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The Journey of the Avian Influenza Virus H5N1 Through 30 Years of Evolutionary Events, Geographical Locations, and Animal Species: A Review

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Abstract

Influenza viruses have caused outbreaks and pandemics throughout human history and have until Covid-19 been considered the group of viruses with the largest potential for pandemics. Avian influenza viruses cause zoonotic diseases, including birds, mammals, and humans. This review focuses on H5N1 because it is highly pathogenic and generated the most common clades among the current ones (*e.g.* 2.3.4.4). Since the first goose that was infected in Guangdong, China by A/Goose/Guangdong/1/96 (H5N1) in 1996, H5N1 has undergone many events of reassortment with other influenza viruses and accumulated many amino acid substitutions on the 10 proteins that are encoded by the H5N1 genome. The review will follow H5N1 through examples of such evolutionary events that permitted the virus to spread across the world, as well as through many animal species. Hallmark mutations that permit or prevent the selective binding to receptors on bird or mammal host cells will be identified. The journey through the NorthAtlantic fly way from Europe to North and South America, culminating in the first reported death from H5N1 in the United States will be explained. The review concludes with the current state of vaccines and anti-virals and an assessment by the author of the current situation.

Contents

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1 Introduction

Influenza viruses can be classified into four genera (A, B, C, and D) based on their proteins and host specificities, avian influenza viruses belong to the A genus (Nakhaie et al., 2017). Based on their hemagglutinin (HA) and neuraminidase (NA) glycoproteins, influenza A viruses can be further divided into sub-types (*e.g.* H5N1, H1N1, H7N9). Avian influenza viruses have caused human illness all the way through the middle ages until today. A classic example is the Spanish Flu at the end of World War I in 1918, which was caused by H1N1 and originated in birds (Berche, 2022). Most cases of influenza today are caused by the H5 and H7 sub-types of viruses, which also have the highest pandemic potential (Sutton, 2018). Avian influenza viruses (AIV) can either exhibit low pathogenicity (LPAIV) or high pathogenicity (HPAIV) in chicken (Scheibner et al., 2023). Most highly pathogenic among the HPAIV viruses are H5N1 and H5N8 (Takadate et al., 2023), as well as H9N2. This is why vaccine development against these three HPAIV has been strong the last few decades (Gomaa et al., 2019; Hegazy et al., 2021; Panickan et al., 2022). Since the most common and most pathogenic clades of AIV are part of H5N1, this review focuses on the development of such clades in this AIV.

In order for H5N1 to cause infection in a host, it will have to bind to a host specific receptor. Of special importance is the linkage between sialyloligosaccharides (SA) and galactose (Gal) on the surface of a bird or mammalian host cell (Matrosovich et al., 2004). The linked molecule is a glycan on the surface of some cells that permits influenza viruses to bind, but does not appear to have a function in the absence of viruses (Walther et al., 2013). To infect birds, H5N1 would have a higher affinity to the α-2,3 linked molecule, designated SA-α-2,3-Gal. In contrast, a mammalian type H5N1 would preferentially bind SA-α-2,6-Gal (Matrosovich et al., 2004). The distribution of these receptors throughout the host has implications for the initial stages of infection, as well as replication of the virus. The bird-type SA-α-2,3-Gal receptor is expressed in birds only on intestinal and respiratory cells, a smaller number of bird species also express the SA-α-2,6-Gal receptor (Costa et al., 2012; Gambaryan et al., 2002). As a consequence, birds usually suffer from intestinal and respiratory infections (Costa et al., 2012). Mammals can also express the SA-α-2,3-Gal receptor, but typically only in the lower respiratory tract, whereas SA-α-2,6-Gal is expressed in the upper respiratory tract. Infection of the upper respiratory tract could result in aerosol driven mammal to mammal including human to human transmission (Costa et al., 2012; Gambaryan et al., 2002; Matrosovich et al., 2004). Interestingly, dairy cattle (*Bos taurus*) express both the SA-α-2,3-Gal and the SA-α-2,6-Gal receptors in their mammary glands, as well as in their cerebrum (Kristensen et al., 2024), which explains the high replication rate of the viruses in the udder and the occurrence of viruses in milk. Throughout this review, we will discuss many mutations on HA and their impact on the affinity of the virus to bind to the SA-α-2,3-Gal and/or the SA-α-2,6-Gal receptors.

Since the first report of a human case of H5N1 in 1997, the virus has conquered the world in terms of geography and species diversity (Krammer & Schultz-Cherry, 2023). Originating in birds in Asia, the virus now infects many different species, including terrestrial and marine mammals, as well as humans (CDC, 2022). This brings attention to the urgent need for a comprehensive understanding of the molecular biology, evolution, and mutational mechanisms of the virus. Chapter I of this review provides a brief overview of the genome and biology of H5N1, Chapters II to IV detail the progression of H5N1 through the different clades, and Chapter V will summarize reassortments and amino acid substitutions of the predominant 2.3.4.4 clade of today. Chapter VI will indicate important mutational events that permit transmission to humans and possibly transmission among humans and Chapter VII will give examples of current prevention and treatment options. The review

will emphasize how the varies mutations permitted the spread of the virus throughout the world and the animal kingdom all the way to humans. The overall conclusion of this review is that there is an urgent need for monitoring and the development of preventative and treatment techniques, but no need for a worldwide panic, yet.

