Invest Clin 66(4): 467- 478, 2025 https://doi.org/10.54817/IC.v66n4a09

The ongoing panzootic of avian Influenza A (H5N1) and its potential pandemic threat.

Flor Helene Pujol[1] and José Esparza[2]

[1]Laboratorio de Virología Molecular, CMBC, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela.
[2]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, USA.

Keywords: Avian Influenza; Pandemics; Panzootic; Influenza A Virus, H5N1 Subtype.

Abstract. The influenza virus is one of the most significant pathogens responsible for respiratory infections and is the human pathogen most frequently associated with epidemics and pandemics. The epidemiological record of influenza suggests that future pandemics caused by this virus are inevitable, even though their timing, origin, and severity remain uncertain. This review focuses on the ongoing panzootic of avian influenza A (H5N1), which is currently spreading across much of the globe. The ongoing panzootic of Influenza A (H5N1) clade 2.3.4.4b has spread rapidly worldwide and is causing concern. The virus has already crossed species barriers, infecting multiple mammalian hosts and causing human cases with varying degrees of severity. While sustained human-to-human transmission has not yet occurred, an increasing frequency of spillover events and the emergence of genotypes with mutations associated with mammalian adaptation are of concern. We assess the potential for this panzootic to evolve into a pandemic and examine the critical measures needed for preparedness and prevention, following a One Health approach.

Corresponding author: Flor H. Pujol. Laboratorio de Virología Molecular, CMBC, IVIC, Caracas, Venezuela. Email: fhpujol@gmail.com

La panzootia actual de influenza aviar A (H5N1) y su potencial amenaza pandémica.

Invest Clin 2025; 66 (4): 467 – 478

Palabras clave: Influenza Aviar; Pandemias; Panzootia; Epizootia; Subtipo H5N1 del Virus de la Influenza A.

Resumen. El virus de la influenza es uno de los patógenos más importantes, causante de infecciones respiratorias, y el agente humano más frecuentemente asociado con epidemias y pandemias. El registro epidemiológico de la influenza sugiere que las futuras pandemias causadas por este virus son inevitables, aunque su momento, origen y gravedad siguen siendo inciertos. Esta revisión se centra en la panzootia actual de la influenza aviar A (H5N1), que actualmente se propaga por gran parte del mundo. La panzootia actual del virus de la influenza A (H5N1), del clado 2.3.4.4b, se ha extendido de manera dramática a nivel mundial y está generando gran preocupación. El virus ya ha cruzado las barreras entre especies, provocando infecciones en múltiples hospedadores mamíferos y causando casos humanos con distintos grados de gravedad. Aunque aún no se ha producido una transmisión sostenida de persona a persona, preocupa la creciente frecuencia de eventos de salto interespecie y la aparición de genotipos con mutaciones asociadas a la adaptación en mamíferos. En esta revisión, evaluamos el potencial de esta panzootia para evolucionar hacia una pandemia y examinamos las medidas críticas necesarias para la preparación y la prevención, siguiendo un enfoque de *Una Sola Salud*.

Received: 07-10-2025 Accepted: 17-11-2025

INTRODUCTION

The influenza virus is one of the most significant pathogens responsible for respiratory infections, distinguished not only by the severity of the illnesses it causes but also by its role in numerous historical epidemics and pandemics [1,2].

While aquatic birds are the natural reservoirs of Influenza A viruses (IAV), these viruses can also infect a broad range of mammalian species, including humans. The epidemiological record of influenza suggests that future pandemics caused by this virus are inevitable, even though their timing, origin, and severity remain uncertain. This review focuses on the ongoing panzootic of avian influenza A (H5N1), which is currently spreading across much of the globe. The virus has already crossed species barriers, leading to infections in multiple mammalian hosts and causing human cases with varying degrees of severity [3]. We assess the potential for this panzootic to evolve into a pandemic and examine the critical measures needed for preparedness and prevention.

Avian Influenza

Influenza viruses belong to the family *Orthomyxoviridae* and are enveloped viruses with a segmented, negative-sense RNA genome. Four genera of influenza viruses have been identified: Influenza A, B, C, and D. Influenza A and B viruses possess eight RNA

segments, whereas Influenza C and D viruses typically contain seven segments, although they frequently package eight [4,5]. Influenza A viruses (IAVs) exhibit a broad host range, infecting birds, humans, and various other mammals. In contrast, Influenza B and C viruses primarily infect humans, while Influenza D virus is predominantly found in bovine hosts [4,6]. In humans, influenza disease is caused mainly by viruses from the A and B genera, with IAVs being of particular concern due to their pandemic potential. These genera are distinguished by the antigenic properties of their internal virion proteins, while IAVs are further differentiated into subtypes by the antigenic properties of their two external proteins [4,6].

