Understanding the Zoonotic Potential of Avian Influenza

March 2023

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Introduction

The current outbreaks of highly pathogenic avian influenza (HPAI) have been extraordinary in their magnitude and geographic distribution. Over the last few years, HPAI H5N1 virus has spread widely throughout many parts of the world affecting wild birds and significantly impacting the poultry industry. Spillover of this virus subtype into multiple mammalian species, including human cases, has also been documented. This has raised concerns about the transmission of HPAI virus to humans, which is also known as the zoonotic potential of this virus. This report provides background information on influenza viruses and a summary of the zoonotic potential of avian influenza in general.

Overview of Influenza Viruses

Influenza viruses are members of the family *Orthomyxoviridae* (Current ICTV Taxonomy Release (ICTV), 2021). There are four types of influenza (flu) viruses known: A, B, C and D (Figure 1).

Influenza A viruses infect a wide range of birds and mammals, including humans (Kessler et al., 2021). Almost all influenza A viruses are thought to have originated in wild aquatic birds, the natural animal reservoir. Over time, influenza A viruses have adapted to new hosts, and now several lineages specific to humans, horses, swine, and dogs exist (Kessler et al., 2021). In humans, these include the seasonal influenza A viruses, which circulate every year mostly during the fall and winter in temperate climates such as Canada. Influenza A viruses have also caused several human pandemics throughout history. Influenza A viruses are divided into subtypes based on two proteins on their surfaces: hemagglutinin (HA) and neuraminidase (NA). Presently, 18 HA subtypes and 11 NA subtypes have been identified (World Health Organization, 2018).

Table 1 - Current influenza A species-specific lineages and subtypes

Lineage	Subtype	Reference
Avian	Multiple (HA 1-16 and NA 1-9)	(Kessler et al., 2021)
Human	H1N1	(World Health Organisation (WHO), 2018b)
	H3N2	
Swine	H1N1	(Ma et al., 2008; Mancera Gracia et al., 2020)
	H1N2	
	H3N2	
Equine	H3N8	(Kessler et al., 2021; Oladunni et al., 2021)
Canine	H3N2	(Klivleyeva et al., 2022)
	H3N8	
Bat	H17N10	(Kessler et al., 2021)
	H18N11	

Influenza B viruses primarily infect humans but can also infect other mammals. Although they can cause severe disease, they generally cause milder disease than influenza A viruses. They include the human seasonal influenza B viruses, which usually circulate along with the seasonal influenza A viruses. Influenza B viruses are divided into two lineages: Victoria and Yamagata.

Influenza C viruses also only infect mammals, but rarely cause disease or only mild illness.

Influenza D viruses primarily affect cattle, but can also infect goats, sheep, pigs, and humans and cause mild illness (Su et al., 2017)