Chapter I: H5N1 Genome and Biology

H5N1 is an RNA virus with a negative-sense single RNA strand of approximately 13.5 kb. The genome organization of the influenza viruses was described in 1983 (Lamb & Choppin, 1983) and has since been determined for many of the H5N1 viruses, including viruses that were isolated from humans (Sangsiriwut et al., 2018). The genome is segmented, which permits gene exchange via reassortment (Gong et al., 2021). The resulting reassortants vary in transmissibility and pathogenicity, while exhibiting a host specificity from wild birds and poultry to terrestrial and marine mammals, including humans. See Fig. 1 for genome information of the virus (A), functions of the proteins (B), and a selection of the animal species that the reassortants are capable of infecting (C).

The eight distinct segments of H5N1 encode at least 10 proteins (Fig. 1). Polymerase basic 1 (segment 1, PB2) consists of 757 amino acids, polymerase basic 2 (segment 2, PB1) consists of 759 amino acids, and polymerase acidic (segment 3, PA) consists of 716 amino acids. These three proteins are part of a heteromeric protein complex that constitutes the RNA-dependent RNA polymerase or viral ribonucleotide protein complex (vRNP) that is characteristic for negative-sense RNA viruses (Te Velthuis et al., 2021). A fourth and final component of vRNP is the nucleoprotein (segment 5, NP), which consists of 498 amino acids (Te Velthuis et al., 2021). The vRNP complex is responsible for RNA transcription, replication, and packaging (Te Velthuis & Fodor, 2016).

The two proteins that are responsible for the designation of the influenza viruses are the surface proteins hemagglutinin (segment 4, HA, 568 amino acids) and neuraminidase (segment 6, NA, 499 amino acids) (Sangsiriwut et al., 2018). The Centers for Disease Control and Prevention (CDC; www.cdc.gov) in the United Stated (US) describe at least 18 subtypes of HA and 11 subtypes of NA, leading to a range of designations, including H5N1 (CDC, 2024b). Together, these two proteins are needed for replication and spread of the virus (Kosik & Yewdell, 2019; McAuley et al., 2019). HA is required for the early stages of infection by binding to host sialic acid (SA) receptors, facilitating entry into the cell, and inducing membrane fusion (Kosik & Yewdell, 2019). A switch of the binding specificity of HA from SA-α-2,3-Gal to SA-α-2,6-Gal is expected for the virus to switch from avian/human to human/human transmission (Li & Wang, 2006). Several mutations in the receptor binding site for HA might be able to facilitate such a switch (Herfst et al., 2012). In order to be functioning, the 17 amino acid signal peptide needs to be cleaved of the full length HA during the co-translational process (Braakman et al., 1991). The cleavage site also contributes to host specificity, a sequence of PQRERRRKR/G is expected to facilitate a high affinity of a 2.3.2 clade H5N1 virus to chicken (Xing et al., 2024). NA is required for the final stages of infection. It cleaves the host receptors off the surface of the new viruses, which aids the release of the virus from the infected host cell (McAuley et al., 2019). HA cleavage sites are included in Table 1.

The short M segment encodes for the M1 (segment 7, 252 amino acids) and M2 (segment 7, 97 amino acids) proteins. The oligomeric M1 protein is essential for the assembly of viral components, which leads to the formation of the virions (Hilsch et al., 2014). M2 is a transmembrane protein and proton channel (Pielak & Chou, 2011). It regulates pH equilibrium while the virus enters the host cell and is vital for viral replication. Together, the roles for M1 and M2 are in viral budding, vRNP transport, and maintaining pH balance throughout the life cycle of the virus.

The final two proteins are the non-structural protein NS1 (segment 8, 224 amino acids) (Ji et al., 2021) and NS2 (segment 8, 121 amino acids) (Zhang et al., 2022). NS1 was described as a key protein that protects the virus from the host innate immune system (Ji et al., 2021). The protein is composed of double-stranded RNA binding domain and an effector domain. It inhibits interferon production through the RIG-1 pathway. NS2 negatively regulates the activation of interferon through the regulatory factor IRF7 (Zhang et al., 2022). Together, NS1 and NS2 influence viral replication (de Chassey et al., 2013). All these H5N1 proteins undergo frequent mutational events through a range of mechanisms, which will be discussed in the next few chapters of this manuscript.

Chapter II: the early years of H5N1

During the 20th century, there were three global influenza pandemics in humans, namely in 1918, 1957, and 1968. These were caused by H1N1 (origin Spain), H2N2 (origin Asia), and H3N2 (origin Hong Kong), respectively (Kilbourne, 2006). The first documented cases of H5N1 infection were in 1959 in Scotland among poultry chicken (*Gallus gallus domesticus*) (Chicken/Scotland/59) (Pereira et al., 1965). In 1996, an H5N1 infection was identified in a goose (family Anatidae) in Guangdong, China; this virus was designated A/Goose/Guangdong/1/96 (H5N1). A very similar virus was identified in an outbreak among humans (*Homo sapiens*) in China and Hong Kong in 1997, designated influenza A/Hong Kong/156/97 (H5N1) (Claas et al., 1998). This outbreak resulted in six human deaths. The HA protein of this virus was very similar to that of the A/Goose/Guangdong/1/96 (H5N1) virus, but the NA protein was lacking a 19 amino acid deletion that was previously seen in the Hong Kong viruses (Xu et al., 1999). See Fig. 2 for the evolution of H5N1 through time, animal species, and geographic locations.

These viruses were transmitted to humans from birds and lacked the ability to spread among humans (Claas et al., 1998). Between 2001 and 2004, a series of reassortment events occurred in H5N1 that led to the virus causing outbreaks in poultry chickens and ducks (*Anas platyrhynchos domesticus)* across eight Asian countries (Li et al., 2004). One 2004 outbreak among poultry and humans raised attention in Thailand (Tiensin et al., 2005). During this outbreak, 62 millions of backyard chickens and ducks were killed. The virus also infected 17 humans, of which 12 dies. A small number of domestic cats (*Felis silvestris catus*), as well as captive tigers (*Panthera tigris*) and leopards (*Panthera pardus*) also died.