The segmented nature of the influenza virus genome facilitates frequent reassortment events. This process occurs when a host is co-infected with two different influenza viruses; during replication, genome segments from both viruses can be packaged together into progeny virions [4,7]. Pigs, which are susceptible to multiple influenza virus subtypes, have traditionally been considered key hosts for reassortment and are often referred to as "mixing vessels" [7]. IAVs display substantial antigenic diversity in their two principal surface glycoproteins: hemagglutinin (H) and neuraminidase (N). This variability allows for a binary classification of IAVs into serotypes, comprising 19 H subtypes and 11 N subtypes, with more than 130 distinct subtype combinations identified to date [8,9]. The primary reservoir of this genetic and antigenic diversity is avian IAVs, which have been the source of multiple influenza pandemics—often through reassortment events between avian and human influenza viruses [7].

Human influenza viruses bind preferentially to α-2,6-linked sialic acid receptors on the surface of epithelial cells, whereas avian influenza viruses have a higher affinity for α-2,3-linked sialic acids. These glycosidic linkages play a critical role in host specificity and cross-species transmission of influenza viruses. Consequently, both the variant and structural configuration of sialic acid receptors influence viral binding affinity to the H glycoprotein of influenza viruses [10].

Avian influenza A viruses (AIAVs) can evolve from low pathogenicity (LPAIV) to highly pathogenic forms (HPAIV), a classification based on their virulence in chickens. A defining molecular feature of HPAIV is the presence of a furin cleavage site in the H protein [3]. Proteolytic cleavage of H into HA1 and HA2 subunits is a crucial step in viral replication, triggering a pH-dependent conformational change that exposes the fusion peptide, enabling fusion with endosomal membranes and subsequent release of the viral genome into the host cytoplasm. HPAIVs contain a polybasic cleavage site that allows processing by furin-like proteases, which are expressed in multiple tissues, thereby facilitating systemic viral dissemination [11].

Notably, HPAIVs have emerged multiple times independently from ancestral LPAIVs, but only among viruses of the H5 and H7 subtypes [12]. Since the first emergence of HPAI H5N1 in 1996, nearly 1,000 human infections have been reported across 24 countries, with a case fatality rate of around 50%[13,14].

The influenza pandemics of the past

Over the past three centuries, ten major influenza pandemics have been documented, averaging approximately three per century (Fig. 1). These events have varied widely in intensity, morbidity, and mortality [1].

In the 18th century, three pandemics were recorded. The first, in 1729–1730, had a global reach, with high morbidity but relatively low mortality—a pattern that was repeated in the subsequent 1732–1733 outbreak. The 1781–1782 pandemic was particularly extensive, reportedly infecting 70–80% of the population, though it also exhibited low mortality [1].

The 19th century witnessed two notable pandemics in 1830–1831 and 1833, which differed in severity, with the latter associ-

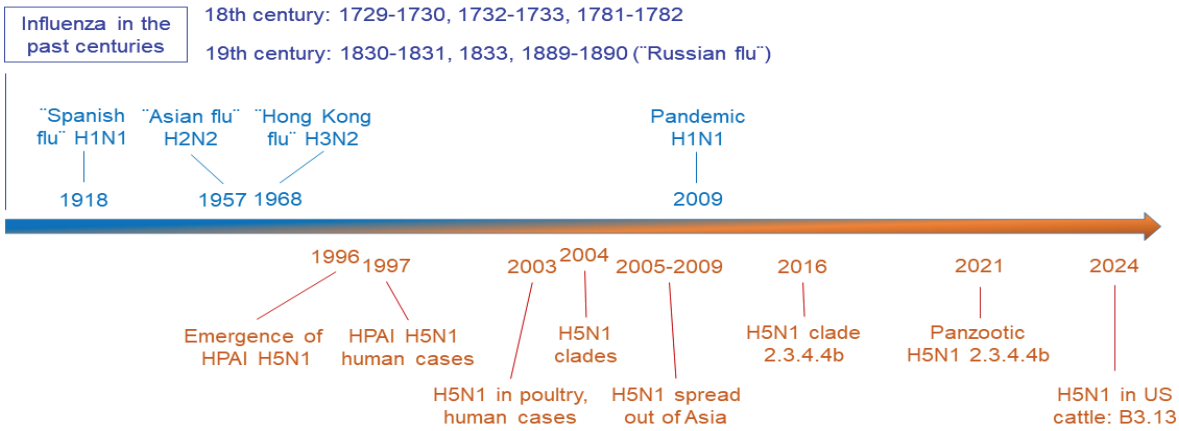

Fig. 1. Major events in human and avian Influenza. The timeline shows the known Influenza pandemics in the past centuries (in blue) and major events in avian influenza over the last two centuries (in orange). The subtype of IAV responsible for the pandemic of the previous two centuries are shown [1,12,18,23,30].

ated with higher mortality. The 1889–1890 pandemic, commonly known as the "Russian flu," was particularly significant because it coincided with the emergence of the germ theory of disease. This conceptual shift—from miasmatic to microbial causation—marked a turning point in public health, paving the way for modern preventive strategies such as improved hygiene and social distancing [1].

The 20th and 21st centuries saw four influenza pandemics, all caused by IAVs. The deadliest of these was the 1918-1919 "Spanish flu," caused by an H1N1 virus. It infected an estimated 500 million people—about 30% of the global population—and resulted in 50 to 100 million deaths over three devastating waves. The impact was magnified by the worldwide disruption and troop movements associated with World War I. Later pandemics included the 1957 "Asian flu" (H2N2) and the 1968 "Hong Kong flu" (H3N2), which were less severe but still responsible for an estimated 1–3 million and 1–4 million deaths, respectively, despite widespread transmission [1].

