 1253–1262. doi: 10.1111/tbed.13779.

Domańska-Blicharz, K., Świętoń, E., Świątalska, A., Monne, I., Fusaro, A., Tarasiuk, K., Wyrostek, K., Styś-Fijoł, N., Giza, A., Pietruk, M., Zecchin, B., Pastori, A., Adaszek, Ł., Pomorska-Mól, M., Tomczyk, G., Terregino, C. and Winiarczyk, S. (2023) 'Outbreak of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus in cats, Poland, June to July 2023', *Eurosurveillance*, 28(31), p. 2300366. doi: 10.2807/1560-7917.ES.2023.28.31.2300366.

Eisfeld, A. J., Biswas, A., Guan, L., Gu, C., Maemura, T., Trifkovic, S., Wang, T., Babujee, L., Dahn, R., Halfmann, P. J., Barnhardt, T., Neumann, G., Suzuki, Y., Thompson, A., Swinford, A. K., Dimitrov, K. M., Poulsen, K. and Kawaoka, Y. (2024) 'Pathogenicity and transmissibility of bovine H5N1 influenza virus', *Nature*, 633(8029), pp. 426–432. doi: 10.1038/s41586-024-07766-6.

Gaidamaka, M. G., Vaganov, G. P., Dromashko, A. S., Shvetskava, B. D. and Fyadina, D. D. (1959) 'Disease of the upper respiratory tract in horses following the human influenza epidemic of 1957', *Bulletin of the World Health Organization*, 20(2–3), pp. 505–508. PMID: 13651931.

Gibbs, E. P. J. and Anderson, T. C. (2010) 'Equine and canine influenza: A review of current events', *Animal Health Research Reviews*, 11(1), pp. 43–51. doi: 10.1017/S1466252310000046.

Ismail, T. M., Sami, A. M., Youssef, H. M. and Abou Zaid, A. A. (1990) 'An outbreak of Equine influenza type 1 in Egypt in 1989', *Veterinary Medical Journal Giza*, 38 (2), pp. 195–206.

Kästner, S. B. R., Haines, D. M., Archer, J. and Townsend, H. G. G. (1999) 'Investigations on the ability of clenbuterol hydrochloride to reduce clinical signs and inflammation associated with Equine influenza A infection', *Equine Veterinary Journal*, 31(2), pp. 160–168. doi: 10.1111/j.2042-3306.1999.tb03810.x.

Khurelbaatar, N., Krueger, W. S., Heil, G. L., Darmaa, B., Ulziimaa, D., Tserennorov, D., Baterdene, A., Anderson, B. D. and Gray, G. C. (2014) 'Little evidence of avian or equine influenza virus infection among a cohort of Mongolian adults with animal exposures, 2010–2011', *PLoS One*, 9(1), p. e85616. doi: 10.1371/journal.pone.0085616.

Landolt, G. A. (2014) 'Equine influenza virus', *Veterinary Clinics of North America: Equine Practice*, 30(3), pp. 507–522. doi: 10.1016/j.cveq.2014.08.003.

Larson, K. R. L., Heil, G. L., Chambers, T. M., Capuano, A., White, S. K. and Gray, G. C. (2015) 'Serological evidence of Equine influenza infections among persons with horse exposure, Iowa', *Journal of Clinical Virology*, 67, pp. 78–83. doi: 10.1016/j.jcv.2015.04.009.

Lim, S.-I., Kim, M. J., Kim, M.-J., Lee, S.-K., Yang, H.-S., Kwon, M., Lim, E. H., Ouh, I.-O., Kim, E.-J., Hyun, B.-H. and Lee, Y.-H. (2023) 'Assessment of equine influenza virus status in the Republic of Korea from 2020 to 2022', *Viruses*, 15(10), p. 2135. doi: 10.3390/v15102135.

Lindh, E., Lounela, H., Ikonen, N., Kantala, T., Savolainen-Kopra, C., Kauppinen, A., Österlund, P., Kareinen, L., Katz, A., Nokireki, T., Jalava, J., London, L., Pitkäpaasi, M., Vuolle, J., Punto-Luoma, A.-L., Kaarto, R., Voutilainen, L., Holopainen, R., Kalin-Mänttäri, L., Laaksonen, T., Kiviranta, H., Pennanen, A., Helve, O., Laamanen, I., Melin, M., Tammiranta, N., Rimhanen-Finne, R., Gadd, T. and Salminen, M. (2023) 'Highly pathogenic avian influenza A (H5N1) virus infection on multiple fur farms in the South and Central Ostrobothnia regions of Finland, July 2023', *Eurosurveillance*, 28(31), p. 2300400. doi: 10.2807/1560-7917.ES.2023.28.31.2300400.