After 2003, the virus migrated out of Asia and a new clade designated 2.2.1 evolved in the poultry population of Egypt, also infecting humans (Table 1) (Arafa et al., 2016). This clade can be further divided into 2.2.1.1 and 2.2.1.1a, as well as 2.2.1.2. By 2013, the HA cleavage site EKRRKKR/GLF became dominant among the 2.2.1.2 strains (Table 1) (Arafa et al., 2016). By 2014, 2.2.1.2 carried mutations in HA at 120, 129Δ, and I151T, which promote increased binding affinity to human receptors (Arafa et al., 2016). Examples of a 2006 outbreak by 2.2.1 (Arafa et al., 2016), a 2007 occurrence of 2.2.1.1 (Younan et al., 2013), and a 2014 outbreak of 2.2.1.2 (Arafa et al., 2015) are included in Table 1 and Fig. 2. A vaccine was developed against 2.2.1.2 using the baculovirus expression system. Initially, it was 70% to 100% effective when administered once or twice, respectively (El-Husseiny et al., 2021). However,10 years later, outbreaks still occurred after an initial decrease upon a rollout of a nationwide vaccination policy (Abdelwhab et al., 2016). This was attributed to the continuous evolution of the virus leading to the continuous emergence and disappearance of clades. During these studies, it was determined that host receptor binding specificity of H5N1 was linked to HA residues 91, 129, 132, 149, 175, 179, 186, 189–190, 218, 221–222 and 224 of the receptor binding domain (Duvvuri et al., 2009).

Chapter III: the 2.3.2.1 clade of H5N1

Instead of introducing the entire fleet of clades and sub-groups, the next two chapters focus on two of the most dominant clades of H5N1 viruses, 2.3.2.1 (Chapter III) and 2.3.4.4 (Chapter IV). The 2.3.2.1 clade evolved at about the same time as the 2.2.1 clade from Egypt. Between 2007 and 2022, 2.3.2.1 spread through bird species of the Anseriformes (ducks, geese, and swans) and Charadriiformes (gulls, waders, and auks) throughout Asia and the Middle East, occasionally branching into mammals (Fig. 2). The classification of the clade into six sub-clades is based upon the HA protein sequence from 274 viruses and designated a through f (Xing et al., 2024). These viruses form 58 genotypes, bases upon mutations and reassortments (Xing et al., 2024). Among the many outbreaks the 2.3.2.1 clade of H5N1 has caused, Fig. 2 lists a 2011 outbreak in Bangladesh involving quails (*Coturnix coturnix*) and ducks (Nooruzzaman et al., 2019) and a 2012 outbreak in Indonesia involving ducks (Dharmayanti et al., 2014). A 2015 outbreaks in China involving housed tigers was caused by 2.3.2.1c and 2.3.4.4e (Hu et al., 2016), a 2016 outbreak in Cameroon among industrial poultry was caused by 2.3.2.1c (Wade et al., 2018). The same clade caused an outbreak among poultry in Lebanon (El Romeh et al., 2017). A study involving protein sequencing of HA determined that all 28 isolates that were tested had the PQRERRRKR/G cleavage site (Xing et al., 2024) (Table 1). The Office International des Epizooties - FAO Network of Expertise of Animal Influenza (OFFLU) lists this cleavage site as indicative of high pathogenicity to chicken (OFFLU, 2022).

Isolates of 2.3.2.1b and 2.3.2.1c, but not 2.3.2.1a isolated from poultry and humans were lethal in a ferret (*Mustela putorius*) model in three of the six tested animals (Pearce et al., 2017). Ferrets are considered a good

model for influenza because their receptor profile in the respiratory tract is similar to that of humans (Belser et al., 2011). In this model, only the 2.3.2.1 isolates caused disease symptoms and death, whereas 2.2.1 isolates from humans only exhibited low virulence (Pearce et al., 2017). The attempt to correlate protein sequences of the isolates tested in the ferret model with actual pathogenicity to the animals gave inconsistent results. As one example of a correlation between protein sequence and pathogenicity, an amino acid that is expected to increase binding to the SA-α-2,6-Gal receptor is in position 133 of HA. In isolates of the 2.2.1 clade, this amino acid was serine (SA-α-2,3-Gal), in isolates of the 2.3.2.1 clade, it was alanine (SA-α-2,6-Gal) (Pearce et al., 2017).

In a similar study, kestrels (*Falco tinnunculus*) were infected with H5N1 of the 2.3.2.1 clade either intranasally or via feeding the birds with infected carcasses (Uno et al., 2020). This study was relevant to prevent outbreaks in raptors, as many of these birds consume carcasses. The infected animals were highly susceptible to the viruses, exhibited signs of depression, a multitude of neurological symptoms, and developed pancreatic lesions. They died 6 days post infection. High viral titers were found in many tissues including feather calamuses (quills) and viruses appeared to replicate in almost all tissues tested (Uno et al., 2020). The conclusion from this study was to remove infected carcasses from fields quickly.

Chapter IV: the 2.3.4.4 clade of H5N1

Starting around 2014, the 2.3.4.4 clade made it across the world. The predominant clade today is 2.3.4.4b, which started around 2020 and spread into Europe and North and South America. Infected animals still include many wild bird species and poultry, but also more species of mammals, including fur farm animals in Europe (*e.g.* minks, *Mustela vison*), seals (order Pinniped) and dolphins (family Delphinidae) in South America, and dairy cattle and poultry farms in North America (Fig. 2). Occasional spill-over events into humans were observed.