The only influenza pandemic of the 21st century to date occurred in 2009, caused by a novel H1N1 virus that originated in North America. Although initially feared to rival the severity of the 1918 pandemic, it ultimately proved relatively mild, with an estimated 125,000 to 400,000 deaths worldwide [1].

The panzootic H5N1 clade 2.3.4.4b

In addition to classification by subtype, IAV genome sequences are further categorized based on phylogenetic relationships, including common ancestry and descendant lineages. These classifications include clades (e.g., 1.1, 2.2, 2.3), subclades (e.g., 2.3.2.1c, 2.3.3.4b), lineages (such as Eurasian and American), and genotypes (e.g., A1, B1, B3.13) [3,15,16]. Clades and subclades are defined primarily through phylogenetic analysis of the hemagglutinin (H) gene, reflecting evolutionary divergence. In contrast, genotypes are determined by the constellation of gene segments present in each strain, offering insight into reassortment events and genomic composition [16].

The subclade 2.3.4.4b emerged in 2016 (Fig. 1) in an H5N8 virus in China and subsequently spread across Asia and into Europe. This virus caused an unexpected epidemic peak in 2020/2021 [17]. The 2.3.4.4b H5N8 rearranged with a LPAI H5N1 around 2020, leading to the 2.3.4.4b H5N1. This vi-

rus caused the wave of 2021/2022, the current panzootic, with several human cases, and was the most devastating avian influenza epidemic [17,18]. After this subclade was introduced into North America, several reassortment events with circulating LPAIVs led to the emergence of new genotypes. At least three genotypes have circulated among infected marine birds in South America since 2022, and these genotypes have also shown infectivity and virulence toward marine mammals [19,20].

Both avian- and human-type receptors are present in swine respiratory epithelia. Swine are generally the intermediate hosts involved in the reassortment and adaptation of AIAVs to mammals before spillover to humans. However, new evolutionary pathways may be considered for the emergence of a pandemic, given the frequent infection of many mammalian species (both marine and terrestrial), particularly cattle in the USA[20,21].

Cattle are typically infected with the Influenza D virus but not with IAVs. However, during the current panzootic, the first documented spillover of Influenza A into cows involved an H5N1 virus of genotype B3.13—originally circulating in wild animals (Fig. 1). This genotype is a reassortant between B3.6 and an LPAIV endemic in the United States. Phylogenetic evidence suggests that a single spillover event was responsible for the emergence of this panzootic clade in cattle [22]. In cattle, the primary site of AIAV replication appears to be the udder, as mammary tissue expresses avian-type α-2,3-linked sialic acid receptors. This raises the possibility of virus transmission through unpasteurized milk. The initial spillover event is believed to have occurred in Texas, USA, with subsequent spread to cattle in other states likely facilitated by contaminated milking equipment [18]. Since February 2024, at least two additional spillover events involving a different genotype, D1.1, have been reported in U.S. cattle [23,24]. Alarmingly, the first fatal human case associated with this panzootic in the United States was linked to infection with this genotype [23].

Most human cases of H5N1 infection during the current panzootic have been mild so far. However, this apparent low pathogenicity may be partly attributed to host-related factors, including the young age of many patients and the presence of pre-existing immunity to N1 and other conserved cellular immunity epitopes [25,26]. In contrast, experimental infection of ferrets with the H5N1 genotype B3.13—currently circulating in U.S. cattle—resulted in high lethality [27]. Another study reported reduced mortality in mice infected with a clade 2.3.4.4b H5N1 virus, despite the strain exhibiting high pathogenicity in chickens [28]. Notably, differences in pathogenicity among genotypes within the panzootic clade have also been observed in both teals and poultry [29].

These findings underscore the potential for this lineage's pathogenicity to evolve further through reassortment and mutation. Comprehensive studies are urgently needed to assess the virulence and transmissibility of these viruses in humans. Regardless of the current severity, it is imperative to implement preparedness and response plans now to mitigate the risk of a future influenza pandemic.

Pandemic risk?

The current panzootic caused by Influenza A-H5N1 has been marked by frequent spillover events into a wide range of terrestrial and marine mammalian species, with around 50 species affected to date [3,30,31]. The United States currently reports the highest number of human cases associated with this outbreak, with 70 confirmed infections between 2024 and the end of April 2025 [13]. However, this number remains below the highest annual record of human H5N1 cases, which occurred in 2015, when 145 cases were reported globally, 136 of them in Egypt alone [13].

A prerequisite for the development of a pandemic is that the panzootic influenza

virus acquires the ability not only to infect humans, but also to sustain efficient human-to-human transmission—something that has not occurred to date. Efficient human-to-human transmission of avian influenza viruses likely requires the cumulative acquisition of several key mutations (Fig. 2) [30-33].