Liu, K. K. (1993) 'Report of an equine influenza outbreak which occurred in Hong Kong', *Bulletin de l'Office Internationale des Épizooties*, 105(2), pp. 116–117.

Morens, D. M. and Taubenberger, J. K. (2010) 'Historical thoughts on influenza viral ecosystems, or behold a pale horse, dead dogs, failing fowl, and sick swine', *Influenza and Other Respiratory Viruses*, 4(6), pp. 327–337. doi: 10.1111/j.1750-2659.2010.00148.x.

Motoshima, M., Okamatsu, M., Asakura, S., Kuribayashi, S., Sengee, S., Batchuluun, D., Ito, M., Maeda, Y., Eto, M., Sakoda, Y., Sodnomdarjaa, R. and Kida, H. (2011) 'Antigenic and genetic analysis of H3N8 influenza viruses isolated from horses in Japan and Mongolia, and imported from Canada and Belgium during 2007–2010', *Archives of Virology*, 156(8), pp. 1379–1385. doi: 10.1007/s00705-011-1000-5.

Muranaka, M., Yamanaka, T., Katayama, Y., Niwa, H., Oku, K., Matsumura, T. and Oyamada, T. (2012) 'Time-related pathological changes in horses experimentally inoculated with equine influenza A virus', *Journal of Equine Science*, 23(2), pp. 17–26. doi: 10.1294/jes.23.17.

Murcia, P. R., Wood, J. L. N. and Holmes, E. C. (2011) 'Genome-scale evolution and phylodynamics of equine H3N8 influenza A virus', *Journal of Virology*, 85(11), pp. 5312–5322. doi: 10.1128/JVI.02619-10.

Nedland, H., Wollman, J., Sreenivasan, C., Quast, M., Singrey, A., Fawcett, L., Christopher-Hennings, J., Nelson, E., Kaushik, R. S., Wang, D. and Li, F. (2018) 'Serological evidence for the co-circulation of two lineages of influenza D viruses in equine populations of the Midwest United States', *Zoonoses and Public Health*, 65(1). doi: 10.1111/zph.12423.

Newton, R., Cooke, A., Elton, D., Bryant, N., Rash, A., Bowman, S., Blunden, T., Miller, J., Hammond, T., Camm, I. and Day, M. (2007) 'Canine influenza virus: Cross-species transmission from horses', *Veterinary Record*, 161(4), pp. 142–143. doi: 10.1136/vr.161.4.142-a.

Oguzie, J. U., Marushchak, L. V., Shittu, I., Lednicky, J. A., Miller, A. L., Hao, H., Nelson, M. I. and Gray, G. C. (2024) 'Avian influenza A (H5N1) virus among dairy cattle, Texas, USA', *Emerging Infectious Diseases*, 30(7), pp. 1425–1429. doi: 10.3201/eid3007.240717.

OIE (World Organisation for Animal Health) (2020) 'OIE Expert Surveillance Panel on Equine Influenza Vaccine Composition — videoconference, 16 April 2020. Conclusions and recommendations', *The Official Bulletin*, 2020(1), pp. 21–25. doi: 10.20506/bull.2020.1.3134.

Oladunni, F. S., Oseni, S. O., Martinez-Sobrido, L. and Chambers, T. M. (2021) 'Equine influenza virus and vaccines', *Viruses*, 13(8), p. 1657. doi: 10.3390/v13081657.

Olguin-Perglione, C. and Barrandeguy, M. E. (2021) 'An overview of Equine influenza in South America', *Viruses*, 13(5), p. 888. doi: 10.3390/v13050888.

Paillot, R. (2014) 'A systematic review of recent advances in Equine influenza vaccination', *Vaccines*, 2(4), pp. 797–831. doi: 10.3390/vaccines2040797.

Paillot, R., Rash, N., Garrett, D., Prowse-Davis, L., Montesso, F., Cullinane, A., Lemaitre, L., Thibault, J.-C., Wittreck, S. and Dancer, A. (2016) 'How to meet the last OIE Expert Surveillance Panel Recommendations on Equine Influenza (EI) Vaccine Composition: A review of the process required for the recombinant Canarypox-based EI vaccine', *Pathogens*, 5(4), p. 64. doi: 10.3390/pathogens5040064.