The ongoing epizootic of H5N1 2.3.4.4b in Europe includes a 2022 die-off of wild migratory waterbirds in the Caspian See in Russia (Sobolev et al., 2023), a 2022 outbreak in farmed minks impacting more than 50,000 animals in Northwest Spain (Agüero et al., 2023), and a small number of infected wild foxes (*Vulpes vulpes*) in Germany in 2023 (Baechlein et al., 2023). A mutation was found in the PB2 gene of viruses from the mink outbreak, T271A (Agüero et al., 2023). At the time, this mutation was considered uncommon but it might have public health implications. The infected foxes in Germany exhibited encephalitis, accompanied by strong cerebral viral replication (Baechlein et al., 2023). These viruses also had a mutation in the PB2 gene, E627K (Baechlein et al., 2023). These three outbreaks are included in Fig. 2.

In a study published in 2024, the mink derived virus was experimentally transmitted into pigs (*Sus scrofa domesticus*) in Spain, pigs suffered from interstitial pneumonia with necrotizing bronchiolitis and exhibited high titers of the virus in their lungs, but did not transmit to other pigs (Kwon et al., 2024). Viruses shed by the pigs carried the E627K mutation on PB2, accompanied by a Q222L mutation in HA. These mutations were by now recognized as crucial to infection of mammals (Kwon et al., 2024).

Further in Europe, infection with 2.3.4.4b was detected in five domestic dogs (*Canus lupus familiaris*) and one cat in Italy in 2023, interestingly, these animals did not show any symptoms (Moreno et al., 2023). Another report of an infected domestic animal came from France in 2023, where a cat living on a duck farm had contracted the virus (Briand et al., 2023). A review about H5N1 2.3.3.4b in Europe summarizes outbreaks in 88 domestic bird and 175 wild birds across 23 countries from the 3rd quarter of 2023 (Adlhoch et al., 2023). The review includes three fatal and one severe infection of humans by 2.3.2.1c in Cambodia (Adlhoch et al., 2023). The here presented list of outbreaks is by no my means comprehensive, but exemplary. The point is that 2.3.4.4b is effective at transmitting across animal species and capable of infecting a wide range of bird and mammalian species, both wild and captive.

Spread into North America was accomplished via Iceland, Greenland, and the NorthAtlantic Flyway to South America (Bevins et al., 2022). One of the first reports was the detection of H5N1 in wild waterfowl in two eastern coastal States of the US in 2021 (Bevins et al., 2022). In late 2021, the virus was detected in bottleneck dolphins (*Tursiops truncates*) in Florida, where it caused neuronal necrosis and inflammation of the brain (Murawski et al., 2024). The highest viral titer was found in the brain of the animals and NA carried an S246N amino acid substitution (Murawski et al., 2024). In late 2021, the virus had also spread to Alaska, where it infected many species of wild birds, poultry, and mammals (Ahlstrom et al., 2024). In 2022/2023, 2.3.4.4b was detected in six crows (*Corvus spec.*) and one red fox on the east coast of Canada (Alkie et al., 2023). This clade made an appearance on the west coast of Canada later in 2023 (Russell et al., 2024). Despite the existence of several

vaccines against 2.3.4.4b H5N1 isolates from poultry (A/Turkey/Indiana/22-003707-003/2022 H5N1 (Lee et al., 2024); A/Turkey/Indiana/3703-003/2022 H5N1 (Spackman et al., 2023), outbreaks on poultry farms in the US are an ongoing problem. However, on October 1, 2024 the US Food and Drug Administration (FDA; www.fda.gov) still assessed the risk to humans from the consumption of chickens and/or eggs as low because HPIV kill the birds so quickly that the entire flock gets destroyed immediately, proper storing and handling further reduces the risk (FDA, 2024). The CDC recommends to cook poultry and eggs to an internal temperature of 165F (CDC, 2024a).

Of special concern is the spill-over of 2.3.4.4b into domestic cattle, starting in Texas and Kansas (Burrough et al., 2024; Oguzie et al., 2024) and then spreading across the US. From cows, the virus can spread to numerous domestic and wild birds and mammals (e.g. racoons, opossums, poultry) and also caused several infections of dairy farm workers (Mostafa et al., 2024). Direct inoculation of the virus into the mammary glands of lactating cows led to necrotizing mastitis in the animals and the conclusion that the dairy cattle clade of 2.3.4.4b can replicate in the udder of cows (Halwe et al., 2024). It was hypothesized that milk is likely the primary route of transmission between cattle (Halwe et al., 2024). A binding study with HA trimers from avian and bovine 2.3.3.4b demonstrated that at this time, binding to the mammalian SA-α-2,6-Gal receptor is still weak (Santos et al., 2024). A study from Canada determined that pasteurization of raw milk is effective at inactivating the virus (Alkie et al., 2025). On December 31, 2024, the CDC still assessed the risk from pasteurized milk as low, but that from raw milk as higher (CDC, 2024a). They recommend an internal cooking temperature of 160F for ground beef and 145F for whole cuts of beef (CDC, 2024a).

In South America, 2.3.4.4b has spilled over into marine mammals, including elephant seals (*Mirounga* spec.) in Argentina, where mammal to mammal spread has been observed (Uhart et al., 2024). Intriguingly, it appears that 2.3.4.4b can spill-back from marine mammals to coastal birds and also one human (Uhart et al., 2024). In Peru, 2.3.4.4b has been detected in wild birds, poultry, and mammals (Cruz et al., 2023). In Brazil, it was found in terns (family Sternidae) (de Araújo et al., 2024). Spill-back into coatis (*Nausua nausua*) has been observed across South America (Rodríguez et al., 2024).