H mutations enhancing affinity for human receptors: Adaptation to the human α-2,6-linked sialic acid receptor is critical. Notably, only four mutations were sufficient to render an Influenza A-H5N1 virus transmissible, via respiratory droplets, in ferrets[34]. More recently, a single mutation, Q226L, in the H of bovine H5N1 (genotype B3.13) was shown to switch viral specificity toward mammalian receptors [35]. Three additional mutations have been identified as key contributors to increased human receptor affinity [32].

PB2 mutations enabling polymerase adaptation to mammalian ANP32 proteins: ANP32A and ANP32B, which serve as host cofactors for the viral polymerase, differ between avian and mammalian species. Several mutations have been characterized that allow avian influenza polymerases to utilize mammalian ANP32 proteins [36] efficiently. Some of these mutations have already been detected at low frequency in viruses infecting cattle [22].

Increased viral stability at low pH: Avian influenza viruses generally exhibit reduced stability in acidic environments such as the human upper respiratory tract, posing a barrier to transmission [33].

Host immune history: Pre-existing immunity to seasonal human influenza viruses, whether from prior infection or vaccination, may influence susceptibility and clinical outcomes following exposure to H5N1 viruses [37].

The eventual emergence of a pandemic influenza virus can result not only from the gradual accumulation of key mutations but also, frequently, from reassortment events[20,21]. Traditional "mixing vessels" for such reassortment have been pigs; however, emerging evidence highlights other hosts with high reassortment potential, including humans, minks, ferrets, seals, dogs, cats, and various bird species—particularly turkeys, chickens, quails, and ducks [20,21]. Interestingly, current data suggest that pigs may be less susceptible to the current panzootic IAV than some other mammals [38,39].

Although several key mutations associated with mammalian adaptation have been detected in viruses isolated from infected mammals during this panzootic [21], none of the sequenced viral isolates to date possess the whole constellation of mutations required for efficient human-to-human trans-

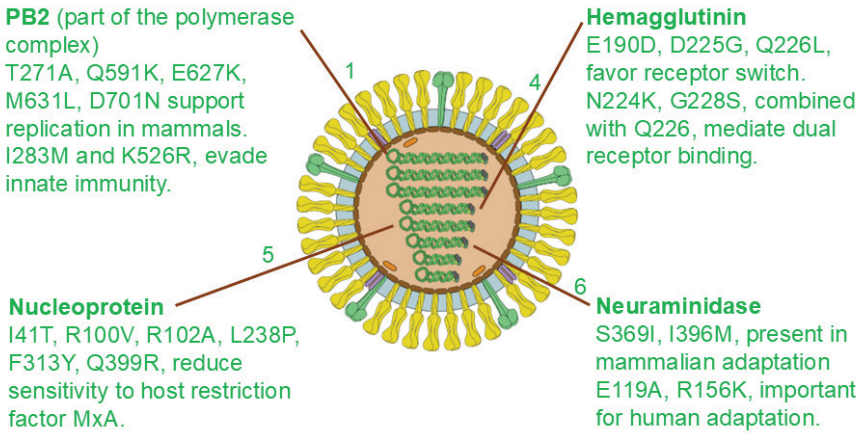

Fig. 2. Major mutations in four of the AIAV associated with efficient mammalian transmission and replication. Mutations in four viral proteins, potentially related to human adaptation, are shown [22,30,32-36].

mission. Nevertheless, the extensive geographic spread of the panzootic clade and its ability to infect a broad range of mammalian hosts raise significant concerns about the potential emergence of a pandemic strain [40].

Preparedness

According to the World Health Organization [41], influenza virus activity is classified into six distinct phases, progressing from circulation limited to animals to widespread human transmission at the global level (Table 1). This framework is divided into two overarching stages: Phases 1–3, which focus on preparedness, and Phases 4–6, which emphasize response and mitigation.

In Phase 1, no influenza viruses circulating among animals have been reported to cause infections in humans. Phase 2 is characterized by an animal influenza virus - circulating among domesticated or wild animals - that has caused human illness and is therefore considered a potential pandemic threat. Phase 3 occurs when an animal or human-animal reassortant influenza virus causes sporadic cases or small clusters of disease in people but does not lead to sustained human-to-human transmission or community-level outbreaks. In Phase 4, human-to-human transmission results in localized community outbreaks, indicating a significant increase in pandemic risk. Phase 5 is marked by community-level outbreaks in at least two countries within one WHO region. In contrast, Phase 6 -defined by community outbreaks in multiple WHO regions - signifies the onset of a global pandemic [41].

Although the global spread of the A (H5N1) panzootic remains a serious concern, no sustained human-to-human transmission has been reported as of May 2025. Consequently, the human influenza epidemic remains classified as Phase 3, highlighting the urgent need to bolster preparedness efforts and prevent escalation to a pandemic. To mitigate this risk, countries must develop and implement coordinated response strategies at both national and international levels, emphasizing proactive preventive measures. Since 2021, the World Health Organization (WHO) has been working with Member States to draft a global treaty on pandemic prevention, preparedness, and response. This treaty was formally adopted by consensus at the 78th World Health Assembly in May 2025. It establishes guidelines for the timely sharing of epidemiological data, genomic analysis of emerging pathogens, and critical information related to potential vaccines and treatments [42].