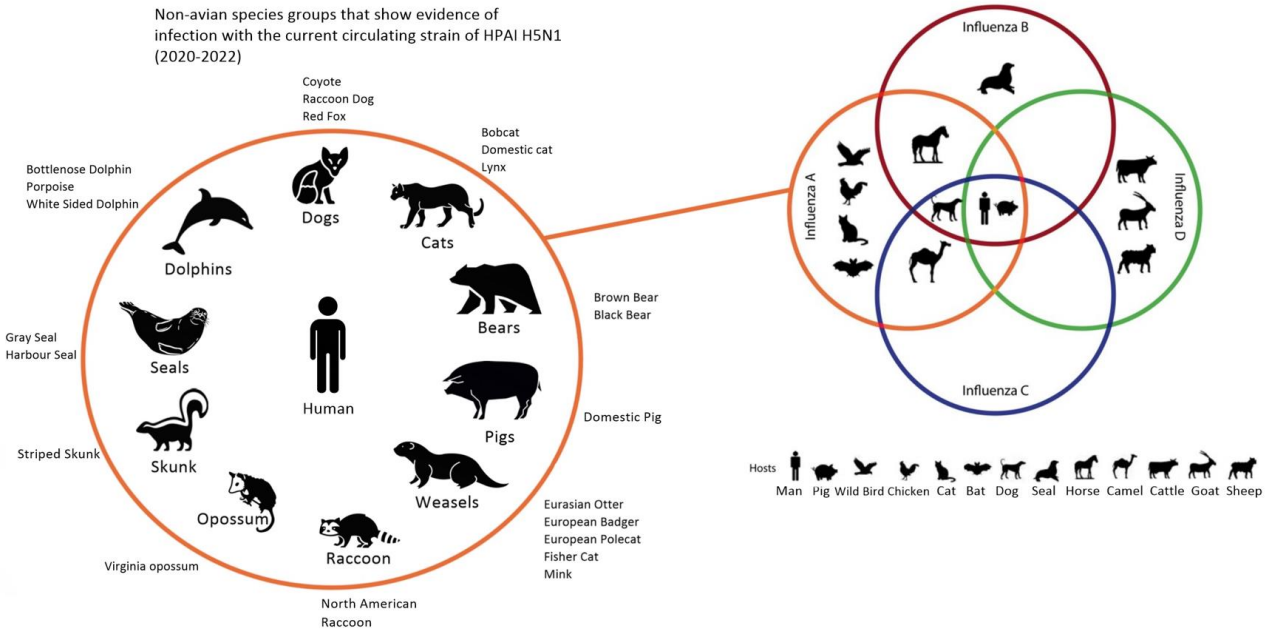

Figure 1. Mammal families that show evidence of infections with influenza viruses (types A, B, C, & D) (Modified from Bailey et al., 2018).

Avian Influenza Viruses

Avian influenza viruses (AIVs) are influenza A viruses that affect birds. AIVs spread naturally within wild aquatic birds (such as ducks, geese, gulls, and shorebirds) worldwide. Infected birds shed virus in their saliva, respiratory secretions, and feces. Generally, wild waterfowl such as ducks do not get sick. However, AIVs can infect many different species of birds, some of which can develop severe disease and die. Domestic poultry flocks are particularly vulnerable to infection (CFSPH, 2023).

Low and Highly Pathogenic Avian Influenza

AIVs are further classified as Highly Pathogenic Avian Influenza (HPAI) and Low Pathogenic Avian Influenza (LPAI) virus strains. The classification is based upon their ability to cause disease in chickens and differences in their HA (Luczo et al., 2015). Generally, HPAI viruses cause severe disease and death in chickens whereas LPAI viruses cause mild disease. The development of disease in other animals is complex and depends on the species and characteristics of the virus. To date, all HPAI viruses have been H5 or H7 subtypes.

Most AIVs that spread in wild birds are LPAI viruses. There are many different LPAI subtypes, but the LPAI H5 and H7 subtypes are of particular concern as they are able to evolve from low pathogenic to highly pathogenic after they infect domestic birds (Monne et al., 2014). Outbreaks due to mutation from LPAI-to-HPAI were reported in Canada (H7N3) in 2004 (Pasick et al., 2005) and recently in the United States in Indiana (H7N8) in 2016 (Lee et al., 2017a) and Tennessee (H7N9) in 2017 (Lee et al., 2017b).

Zoonotic Potential of Avian Influenza viruses

The ability of AIVs to infect mammals, including humans, is complex and not fully understood. Many characteristics (viral and host) have been identified that contribute to host specificity and the zoonotic potential of AIVs, as explained below.

Genome Structure and Viral and Host Characteristics

Influenza A viruses are enveloped, segmented RNA viruses (Bouvier & Palese, 2008). The viral genome is made up of 8 gene segments encoding up to 17 viral proteins (Chauhan & Gordon, 2022). A high-level summary of the genes and their protein functions is provided in Figure 2.

Several different gene segments/proteins may contribute to the zoonotic potential of avian influenza viruses, including HA and NA, the polymerase proteins (PB1, PB2, PA), and other proteins such as the nucleoprotein (NP) and non-structural protein (NS).