Parrish, C. R., Murcia, P. R. and Holmes, E. C. (2015) 'Influenza virus reservoirs and intermediate hosts: dogs, horses, and new possibilities for influenza virus exposure of humans', *Journal of Virology*, 89(6), pp. 2990–2994. doi: 10.1128/JVI.03146-14.

Pusterla, N., Kass, P. H., Mapes, S., Johnson, C., Barnett, D. C., Vaala, W., Gutierrez, C., McDaniel, R., Whitehead, B. and Manning, J. (2011) 'Surveillance programme for important equine infectious respiratory pathogens in the USA', *Veterinary Record*, 169(1), pp. 12–12. doi: 10.1136/vr.d2157.

Pusterla, N., Kass, P. H., Mapes, S., Wademan, C., Akana, N., Barnett, C., MacKenzie, C. and Vaala, W. (2015) 'Voluntary surveillance program for equine influenza virus in the United States from 2010 to 2013', *Journal of Veterinary Internal Medicine*, 29(1), pp. 417–422. doi: 10.1111/jvim.12519.

Rodriguez, L., Reedy, S., Nogales, A., Murcia, P. R., Chambers, T. M. and Martinez-Sobrido, L. (2018) 'Development of a novel equine influenza virus live-attenuated vaccine', *Virology*, 516, pp. 76–85. doi: 10.1016/j.virol.2018.01.005.

Sack, A., Cullinane, A., Daramragchaa, U., Chuluunbaatar, M., Gonchigoo, B. and Gray, G. C. (2019) 'Equine influenza virus — A neglected, reemergent disease threat', *Emerging Infectious Diseases*, 25(6), pp. 1185–1191. doi: 10.3201/eid2506.161846.

Sajid, M., Ahmad, M.-D., Khan, M. A., Anjum, M. A. and Mushtaq, M. H. (2013) 'Investigation of equine influenza virus in two geographical regions of Pakistan', *Tropical Animal Health and Production*, 45(2), pp. 693–694. doi: 10.1007/s11250-012-0247-5.

Sarasola, P., Taylor, D., Love, S. and McKellar, Q. (1992) 'Secondary bacterial infections following an outbreak of equine influenza', *Veterinary Record*, 131(19), pp. 441–442. doi: 10.1136/vr.131.19.441.

Scholtens, R. G., Steele, J. H., Dowdle, W. R., Yarbrough, W. B. and Robinson, R. Q. (1964) 'U.S. epizootic of Equine influenza, 1963', *Public Health Reports (Washington, D.C.: 1896)*, 79(5), pp. 393–402. PMID: 14153655.

Shittu, I., Meseko, C. A., Sulaiman, L. P., Inuwa, B., Mustapha, M., Zakariya, P. S., Muhammad, A. A., Muhammad, U., Atuman, Y. J., Barde, I. J., Zecchin, B., Quaranta, E. G., Shamaki, D., Alabi, O., Monne, I., Fusaro, A. and Joannis, T. M. (2020) 'Fatal multiple outbreaks of Equine influenza H3N8 in Nigeria, 2019: The first introduction of Florida clade 1 to West Africa', *Veterinary Microbiology*, 248, p. 108820. doi: 10.1016/j.vetmic.2020.108820.

Singh, G. (1994) 'Characterization of A/eq-1 virus isolated during the equine influenza epidemic in India', *Acta Virologica*, 38(1), pp. 25–26. PMID: 8067310.

Singh, R. K., Dhama, K., Karthik, K., Khandia, R., Munjal, A., Khurana, S. K., Chakraborty, S., Malik, Y. S., Virmani, N., Singh, R., Tripathi, B. N., Munir, M. and Van Der Kolk, J. H. (2018) 'A comprehensive review on equine influenza virus: Etiology, epidemiology, pathobiology, advances in developing diagnostics, vaccines, and control strategies', *Frontiers in Microbiology*, 9, p. 1941. doi: 10.3389/fmicb.2018.01941.

Skelton, R. M. and Huber, V. C. (2022) 'Comparing influenza virus biology for understanding influenza D virus', *Viruses*, 14(5), p. 1036. doi: 10.3390/v14051036.

Su, S., Wang, L., Fu, X., He, S., Hong, M., Zhou, P., Lai, A., Gray, G. and Li, S. (2014) 'Equine Influenza A (H3N8) virus infection in cats', *Emerging Infectious Diseases*, 20(12), pp. 2096–2099. doi: 10.3201/eid2012.140867.