Table 1. World Health Organization pandemic phases descriptions.

Phase	Description	Estimated probability of pandemia
1	No animal influenza virus reported as causing human cases	Uncertain
2	An animal influenza virus has caused infection in humans	Uncertain
3	An animal or human-animal reassortant virus has caused a small number of human cases but has not resulted in significant human-to-human transmission	Uncertain
4	Human-to-human transmission able to sustain community-level outbreaks	Medium to high
5	Community-level outbreaks in at least two countries in one WHO region	High to certain
6	Community-level outbreaks in an additional WHO region	Pandemic in progress

Adapted from World Health Organization [41].

National preparedness plans should incorporate robust communication strategies to promote public adherence to non-pharmaceutical interventions (NPIs). For influenza, these include individual-level protective behaviors such as staying home when symptomatic, covering coughs and sneezes, and practicing frequent hand hygiene. At the community level, recommended measures may include temporary school closures, suspending childcare services, and canceling mass gatherings during periods of heightened transmission. When a novel pandemic influenza virus emerges, the combined implementation of NPIs and antiviral therapies can significantly reduce transmission rates, particularly in the early stages before a vaccine becomes widely available [41-44].

Pandemic preparedness for influenza depends heavily on early detection of novel strains, particularly those that originate in animal reservoirs through spillover events. Over the past two decades, repeated zoonotic influenza outbreaks have underscored the vital importance of sustained surveillance in both human and animal populations, especially among birds and swine. As noted earlier in this paper, the most pressing current threat is the ongoing panzootic of avian influenza A (H5N1). However, other avian influenza subtypes continue to circulate in more localized contexts. Genetic reassortment during coinfections plays a critical role in the emergence of pandemic-capable strains. While the surface glycoproteins H and N are traditionally viewed as central to influenza infectivity and host specificity, increasing evidence indicates that internal viral genes -particularly those encoding the polymerase complex- also significantly contribute to virulence and disease severity [44]. Since 1952, the World Health Organization has led a global initiative to monitor circulating influenza viruses, enabling a coordinated international response to emerging influenza threats [45].

Given that a future pandemic influenza virus is likely to emerge from an avian influenza strain affecting poultry, control strategies must integrate biosecurity measures, epidemiological surveillance, targeted culling, and vaccination with strain-specific immunogens [46-48]. Culling should be prioritized in outbreaks involving HPAI strains, mainly when rapid transmission occurs within poultry populations. Protective measures for farm workers are also essential to minimize the risk of zoonotic transmission. A similar level of urgency applies to controlling H5N1 infections in other livestock species, such as cattle [49].

During the preparedness phase, early interventions should focus on securing vaccine and antiviral stockpiles [48-51] and on expanding the healthcare system capacity to ensure effective clinical management during a pandemic. Notably, at least three experimental vaccines targeting early H5N1 influenza virus strains have demonstrated the ability to elicit cross-reactive binding and cross-neutralizing antibodies against the HPAI clade 2.3.4.4b in humans, suggesting they could serve as interim protective tools while updated vaccines are developed[52]. Furthermore, a novel H5-based mRNA vaccine has recently been shown to generate a robust adaptive immune response in cattle[53]. Nevert heless, the final formulation of a pandemic influenza vaccine must be tailored to the specific viral strain in circulation at the time of emergence to ensure optimal efficacy.

CONCLUSIONS

The unprecedented epidemiology of the panzootic AIAV (H5N1) clade 2.3.4.4b has already resulted in devastating impacts on poultry and wild mammal populations. It now poses an emerging threat to livestock, further complicating the pre-pandemic landscape. Although several human infections have been reported, the mortality rate to date has been lower than in previous outbreaks. While it remains impossible to predict with certainty when or how a new influ-

enza pandemic might emerge, the current spread of AIAV (H5N1) is deeply concerning and demands urgent preparedness efforts. A coordinated One Health approach—integrating human, animal, environmental health, and, evidently, vaccines—is essential to address this evolving threat effectively. This requires strong collaboration among animal, environmental, and public health sectors to assess risks, strengthen early prevention, coordinate outbreak responses, and develop effective countermeasures. To reduce future pandemic risks, urgent action is needed, not only to protect people at the highest risk of zoonotic infection (such as farm workers) but also to prevent transmission among wild and domestic animals and humans. Efforts should target the underlying factors that enable such outbreaks and ensure rapid risk assessment and response to every zoonotic event [54].