A few select examples of the continuous existence of H5N1 in Asia are included in Fig. 2 (Heo et al., 2024; Ke et al., 2022; Yang et al., 2023). An example of 2.3.4.4e comes from Japan where cranes (family Gruidae) and raptors (order Falconiformes) got infected (Soda et al., 2022). The two most recent cases of bird flu that could not be classified beyond H5N1 yet are a harbor seal (*Phoca vitulina*) and a flamingo (*Phoenicopterus roseus*) at the Lincoln Zoo in Chicago on January 16, 2025 (Wetly, 2025) and two muted swans (*Cygnus olor*) in Baldeney See in Essen, Germany (WDR, 2025). Also, on January 17, the CDC reported the 67th human case of bird flu in the US and recommends faster testing for bird flu in hospitals (Edwards, 2025; Charostad et al., 2023; Hu et al., 2013; Kwon et al., 2022).

Altogether, the virus has by now conquered the world and is infecting an amazing array of bird and mammal species. This phenotypic diversity is enabled by changes through amino acid substitutions in crucial proteins, as well as reassortments of entire genome segments.

Chapter V: mutations of the 2.3.4.4b clade

The success of the 2.3.4.4b clade has been made possible by two evolutionary mechanisms, reassortment and antigenic drift. As mentioned in Chapter I, reassortment is a consequence of the segmented genome of the influenza viruses (Gong et al., 2021). What this means is that two viruses co-infecting the same single host can exchange entire segments of the genome, the outcome of which is a novel hybrid or reassortant (Kawaoka et al., 1989; Peacock et al., 2024). This can result in new combinations of HA and NA, leading to novel designations. For example, a reassortant designated H5N6 now has the type 5 HA and the type 6 NA (see Table 1 for examples of such reassortants in 2.3.4.4). The current clade designations are 2.3.4.4b (BB) for the European viruses, 2.3.4.4b (B3.2) for the South American viruses and 2.3.4.4b (B 3.13) for the US dairy cattle viruses (Peacock et al., 2024).

Fig. 3 was modified from Peacock and coworkers (Peacock et al., 2024) and explains the reassortments leading to H5N1 2.3.4.4b (BB) found in European fur farm animals, such as minks. The first reassortment includes 2.3.4.4b H5N8 and a Eurasian LPAI to generate 2.3.4.4b H5N1. This event may have current either in Europe or Asia around 2020 (Fusaro et al., 2024; Xie et al., 2023). The event integrated segments 4 (HA) and 6 (NA) into H5N8 (Fig. 3). Subsequent reassortments within Europe included the integration of segments 1 and

3 (vRNAP) from a Eurasian LPAI to form 2.3.4.4b (AB) and the integration of three segments from gull-adapted H13/H16 into 2.3.4.4b (AB) to form 2.3.4.4b (BB) (Byrne et al., 2023; Fusaro et al., 2024). This latter clade is the virus that caused outbreaks on European fur farms, including those in Spain (Agüero et al., 2023) and Finland (Kareinen et al., 2024).

Fig. 4 is the continuation of H5N1 2.3.4.4b in the Americas. The clade that was moved to the Americas via the NorthAtlantic Flyway was 2.3.4.4b H5N1. It then reassorted multiple times with American LPAI to generate 2.3.4.4b B3.13, which infected dairy cattle in North America (Oguzie et al., 2024). An intermediate of these reassortments was 2.3.4.4b B3.2, the clade that infected South American mammals.

Antigenic drift is the second mechanism that contributes to the evolution of 2.3.4.4b. This refers to the introduction of amino acid substitutions into assorted areas of proteins, often but not always impacting the surface proteins and recognition of the host receptors. A special feature within HA is the cleavage site. A summary of HA cleavage sites across influenza A viruses is provided by OFFLU (OFFLU) (OFFLU, 2022). A major distinction is made between mono-basic cleavage sites, which have a single basic amino acid right before the cleavage site (*e.g.* PEKQTR/G) and multi-basic cleavage sites, which are characterized by several basic amino acids before the cleavage site (e.g. PQRERRRKK/G). Mono-basic cleavage sites are cleaved by few cellular proteases, which limits the virus to the respiratory and intestinal systems of birds, viruses are of low pathogenicity to birds (LPAI). Mono-basic cleavage sites also occur in mammals and permit cleavage by specific proteases in the respiratory tract (*e.g.* trypsin). Multi-basic cleavage sites permit cleavage by a wider range of proteases (*e.g.* furin) (Stieneke-Gröber et al., 1992), which makes the virus more pathogenic to birds (HPAI), permitting infection of multiple organs. They also occur in mammals on a small number of occasions, where they can lead to systemic infections.

As one example of the impact on the sequence of the HA cleavage site on proteolytic cleavage of HA, and the resulting pathogenicity to the host, a cleavage site of RRRKKR/G (poly-basic) was experimentally modified to RETR/G (mono-basic) (Horimoto & Kawaoka, 1994). Viruses with the mutated cleavage site could no longer be cleaved by proteases in chicken embryo fibroblasts and were avirulent in chickens (Horimoto & Kawaoka, 1994). A structural study from 2007 determined that the consensus sequence of QRERRRKKR/G over 651 H5N1 viruses resided within the cleavage site loop where it was well exposed to proteases (Guo et al., 2007).