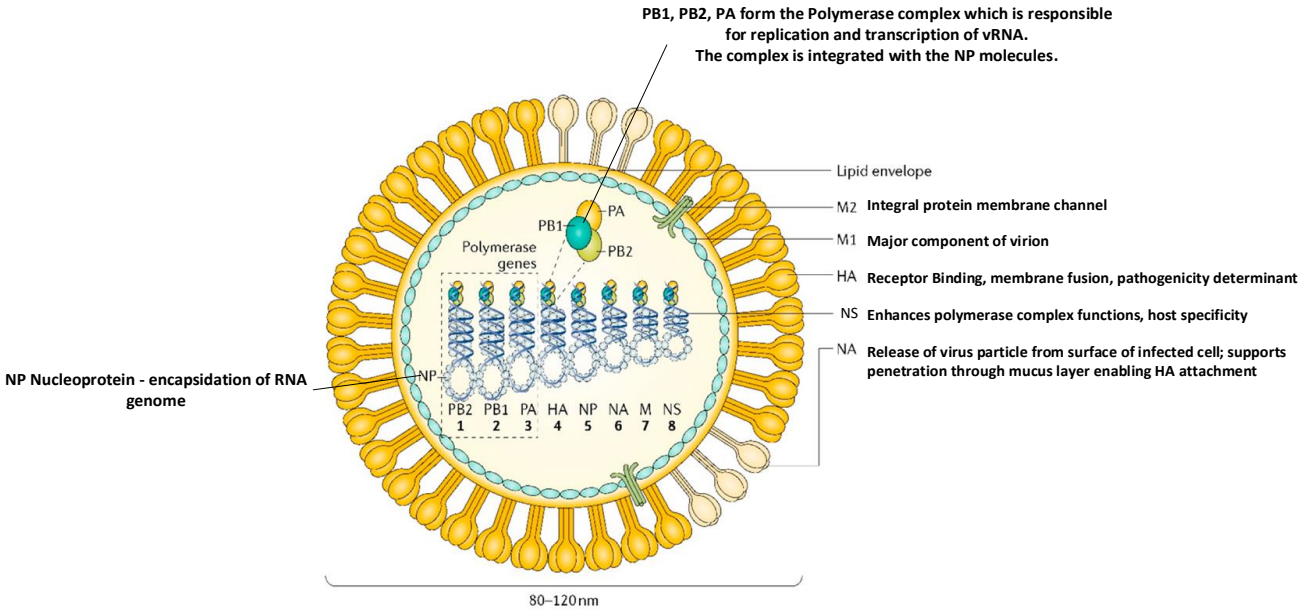

Figure 2. The genome structure including gene segments. (Modified from Krammer et al., 2018)

Surface Proteins (HA and NA)

Influenza A viruses use their HA to bind to sialic acid receptors on the surface of cells lining the respiratory or intestinal tracts of the infected host (Kuchipudi et al., 2021). The match between the host cell receptor and the viral HA is one of the most important determinants of host specificity (Matrosovich et al., 2015).

AIVs more easily bind to alpha 2,3 sialic acid ("avian type") receptors that are located mostly in the intestinal tract of birds (Figure 3). Human influenza A viruses more easily bind to a different type of receptor, alpha 2,6 sialic acid ("human type") receptors, that are located in the respiratory tract of people (Li et.,2019). Humans also have some "avian-type" receptors in their respiratory tract, however, there are fewer and other factors affect the ability of AIVs to bind to these receptors. This means that although humans can be infected directly with some AIVs, this rarely occurs. There is only one amino acid difference between the HA protein in AIVs and human influenza A viruses that affects their ability to bind to either "avian-type" or "human-type" receptors, however, a change in this amino acid does not occur often (Bateman et al., 2008).

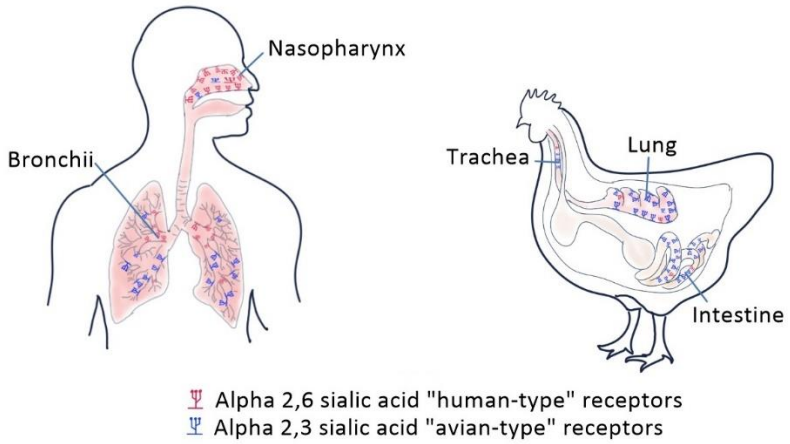

Figure 3. The presence and distribution of sialic acid (SA) receptors in humans and chickens.