ORCID number of authors

- Flor H. Pujol (FHP): 0000-0001-6086-6883
- José Esparza (JE): 0000-0002-2305-6264

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. **Esparza J**. Lessons from history: What can we learn from 300 years of pandemic flu that could inform the response to COVID-19? Am J Public Health. 2020; 110: 1160-1. *https://doi.org/10.2105/AJPH.2020.305761.*
2. **Taubenberger JK, Kash JC**. Influenza virus evolution, host adaptation, and pandemic formation. Cell Host Microbe. 2010; 7: 440-51. *https://doi.org/10.1016/j.chom.2010.05.009.*
3. **Butt SL, Nooruzzaman M, Covaleda LM, Diel DG**. Hot topic: Influenza A H5N1 virus exhibits a broad host range, including dairy cows. JDS Commun 2024; 5: S13-S19. *https://doi.org/10.3168/jdsc.2024-0638.*
4. **Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, et al**. Influenza. Nat Rev Dis Primers 2018; 4: 3. *https://doi.org/10.1038/s41572-018-0002-y.*
5. **Nakatsu S, Murakami S, Shindo K, Horimoto T, Sagara H, Noda T, Kawaoka Y**. Influenza C and D Viruses Package Eight Organized Ribonucleoprotein Complexes. J Virol 2018; 92: e02084-17. https://doi.org/10.1128/JVI.02084-17.
6. **Sreenivasan CC, Sheng Z, Wang D, Li F**. Host Range, Biology, and Species Specificity of Seven-Segmented Influenza Viruses-A Comparative Review on Influenza C and D. Pathogens 2021; 10: 1583. *https://doi.org/10.3390/pathogens10121583.*
7. **Steel J, Lowen AC**. Influenza A virus reassortment. Curr Top Microbiol Immunol, 2014; 385: 377-401. *https://doi.org/10.1007/82_2014_395.*
8. **Raza MA, Ashraf MA, Amjad MN, Din GU, Shen B, Hu Y**. The peculiar characteristics and advancement in diagnostic methodologies of influenza A virus. Front Microbiol, 2025; 15: 1435384. *https://doi.org/10.3389/fmicb.2024.1435384.*
9. **Karakus U, Mena I, Kottur J, El Zahed SS, Seoane R, Yildiz S, et al**. H19 influenza A virus exhibits species-specific MHC class II receptor usage. Cell Host Microbe. 2024; 32: 1089-1102. e10. *https://doi.org/10.1016/j.chom.2024.05.018*
10. **Zhao C, Pu J**. Influence of Host Sialic Acid Receptors Structure on the Host Specificity of Influenza Viruses. Viruses. 2022; 14: 2141. *https://doi.org/10.3390/v14102141.*
11. **de Bruin ACM, Spronken MI, Bestebroer TM, Fouchier RAM, Richard M**. Conserved Expression and Functionality of Furin between Chickens and Ducks as an Activating Protease of Highly Pathogenic Avian Influenza Virus Hemagglutinins. Microbiol Spectr, 2023; 11: e0460222. *https://doi.org/10.1128/spectrum.04602-22.*

12. **Lycett SJ, Duchatel F, Digard P**. A brief history of bird flu. Philos Trans R Soc Lond B Biol Sci. 2019; 374(1775): 20180257. *https://doi.org/10.1098/rstb.2018.0257.*
13. **Centers for Disease Control and Prevention**. Past Reported Global Human Cases with Highly Pathogenic Avian Influenza A (H5N1) (HPAI H5N1) by Country, 1997-2025. 2025.Accessed on September 25, 2025. *https://www.cdc.gov/bird-flu/php/avian-flu-summary/chart-epi-curve-ah5n1.html.*
14. **Charostad J, Rezaei Zadeh Rukerd M, Mahmoudvand S, Bashash D, Hashemi SMA, Nakhaie M, Zandi K.** A comprehensive review of highly pathogenic avian influenza (HPAI) H5N1: An imminent threat at doorstep. Travel Med Infect Dis, 2023; 55: 102638. *https://doi.org/10.1016/j.tmaid.2023.102638.*
15. **Elsmo EJ, Wünschmann A, Beckmen KB, Broughton-Neiswanger LE, Buckles EL, Ellis J, et al**. Highly Pathogenic Avian Influenza A(H5N1) Virus Clade 2.3.4.4b Infections in Wild Terrestrial Mammals, United States, 2022. Emerg Infect Dis. 2023; 29: 2451-2460. *https://doi.org/10.3201/eid2912.230464.*
16. **Youk S, Torchetti MK, Lantz K, Lenoch JB, Killian ML, Leyson C, et al.** H5N1 highly pathogenic avian influenza clade 2.3.4.4b in wild and domestic birds: Introductions into the United States and reassortments, December 2021-April 2022. Virology. 2023; 587: 109860. *https://doi.org/10.1016/j.virol.2023.109860.*
17. **Zeng J, Du F, Xiao L, Sun H, Lu L, Lei W, et al**. Spatiotemporal genotype replacement of H5N8 avian influenza viruses contributed to H5N1 emergence in 2021/2022 panzootic. J Virol, 2024; 98: e0140123. *https://doi.org/10.1128/jvi.01401-23.*
18. **Peacock TP, Moncla L, Dudas G, VanInsberghe D, Sukhova K, Lloyd-Smith JO, et al**. The global H5N1 influenza panzootic in mammals. Nature. 2025; 637(8045): 304-313. *https://doi.org/10.1038/s41586-024-08054-z.*
19. **Bruno A, de Mora D, Olmedo M, Garces J, Marzal A, García-Bereguiain MA**. Highly pathogenic avian influenza A (H5N1) virus outbreaks in South America in 2022–2024: a comprehensive review of an ongoing panzootic. Virology. 2025; 610: 110602. *https://doi.org/10.1016/j.virol.2025.110602.*
20. **Ruiz-Saenz J, Martinez-Gutierrez M, Pujol FH.** Multiple introductions of highly pathogenic avian influenza H5N1 clade 2.3.4.4b into South America. Travel Med Infect Dis. 2023; 53: 102591. *https://doi.org/10.1016/j.tmaid.2023.102591.*
21. **Abdelwhab EM, Mettenleiter TC**. Zoonotic Animal Influenza Virus and Potential Mixing Vessel Hosts. Viruses. 2023; 15: 980. *https://doi.org/10.3390/v15040980.*
22. **Nguyen TQ, Hutter CR, Markin A, Thomas M, Lantz K, Killian ML, et al**. Emergence and interstate spread of highly pathogenic avian influenza A(H5N1) in dairy cattle in the United States. Science. 2025; 388(6745): eadq0900. *https://doi.org/10.1126/science.adq0900.*
23. European Food Safety Authority; European Centre for Disease Prevention and Control; European Union Reference Laboratory for Avian Influenza; Alexakis L, Buczkowski H, Ducatez M, Fusaro A, Gonzales JL, Kuiken T, et al. Avian influenza overview December 2024-March 2025. EFSA J. 2025; 23: e9352. *https://doi.org/10.2903/j.efsa.2025.9352.*
24. **Zhang G, Shi Y, Ge H, Wang Y, Lu L, Jiang S, Wang Q**. Genomic signatures and host adaptation of H5N1 clade 2.3.4.4b: A call for global surveillance and multi-target antiviral strategies. Curr Res Microb Sci. 2025; 8: 100377. *https://doi.org/10.1016/j.crmicr.2025.100377.*
25. **Krammer F, Hermann E, Rasmussen AL**. Highly pathogenic avian influenza H5N1: history, current situation, and outlook. J Virol, 2025; 99: e0220924. *https://doi.org/10.1128/jvi.02209-24.*
26. **Sidney J, Kim A-R, de Vries RD, Peters B, Meade PS, Krammer F, et al**. Targets of influenza human T-cell response are mostly conserved in H5N1. mBio. 2025; 16: e0347924. *https://doi.org/10.1128/mbio.03479-24.*