Table 1 includes examples cleavage sites from H5N1 and reassortants derived from H5N1. The HA cleavage site from the original LPAI H5N1 virus is given as PQRETR/G, which is mono-basic (Squires et al., 2012). The cleavage sites for 2.2 was PQEKRRKKR/G, which has five basic amino acids (Arafa et al., 2016). HPAI 2.3.2.1 was reported with a PQRERRRKR/G cleavage site for HA (Xing et al., 2024).

The HA cleavage sites of 2.3.4.4 are more diverse than those of previous clades (Table 1). As one example, a reassortant of 2.3.4.4 was reported from China, consisting of the HA gene from 2.3.4.4b and the NA gene from 2.3.4.4c H5N6 to form 2.3.4.4b H5N8 (Zhang et al., 2021). The HA cleavage site for these viruses was determined to be RERRRKR/G (Zhang et al., 2021). Among 98 of birds from Russia, a signature cleavage site of PLREKRRKR/G was determined (Glazunova et al., 2024). The viruses were attributed to 2.3.4.4 without providing the a-h designation. The HA cleavage site of a virus that was recovered from a human patient was determined to be PLRERRRKKR/G (He et al., 2018). This virus was a triple reassortant of H5N6, H7N9, and H10N8 and classified as 2.3.4.4. The virus was considered highly pathogenic to humans.

2.3.4.4b BB from Spain that originated from gulls (family Larinae) and infected fur farm animals in 2022 had the mono-basic HA cleavage site of PEKQTR/G (Agüero et al., 2023). The South American 2.3.4.4b B3.2 that infects marine mammals has the same mono-basic HA cleavage site (Uhart et al., 2024) and so does the South American 2.3.4.4b B3.2 from coatis (Rodríguez et al., 2024) and the North American 2.3.4.4b B3.13 from cattle (Burrough et al., 2024). As was shown in Figs. 3 and 4, these viruses are reassortants of an LPAI which might have provided the mono-basic HA cleavage site into an existing lade of HPAIV 2.3.4.4b.

The next paragraphs will summarize a small number of amino acid substitutions across the H5N1 proteome that impact the transition from bird to mammalian hosts. Among the mutations that effect binding of HA to bird or mammalian host receptors, T160L has been shown to have dual binding ability to the SA-α-2,3-Gal receptor of birds and the SA-α-2,6-Gal receptor of mammals (Gao et al., 2018). This was attributed to a lack of glycosylation at site 158. The N193D substitution in the HA receptor binding domain increased affinity towards SA-α-2,6-Gal in 2.3.4.4 isolates from migratory birds in South Korea, accompanied by increased pathogenicity in both, birds and mammals (Jang et al., 2024). An interesting study came from South Dakota where ten cats had died in a rural residence in 2024 from infection with H5N1 2.3.4.4b (Chothe et al., 2024). A T143A mutation

was found in HA where it impacted infectivity and immune evasion. These cats co-expressed the SA-α-2,3-Gal and the SA-α-2,6-Gal receptor, which gave rise to the suggestion that cats might serve as an effective host for the reassortment of bird and mammalian H5N1.

The E627K amino acid substitution in PB2 is a hallmark mutation linked to mammalian adaptation by permitting viral replication at the somewhat lower temperatures that are found in mammals, relative to birds. E627K was found in 2.3.4.4b B3.13 that were isolated from cattle in North America (Halwe et al., 2024; Singh et al., 2024), B3.2 that were isolated from coatis in South America (Rodríguez et al., 2024), farmed fur animals in Finland (Kareinen et al., 2024), and three wild foxes infected with 2.3.4.4b in The Netherlands in 2021/2 (Bordes et al., 2023). In the latter case, the viruses carried both, the 627E variant from birds and the 627K variant from mammals. It was suggested that the mutation might have occurred in these specific animals (Bordes et al., 2023).

The sea elephants from Argentina exhibited eight amino acid substitutions across the genome of 2.3.4.4b B3.2 (Uhart et al., 2024). These were L548F in PB1, Q591K and D701N in PB2, A20T, M86I, and M548I in PA, as well as R21Q and I226T in NS1. Seven of these were predominant in viruses isolated in Brazil and Uruguay, it was suggested that the viruses had probably spread from mammal to mammal in these two countries (Uhart et al., 2024). Mammal to mammal spread was further supported by the existence of two of the above mutations, D701N and Q591K (Peacock et al., 2023), across 2.3.4.4b viruses isolated from Peru (Leguia et al., 2023), Argentina (Uhart et al., 2024), Uruguay (Tomás et al., 2024), and Brazil (de Araújo et al., 2024). These viruses also formed a single clade on the evolutionary tree, which was found as B3.2 in a variety of South American marine mammals (*e.g.* sea lions dolphins, sea otters, elephant seals), as well as one human (Peacock et al., 2024).

The outbreak of 2.3.4.4b B3.13 in cattle that also included cats and several farm workers (conjunctivitis) included mammal to mammal spread, presumably through contaminated milking equipment (Eisfeld et al., 2024). One most recently isolated virus from cows was able to bind to both, the SA-α-2,3-Gal receptor of birds and the SA-α-2,6-Gal receptor, a feature that was not observed for previously isolated viruses (Eisfeld et al., 2024). Two amino acid substitutions that are characteristic for 2.3.4.4b B3.13 (Peacock et al., 2024) are M631L in PB2 which impacts dimerization of the vRNP (Sheppard et al., 2023) and K497R in PA (Yamayoshi et al., 2018). The Finland (Kareinen et al., 2024) and Spain (Agüero et al., 2023) outbreaks on fur farms were caused by 2.3.4.4b BB and included viruses with mutations in T271A of PB2, which had previously been shown to increases polymerase activity in mammalians (Bussey et al., 2010). Altogether, mutational events by reassortment or antigenic drift impact varies functions of the virus, including cleavage of HA, binding specificity to the host receptors, replication, and pathogenicity.