The ability of an influenza A virus to replicate in a host and spread to other hosts also depends on the pH within the host and in the environment (Joseph et al., 2017). The pH affects the stability of HA surface proteins. HAs of AIVs are generally more stable at a higher pH than mammalian influenza A viruses. This allows AIVs to replicate more efficiently in the intestinal tract of birds and spread through aquatic environments.

The main function of NA surface protein is to destroy the SA receptors on the host cell after infection, which releases new viruses and prevents re-infection of that cell (Joseph et al., 2017; Mostafa et al., 2018). For the virus to spread effectively within a host, the NA needs to be able to destroy the main receptors that the virus binds to. The NA of AIVs more easily destroy the "avian-type" receptors. Influenza A viruses that have adapted to humans more easily bind to "human-type" receptors, therefore, the NA of these adapted viruses have changed to be able to destroy both types of receptors.

Polymerases (PB2, PB1, PA)

Several changes within polymerase basic 2 (PB2) protein can affect the ability of AIVs to cause disease in mammals. One of the most important changes involves the temperature sensitivity of the virus (Joseph et al., 2017; Mostafa et al., 2018). Human influenza A viruses are adapted to the upper respiratory tract and have an optimum temperature of 33°C, whereas AIVs are adapted to the intestinal tract and prefer higher temperatures (>37°C). A single amino acid change in PB2 can enable AIV's to replicate at the lower temperatures in the upper respiratory tract, thus broadening the host range (Li et.,2019).

The other two polymerase proteins, polymerase basic 1 (PB1) and polymerase acidic (PA), appear to also have a role in the host specificity of influenza A viruses, although this role is not completely understood.

Other Proteins (NP, NS)

Nucleoprotein (NP) helps influenza A viruses to replicate in a host cell (Zimmermann et al., 2011). Mammals have some mechanisms of immunity to counteract the function of NP. One of these antiviral proteins, called myxovirus resistance A (MxA), interferes with NP functioning (Joseph et al., 2017; Mostafa et al., 2018). AIVs are more sensitive to MxA than human influenza viruses. Changes in the NP of AIV that make it less sensitive to MxA may allow AIV to replicate more easily in mammals.

Changes in the non-structural (NS) protein may also allow AIVs to replicate more efficiently in mammals. Experimentally, these changes possibly increase the polymerase activity, enabling the virus to replicate in the respiratory tract of humans which have a lower temperature compared to the core body temperature (Reuther et al., 2014).

Evolution of Avian Influenza Viruses

Changes to the AIV genome can occur in one of two ways: antigenic drift or antigenic shift.

Mutation (Antigenic Drift)

Antigenic drift refers to mutations that accumulate in individual genes during viral replication that occurs over time.

As the viral polymerase protein cannot fix errors that arise during viral replication (called proofreading), mutations occur fairly regularly; however, these mutations often have no effect on virus behaviours. In some instances, these mutations can alter the functions of viral proteins and enhance the infectious potential for influenza viruses.

New AIV strains arise continually due to antigenic drift. The more influenza infections there are in a population, the greater chance there is for new variants to arise.

Reassortment (Antigenic Shift)

When two different influenza A viruses co-infect a cell, the segmented nature of the viral genome allows for the swapping of one or more gene segments between the two viruses (Steel & Lowen, 2014). This process is called reassortment or antigenic shift. The new virus generated is called a reassortant virus. Reassortment occurs frequently within influenza A viruses and can result in rapid changes in the characteristics of the virus. Reassortment has created enormous viral

diversity and plays an important role in the evolution of influenza A viruses including AIV and expanded host ranges for these viruses.