27. **Gu C, Maemura T, Guan L, Eisfeld AJ, Biswas A, Kiso M, et al.** A human isolate of bovine H5N1 is transmissible and lethal in animal models. Nature. 2024; 636(8043): 711-8. *https://doi.org/10.1038/s41586-024-08254-7.*
28. **Mahmoud SH, Khattab MS, Yehia N, Zanaty A, Arafa AES, Khalil AA**. Pathogenicity of Highly Pathogenic Avian Influenza A/H5Nx Viruses in Avian and Murine Models. Pathogens. 2025; 14: 149. *https://doi.org/10.3390/pathogens14020149.*
29. **Alkie TN, Embury-Hyatt C, Signore AV, Baldwin F, Hisanaga T, Xu W, et al**. Comparative pathogenicity of three A(H5N1) clade 2.3.4.4b HPAI viruses in blue-winged teal and transmission to domestic poultry. mSphere. 2025; 10: e0002125. *https://doi.org/10.1128/msphere.00021-25.*
30. **Capelastegui F, Goldhill DH**. H5N1 2.3.4.4b: a review of mammalian adaptations and risk of pandemic emergence. J Gen Virol. 2025; 106: 002109. *https://doi.org/10.1099/jgv.0.002109.*
31. **Xie Z, Yang J, Jiao W, Li X, Iqbal M, Liao M, Dai M.** Clade 2.3.4.4b highly pathogenic avian influenza H5N1 viruses: knowns, unknowns, and challenges. J Virol. 2025; 99: e0042425. *https://doi.org/10.1128/jvi.00424-25.*
32. **Good MR, Suja D, Guthmiller JJ.** The sweet side of H5N1 influenza virus infection. PLoS Pathog. 2025; 21: e1012847. *https://doi.org/10.1371/journal.ppat.1012847.*
33. **Long JS, Mistry B, Haslam SM, Barclay WS**. Host and viral determinants of influenza A virus species specificity. Nat Rev Microbiol. 2019; 17: 67–81. *https://doi.org/10.1038/s41579-018- -0115-z.*
34. **Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, Shinya K, et al.** Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. Nature; 2012; 486(7403): 420-428. *https://doi.org/10.1038/nature10831.*
35. **Lin TH, Zhu X, Wang S, Zhang D, McBride R, Yu W, et al**. A single mutation in bovine influenza H5N1 hemagglutinin switches specificity to human receptors. Science. 2024; 386(6726): 1128-34. *https://doi.org/10.1126/science.adt0180.*
36. **Peacock TP, Sheppard CM, Lister MG, Staller E, Frise R, Swann OC, et al.** Mammalian ANP32A and ANP32B Proteins Drive Differential Polymerase Adaptations in Avian Influenza Virus. J Virol. 2023; 97: e0021323. *https://doi.org/10.1128/jvi.00213-23.*
37. **Garretson TA, Liu J, Li SH, Scher G, Santos JJS, Hogan G, et al**. Immune history shapes human antibody responses to H5N1 influenza viruses. Nat Med. 2025; 31: 1454-8. *https://doi.org/10.1038/s41591-025-03599-6.*
38. **Kaplan BS, Torchetti MK, Lager KM, Webby RJ, Vincent AL.** Absence of clinical disease and contact transmission of HPAI H5NX clade 2.3.4.4 from North America in experimentally infected pigs. Influenza Other Respir Viruses. 2017; 11: 464-470. *https://doi.org/10.1111/irv.12463.*
39. **Kwon T, Trujillo JD, Carossino M, Machkovech HM, Cool K, Lyoo EL, et al.** Pathogenicity and transmissibility of bovine-derived HPAI H5N1 B3.13 virus in pigs. Emerg Microbes Infect. 2025; 14: 2509742. *https://doi.org/10.1080/22221751.2025.2509742.*
40. **Krammer F, Schultz-Cherry S**. We need to keep an eye on avian influenza. Nat Rev Immunol. 2023; 23: 267-8. *https://doi.org/10.1038/s41577-023-00868-8.*
41. **WHO Global Influenza Programme & World Health Organization**. Pandemic influenza preparedness and response: a WHO guidance document. 2009. World Health Organization. Accessed on May 25, 2025. *https://iris.who.int/handle/10665/44123.*
42. **World Health Organization**. Intergovernmental Negotiating Body to draft and negotiate a WHO convention, agreement or other international instrument on pandemic prevention, preparedness and response. 2025. Accessed on May 5, 2025. *https://apps.who.int/gb/ebwha/pdf_files/WHA78/A78_10-en.pdf.*