Chapter VI: human cases of H5N1

Human cases of H5N1 have been reported infrequently since A/HongKong/156/97 (H5N1) the first one in 1997 (Claas et al., 1998). The January 3, 2025 report from the World Health Organization (WHO; www.who.int) listed a total of 261 human cases of H5N1 infection from five countries within the Western Pacific region between January 1, 2003 and December 12, 2024 (WHO, 2025). Of these, 142 were fatal. H5N6 caused 93 laboratory confirmed human cases since 2014, of which 57 were fatal. At this time, the WHO still considers the risk low because of a lack of ability of the viruses to transmit between humans. They do recommend continued vigilance, though. By January 17, 2025, the CDC reported 67 confirmed human cases in the US from 2024, including one death from Louisiana just a few days earlier. Of these, 40 cases were from dairy herds, 23 from poultry farms, 1 of other animal exposure and 2 of exposure source unknown. The CDC also considers the risk to the public at this time still as low, but provides surveillance.

The virus from a human case in Chile carried the Q591K and D701N substitution on PB2 that were characteristic for 2.3.4.4b B3.2 from mammals in South America (Rimondi et al., 2024). A virus from a dairy farm associated human case in the US from March 2024 carried the E627 K mutation in PB2 that is characteristic of 2.3.4.4b B3.13 from US cattle (Hu et al., 2024). An article that was published in Science in 2023 had postulated this E627K mutation as essential for H5N1 to infect and replicate in humans (Kupferschmidt, 2023), a step that has been accomplished already multiple times since the Spanish Flu in 1918. The same Science article postulated a pair of amino acids substitutions in PB2 as needed and less likely to occur together because of the close proximity of the two amino acids. These are Q226L and G228S, impacting receptor binding specificity (Kupferschmidt, 2023). One of these, Q226L, has since been found in H5N1 2.3.4.4b 3.13 (A/Texas/37/2024,

Texas) that caused the human death by bird flu in the US (Lin et al., 2024). An additional A224L substitution in this virus further enhanced the binding specificity to the human receptor. Once more, it was highlighted that continuous surveillance of the H5N1 2.3.4.4b viruses and their mutations was needed.

Other mutations that may be beneficial for transmission to humans, as well as human to human transmission include L204M in NA. This amino acid substitution was found among others in several human derived H5N1, which and exhibited differences in virus fitness (Scheibner et al., 2023). The L240M mutation also enhanced viral replication in human airways (Scheibner et al., 2023). 2.3.4.4b from minks and seals still carried the T271A mutation in PB2 when experimentally transmitted to ferrets and permitted direct contact and airborne transmission in these animals (Restori et al., 2024). This was not a virus isolated from humans, but the ease of transmission does indicate an increased potential to cause pandemics.

Chapter VII: prevention and treatment

While there appears to be agreement that the risk to the public is currently still to be assessed as low, there is also agreement that the situation needs monitoring. This raises the question what other precautions can be taken should an H5N1 pandemic occur. This gets us to the development and testing of vaccines and anti-virals for humans.

Vaccines have been mentioned infrequently throughout this review, but these were developed against some of the older clades of H5N1 and often rather clade specific (*e.g.* 2.2.1 (Grund et al., 2011)). This chapter will provide more examples of current vaccines and vaccines in development. On January 9, 2025, Clinicaltrials.gov listed 179 trials under vaccines for bird flu, of which 177 are against H5N1, possibly among others (*e.g.* H7N9). Only one of these is a phase III trial, NCT01382329 from Japan, for a vero cell derived whole virus vaccine against H5N1. The large number of phase I and II trials are indicative of a large amount of effort into the development of novel vaccines, though it will take a while until these vaccines can be approved, manufactured and distributed.

The mechanisms of function of these vaccines include inactivated virus vaccines (*e.g.* NCT02171819, phase I, (Phan et al., 2016); NCT00417560 phase I/II, completed), live and replication competent recombinant adenovirus type 4 vaccines (*e.g.* Ad4-H5-Vtn, NCT01443936, phase I, (Matsuda et al., 2021), subunit virion vaccines (*e.g.* NCT01086657, phase I, (Andrews et al., 2022)), and recombinant H5 protein vaccines (*e.g.* NCT01657929, phase I, completed). Many of these vaccines focus on the HA protein because this is the primary immunogen for influenza viruses (Nichol & Treanor, 2006). Since activation of CD4+ and CD8+ T lymphocytes plays a crucial role in the immune response as well, it is advised to generate vaccines that stimulate the innate and humoral parts of the immune response (Janssens et al., 2022). This is the case for live attenuated vaccines, but not for inactivated virus vaccines (Janssens et al., 2022).

Of special interest are also mRNA vaccines after their success with SARS CoV-2 (Teo, 2022). This technology uses a liquid nanoparticle to deliver mRNA into the cytosol of the host cell (Pardi et al., 2015). Moderna who had developed one of the SARS CoV-2 vaccines (mRNA 1273) (Gilbert et al., 2022) was just awarded $ 176 million to develop an improved mRNA H5N1 vaccine. Moderna already has two mRNA vaccines against influenza viruses H7N9 and H10N8, VAL-339851 and VAL-506440, respectively (Feldman et al., 2019). GlaxoSmithKline is recruiting for a phase I/II trial to evaluate safety, reactogenicity, and immunogenicity of an mRNA H5N1 vaccine (NCT06382311).