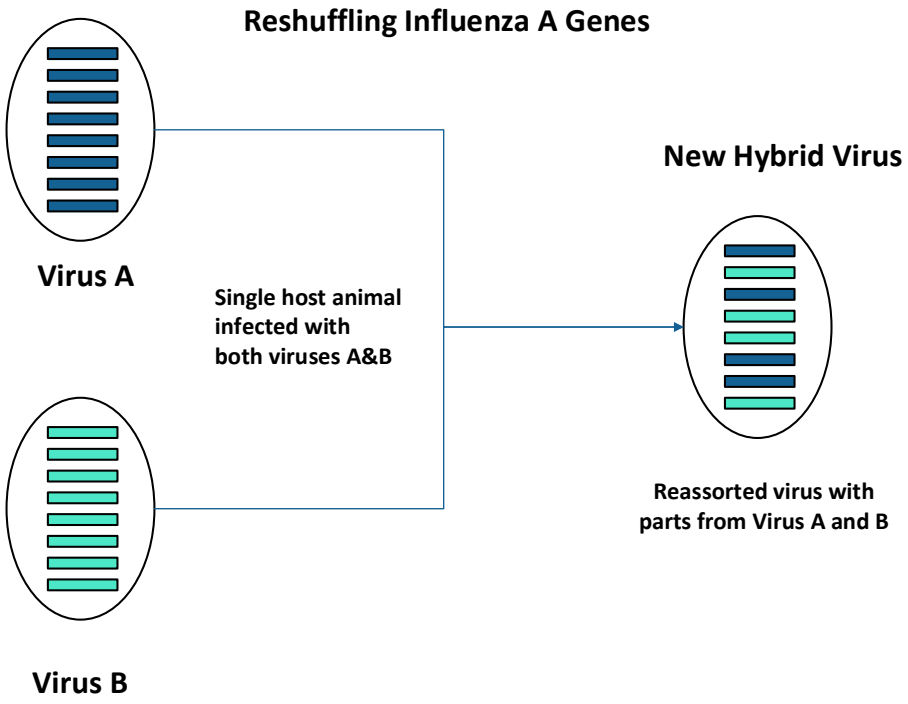

Figure 4: An example of a reassortant virus from virus A and virus B

Pigs as Mixing Vessels

Swine are susceptible to infection with both avian and human influenza A viruses because they have both "avian-type" and "human-type" receptors in their respiratory tract (Ma et al., 2008). New influenza viruses can be created by reassortment of viral gene segments between avian, swine, and human influenza A viruses if pigs become concurrently infected with multiple viruses. AIVs can be transmitted to pigs from domestic poultry (Meseko et al., 2018) and human influenza A viruses can also be transmitted to pigs (Ma et al., 2008). Pigs are therefore generally considered to be a "mixing vessel" for influenza A viruses (Figure 5).

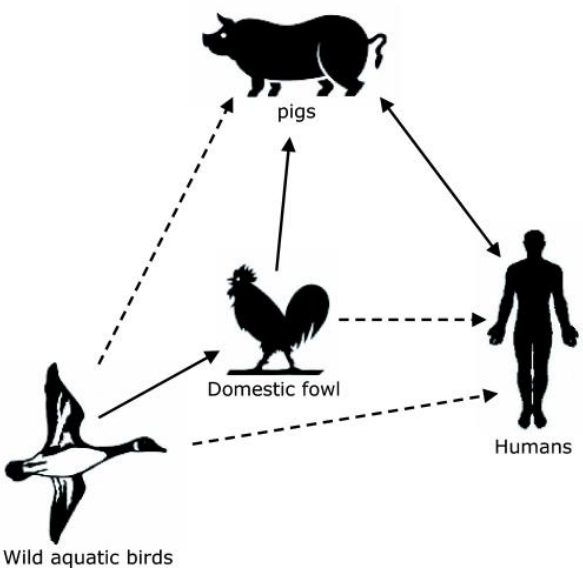

Figure 5. The pig as a "mixing vessel" for influenza A viruses. Solid lines: frequent and/or confirmed transmission events. Dotted lines: possible and/or occasional transmission events. (Ma et al., 2008).

Zoonotic Avian Influenza Viruses

Rarely, humans are infected with AIVs directly. To date, the HPAI H5N1 and H7N9 subtypes have been responsible for most severe human illness from AIVs worldwide.

In 1997, an H5N1 influenza virus was directly transmitted from birds in live poultry markets in Hong Kong to humans. Eighteen people were infected in this outbreak, six of whom died. Outbreaks of HPAI H5N1 virus were reported in poultry in Hong Kong earlier that same year. By 2005, this HPAI virus had spread from Asia to Europe and Africa and became endemic in poultry populations in some countries. The first report of a human infection with aH5N1 virus in the Americas was in Canada on January 8, 2014 and occurred in a traveler who had recently returned from China.