Tammiranta, N., Isomursu, M., Fusaro, A., Nylund, M., Nokireki, T., Giussani, E., Zecchin, B., Terregino, C. and Gadd, T. (2023) 'Highly pathogenic avian influenza A (H5N1) virus infections in wild carnivores connected to mass mortalities of pheasants in Finland', *Infection, Genetics and Evolution*, 111, p. 105423. doi: 10.1016/j.meegid.2023.105423.

Tu, J., Zhou, H., Jiang, T., Li, C., Zhang, A., Guo, X., Zou, W., Chen, H. and Jin, M. (2009) 'Isolation and molecular characterization of equine H3N8 influenza viruses from pigs in China', *Archives of Virology*, 154(5), pp. 887–890. doi: 10.1007/s00705-009-0381-1.

Vaala, W., Barnett, D. C., James, K., Chappell, D., Craig, B., Gaughan, E., Bain, F., Barnum, S. M. and Pusterla, N. (2019) 'Prevalence factors associated with equine influenza virus infection in equids with upper respiratory tract infection from 2008 to 2019', *Association of Equine Practitioners Proceedings*, 65, pp. 385–386. Available at: https://aaep.org/wp-content/uploads/2024/02/Proceedings-65th-Annual-Convention-2019.pdf.

Waddell, G. H., Teigland, M. B. and Sigel, M. M. (1963) 'A new influenza virus associated with equine respiratory disease', *Journal of the American Veterinary Medical Association*, 143, pp. 587–590. PMID: 14077956.

Waghmare, S. P., Mode, S. G., Kolte, A. Y., Babhulkar, N., Vyavahare, S. H. and Patel, A. (2010) 'Equine influenza: An overview', *Veterinary World*, 3(4), pp. 194–197. Available at: https://www.veterinaryworld.org/Vol.3/April/Equine%20Influenza.pdf.

Webster, R. G. and Yuanji, G. (1991) 'New influenza virus in horses', *Nature*, 351(6327), pp. 527–527. doi: 10.1038/351527a0.

WHO (World Health Organization) (2023). *Influenza A(H5N1) in Cats — Poland*. Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON476.

Xie, T., Anderson, B., Daramragchaa, U., Chuluunbaatar, M. and Gray, G. (2016) 'A review of evidence that equine influenza viruses are zoonotic', *Pathogens*, 5(3), p. 50. doi: 10.3390/pathogens5030050.

Yondon, M., Heil, G. L., Burks, J. P., Zayat, B., Waltzek, T. B., Jamiyan, B., McKenzie, P. P., Krueger, W. S., Friary, J. A. and Gray, G. C. (2013) 'Isolation and characterization of H3N8 equine influenza A virus associated with the 2011 epizootic in Mongolia', *Influenza and Other Respiratory Viruses*, 7(5), pp. 659–665. doi: 10.1111/irv.12069.

Yondon, M., Zayat, B., Nelson, M. I., Heil, G. L., Anderson, B. D., Lin, X., Halpin, R. A., McKenzie, P. P., White, S. K., Wentworth, D. E. and Gray, G. C. (2014) 'Equine influenza A (H3N8) virus isolated from Bactrian camel, Mongolia', *Emerging Infectious Diseases*, 20(12), pp. 2144–2147. doi: 10.3201/eid2012.140435.

Yu, J., Li, F. and Wang, D. (2021) 'The first decade of research advances in influenza D virus', *Journal of General Virology*, 102(1), p. 001529. doi: 10.1099/jgv.0.001529.

Zhang, P., Sun, Y., Tan, C., Ling, M., Li, X., Wang, W. and Cong, Y. (2021) 'Preparation and evaluation of virus-like particle vaccine against H3N8 subtype equine influenza', *Microbial Pathogenesis*, 157, p. 104885. doi: 10.1016/j.micpath.2021.104885.

Zhu, H., Damdinjav, B., Gonzalez, G., Patrono, L. V., Ramirez-Mendoza, H., Amat, J. A. R., Crispell, J., Parr, Y. A., Hammond, T., Shiilegdamba, E., Leung, Y. H. C., Peiris, M., Marshall, J. F., Hughes, J., Gilbert, M. and Murcia, P. R. (2019) 'Absence of adaptive evolution is the main barrier against influenza emergence in horses in Asia despite frequent virus interspecies transmission from wild birds', *PLoS Pathogens*, 15(2), p. e1007531. doi: 10.1371/journal.ppat.1007531.