43. **Qualls N, Levitt A, Kanade N, Wright-Jegede N, Dopson S, Biggerstaff M, et al**. Community Mitigation Guidelines to Prevent Pandemic Influenza – United States, 2017. MMWR. 2017; 66: 1-34. *https://www.cdc.gov/mmwr/volumes/66/rr/rr6601a1.htm.*
44. **Harrington WN, Kackos CM, Webby RJ.** The evolution and future of influenza pandemic preparedness. Exp Mol Med. 2021; 53: 737-749. *https://doi.org/10.1038/s12276-021-00603-0.*
45. **Ziegler T, Mamahit A, Cox NJ**. 65 tears of influenza surveillance by a World Health Organization-coordinated global network. Influenza Other Respir Viruses. 2018; 12: 558-565. *https://doi.org/10.1111/irv.12570.*
46. **Cargnin Faccin F, Perez DR**. Pandemic preparedness through vaccine development for avian influenza viruses. Hum Vaccin Immunother. 2024; 20: 2347019. *https://doi.org/10.1080/21645515.2024.2347019.*
47. **Centers for Disease Control and Prevention**. Highly Pathogenic Avian Influenza A(H5N1) Virus: Interim Recommendations for Prevention, Monitoring, and Public Health Investigations. 2024. Accessed on May 4, 2025. *https://www.cdc.gov/bird-flu/prevention/hpai-interim-recommendations.html.*
48. **Cohen J.** Bird Shots. Science. 2023; 380(6640): 24-7. *https://doi.org/10.1126/science.adi1004.*
49. **Lewis N, Beer M**. Stop H5N1 influenza in US cattle now. Science. 2024; 385(6705): 123.2024. *https://doi.org/10.1126/science.adr5866.*
50. **Medina-Armenteros Y, Cajado-Carvalho D, das Neves Oliveira R, Apetito Akamatsu M, Lee Ho P.** Recent Occurrence, Diversity, and Candidate Vaccine Virus Selection for Pandemic H5N1: Alert Is in the Air. Vaccines. 2024; 12: 1044. *https://doi.org/10.3390/vaccines12091044.*
51. **Jones JC, Yen HL, Adams P, Armstrong K, Govorkova EA.** Influenza antivirals and their role in pandemic preparedness. Antiviral Res. 2023; 210: 105499. *https://doi.org/10.1016/j.antiviral.2022.105499.*
52. **Khurana S, King LR, Manischewitz J, Posadas O, Mishra AK, Liu D, et al.** Licensed H5N1 vaccines generate cross-neutralizing antibodies against highly pathogenic H5N1 clade 2.3.4.4b influenza virus. Nat Med. 2024; 30: 2771-2776. *https://doi.org/10.1038/s41591-024-03189-y.*
53. **Souza CK, Santos JJS, Boggiatto P, Sterle H, Arruda B, Palmer MV, et al.** H5 influenza virus mRNA-lipid nanoparticle (LNP) vaccination elicits adaptive immune responses in Holstein calves. 2025. bioRxiv preprint. */https://doi.org/10.1101/2025.05.01.651548.*
54. **Koopmans MPG, Barton Behravesh C, Cunningham AA, Adisasmito WB, Almuhairi S, Bilivogui P, et al.** The panzootic spread of highly pathogenic avian influenza H5N1 sublineage 2.3.4.4b: a critical appraisal of One Health preparedness and prevention. Lancet Infect Dis. 2024; 24: e774-e781. *https://doi.org/10.1016/S1473-3099(24)00438-9.*