The H5N1 virus continued to evolve and reassort with other avian influenza viruses. In October 2020, the H5N1 virus associated with the current outbreak first emerged in the Netherlands (Cui et al., 2022). Since then, this HPAI H5N1 virus has caused numerous outbreaks in poultry and wild birds. In December 2021, this HPAI H5N1 virus spread to North America through wild birds where it has continued to evolve and reassort with LPAI viruses (Kandeil et al., 2022).

There have been numerous reports of mammals being infected in several different countries with the current HPAI H5N1 virus (Appendix A). Unusually, many of these cases have had severe disease affecting the brain (Kandeil et al., 2022). Some adaptation to mammals has been reported in some of these cases (European Food Safety Authority, 2022). There has also been a report of likely spread between mammals on a mink farm in Spain (Agüero et al., 2023).

To date, there have been seven human cases associated with this current HPAI H5N1 virus. An asymptomatic case was found in England in January 2022 following identification of the virus in a duck flock kept at the residence (Oliver et al., 2022). A case with mild symptoms who had close contact with H5N1 infected poultry was identified in the USA in April 2022 (WHO, 2022). In October 2022, two asymptomatic cases in poultry workers were reported from Spain who had exposure to infected poultry, although these are now thought to be environmental contamination only (ProMED, 2023). Also in October 2022, a severe case with exposure to backyard poultry was reported from Viet Nam and a fatal case also with exposure to backyard poultry was reported from China (The Centre for Health Protection (CHP), 2022; (WHO), 2022). In January 2023, Ecuador confirmed a severe case of HPAI H5N1 in a child who had contact with sick backyard poultry(WHO, 2023, PAHO, 2023). Recently, in February 2023 an 11-year-old girl in Cambodia died from a different type (clade) of H5N1 in the country's first known human H5N1 infection since 2014 (Health Cambodia, 2023).

Worldwide, beginning January 2003 to November 11, 2022, 868 cases of human infection with avian influenza A(H5N1) virus have been found in 21 countries. Four hundred and fifty seven of these 868 cases were fatal (case fatality rate [CFR] of 53%). Indonesia, Vietnam, and Egypt have reported the highest number of human HPAI H5N1 cases to date.

In 2013, human infections with A(H7N9) virus were reported for the first time in China and associated with close contact with poultry. Since then, both small outbreaks and sporadic infections have occurred. The most recent case was reported in March 2019. From 2013 to 2019, there have been 1568 laboratory-confirmed human cases, including at least 615 deaths (CFR of 39%), almost all in China (WHO), 2019).

Risk Factors for Human Infection

The main risk factor for human infection with AIVs is either by direct or indirect exposure to infected live or dead birds and contaminated areas like infected farms and live bird markets. Generally, slaughtering, defeathering and handling carcasses of infected poultry present risks. A small number of human cases of influenza H5N1 virus have been associated with the consumption of dishes made with raw, contaminated poultry blood. However, once cooked thoroughly, food does not pose a risk. Being in close contact with human cases has also been found to be a risk factor for infection with HPAI H5N1 virus. For some HPAI Asian strain H5N1 human cases, the source of exposure is unknown (WHO), 2018a).

The Role of Avian Influenza Viruses in Past Pandemics

An influenza pandemic can occur if a new influenza virus emerges with the ability to cause continued human-to-human transmission (Taubenberger et al., 2007). If the human population has little to no immunity against the new virus a pandemic virus can spread rapidly and globally with increasing international travels.

AIVs have been key contributors to the emergence of human influenza pandemics throughout the 20th century. The largest known influenza pandemic to date, the 1918 influenza, was caused by an H1N1 virus, which spilled over from an avian species to humans, either directly or through an intermediate host (Kessler et al., 2021). Changes to the virus' HA protein, which allowed it to bind to human-type receptors as well as to its PB2 and PA proteins, likely enabled avian to human transmission (Chauhan & Gordon, 2022; Gamblin et al., 2004; Taubenberger et al., 2005). Following the 1918 pandemic, the H1N1 virus became endemic in the human population.

In 1957, the H2N2 pandemic virus appeared, which was a reassortant of an avian H2N2 virus and a descendant of the 1918 H1N1 strain (Kessler et al., 2021). In 1968, the H3N2 pandemic occurred, due to the H2N2 virus reassorting with an avian H3Nx virus (WHO 2018). Descendants of this H3N2 virus continue to circulate seasonally in humans today.

The most recent human influenza pandemic was in 2009 and was caused by the zoonotic transmission of a recombinant H1N1 swine influenza virus, with gene segments from several different avian, swine and human viruses. This virus strain also continues to circulate in people presently as a seasonal H1N1 influenza strain.

Conclusion

Over the past century, evidence showed that AIVs have the potential to infect humans and cause disease. Fortunately, AIVs usually only rarely spread to humans if there is close contact with infected poultry. In most cases, both viral characteristics and host factors prevent AIVs from spreading to people. However, AIVs can evolve through mutation and reassortment with other influenza A viruses, leading to the creation of new strains and subtypes that may more easily infect mammals. If these changes occur to some of the gene segments and resulting proteins that have been found to affect the host specificity of influenza A viruses, these new virus subtypes may then be able to more easily infect and cause disease in humans and spread through the population. In the past, certain AIV subtypes have caused severe disease in humans and have adapted or reassorted to be able to spread efficiently from person-to-person, in some cases causing pandemics. Although the current HPAI H5N1 virus has infected humans in situations where there was close contact with infected poultry, to date, it has not resulted in spread from person-to-person. The World Health Organization (WHO) has assessed the current risk of this HPAI H5N1 virus to humans as low (WHO, 2022) and higher risk for those exposed to poultry. However, the pandemic potential has been assessed as moderate (CDC, 2022). Continued understanding, monitoring, and control of this and other AIV subtypes is important due to the zoonotic potential of AIV.

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Appendix A: Avian Influenza A(H5N1) virus detections in mammal species other than humans related to the currently circulating "Eurasian strain" worldwide, 2020-2023

Animal	Country	Epidemic season	Reference
Red foxes	Sweden; Netherlands; Finland; Estonia; Ireland; Wales; England; Belgium; Norway; Japan; USA; Canada	2020-2021 2021-2022	(National Veterinary Institute Sweden (SVA), 2021) (World Organisation for Animal Health (WOAH), 2022) (World Organisation for Animal Health (WOAH), 2021) Avian influenza of avian origin in non avian wildlife GOV. UK 2023
Common raccoon dog	Japan	2021-2022	(WOAH)
Coyote	USA	2021-2022	(WOAH)
Eurasian otter	Netherlands; Finland; Scotland	2021-2022	(Wageningen University Research (WUR), 2022) Avian influenza of avian origin in non avian wildlife GOV. UK 2023
European badger	Netherlands	2021-2022	(WUR)
European polecat	Netherlands	2021-2022	(WUR)
Ferret	Slovenia	2021-2022	Slovenian National Reference Laboratory for Avian Influenza
Mink	Canada; Spain	2021-2022	(WOAH), CFIA/ECCC Dashboard, 2022; Agüero et al., 2023
Lynx	Finland	2021-2022	(Finnish Food Authority (FFA))
Bobcat	USA	2021-2022	(WOAH)
Fisher cat	USA	2021-2022	(WOAH)
Raccoon	USA, Canada	2021-2022	(WOAH), CFIA/ECCC Dashboard, 2022
Skunks	Canada; USA	2021-2022	CFIA/ECCC Dashboard, 2022,USDA
Grey seals	USA	2021-2022	(WOAH)
Harbour seals	USA; Canada	2021-2022	(WOAH), CFIA/ECCC Dashboard, 2022
Black bear	Canada	2021-2022	(Healthy Wildlife (Canadan Wildlife Health Cooperative), 2022)(Healthy Wildlife, WOAH), CFIA/ECCC Dashboard, 2022
Domestic pigs	Italy	2021-2022	EURL
Virginia opossum	USA	2021-2022	(WOAH)
Porpoise	Sweden	2021-2022	(SVA)
Bottlenose dolphin	USA Peru	2021-2022	(University of Florida Health (UFHealth))
White sided dolphin	Canada	2021-2022	(CFIA/ECCC Dashboard, 2022)
American black bear	USA	2022-2023	(United States Department of Agriculture (USDA), 2023)
Grizzly bear	USA	2022-2023	USDA
Kodiak bear	USA	2022-2023	USDA
Striped skunk	USA	2022-2023	USDA
Domestic cat	France	2022-2023	WAHIS (woah.org) FAO (fao.org)
Sea lions	Peru	2023	University of Minnesota (CIDRAP)

(Modified from European Food Safety Authority (EFSA), 2022)