Among vaccines that are not based on mRNA technology, GlaxoSmithKline together with the National Institute of Allergy and Infection (NIAID) completed a trial on a monovalent vaccine with AS03 adjuvant (NCT01910519) (Wimmers et al., 2021). The inactivated vaccine Audenz was approved by the FDA for people aged 6 months and older who are at higher risk of exposure to the H5N1 (Aldhaeefi et al., 2024). In Europe, Novartis developed a pre-pandemic H5N1 vaccine, named Aflunov (Gasparini et al., 2012). This is a sub-unit vaccine containing HA from H5N1 adjuvanted with MF59 (Del Giudice et al., 2013).

The above are examples of vaccines to be used on humans. There are existing vaccines for birds and mammals. A group of researchers in China has developed a H5N1 virus-like particle vaccine based on the baculovirus expression system (Kong et al., 2024). This vaccine protected chickens against divergent viruses dependent on the adjuvant and vaccine dose (Kong et al., 2024). Mice were protected against H7N9 with a whole-virion MF59-adjuvanted vaccine (Chang et al., 2017), which also caused high antibody titers against HA in ferrets (Hatta et al., 2018). A consortium of institutions has developed an HA encoding mRNA vaccine against

2.3.4.4b H5 influenza viruses that elicited a good antibody and T lymphocyte response in mice and ferrets (Furey et al., 2024). At this time, vaccination is considered a most effective prevention of influenza in humans (Cargnin Faccin & Perez, 2024).

Among the anti-viral treatments (Table 2), much research has been done on existing drugs of the two categories neuraminiase inhibitor (NAIs) and CAP-dependent nuclease inhibitor (CENI). NAIS inhibit the NA protein of all influenza A and B. This prevents the virus from fusing with the host cell membrane. The NAI oseltamivir was approved for medical use in the US in 1999 and has the brand name Tamiflu. The drug can be used for prevention and treatment of influenza, expecially in people of high risk. Administration is by pill or liquid, common side effects include diarrhea and vomitting. Effectiveness of oseltamivir is controversial. In one study, treatment with oseltamivir resulting in an odds ratio of 0.15, relative to the untreated control group (Liem et al., 2009). In a systematic review, oseltamivir could not be associated with a reduced risk of hospitalization. Instead, is was associated with an in increase in gastrointestinal symptoms (Hanula et al., 2024). Examples of mutations in more recent influenza viruses that render oseltamivir less effective are included in Table 2.

Zanamavir under the brand name Relenzo (GlaxoSmithKline) was approved for in the US in 1999, for treatment of influenza (not for prevention). It is administered by oral inhalation. The use of the drug is controversial as well, clinically relevant benefits are small, and may be outweighed by side effects and the risk for mutations on the respective viruses (Michiels et al., 2013). Peramivir under the brand name Rapivab (BioCryst Pharmaceuticals) was approved in the US in 2014 for the treatment of influenza in adults. Administration of the drug can be intramuscular or intraveneously at a dose that is lower than for the older NAIs. Peramivir also has a longer half-life (Wester & Shetty, 2016). In clinical trials, peramivir relieved symptoms in cases of acute, but uncomplicated influenza. This was considered an improvements over older anti-viral drugs used to treat influenza (McLaughlin et al., 2015). Mutations in NA can occur and are included in Table 2. Laninamivir octanoate under the brand name Inavir has been approved to treat influenza in Japan, but not yet in the US. It is inhaled by powder and is long acting. A mutation in H275Y on NA that is resistant towards oseltamivir (de Jong et al., 2005) can still be inactivated by laninamivir (Ikematsu & Kawai, 2011). A meta-study from Japan compared the effectiveness of several of the NAIS against H3N2 and found that peramivir was preferable over laninamivir (Higashiguchi et al., 2018).

Baloxavir under the brand name Xofluza is an example of a CENI (Table 2). The endonuclease activity resides on PA and cleaves the cap off viral RNA transcription. Baloxavir is adminstered as a single dose by mouth. It functions even against some of the other mutation carrying viruses that are resistant to NAIs, but repeated treatment can give rise to mutations in I38T (Andreev et al., 2024; Noshi et al., 2018). Altogether, the existing anti-virals against influenza viruses such as H5N1 are partially effective, but not perfect. Many trials can be found on clinicaltrials.gov that test multiple combinations of NAI or CENI for their effectiveness and safety (NCT05648448) or non NAI/CENI drugs (*e.g.* phenylephrine NCT05118672).

2 Conclusion

In summary, H5N1 is a perfect example of a pathogen that by means of specific mutations can spread across animal species and by means of migratory birds and possibly other animals can spread across the world. The question arises is how this will impact humans in the long run. Currently, human cases have mostly been by direct infection from an animal and not by human to human transmissions. It seems like only one or two mutations are needed from here to enable airborne transmission among humans. Will these mutations happen? It is difficult to predict the future, but seemingly unlikely events happen in evolution more often. At this point in time, the author shares the assessment of the WHO, the CDC, and many researchers that the risk is low. This can change any time, meaning that continued monitoring of H5N1 and other AIV is needed, as well as faster testing in hospitals; together with further research, development, and manufacturing of vaccines and anti-virals.

Acknowledgments
B.M.P is funded by the ND Agricultural Experiment Station.

Table 1: Characteristics of predominant H5N1 clades and reassortants

Clade	Origin[1]	Timeline	HA cleavage site[2]	New clade[3]	Other characteristics[4]	Reference
H5	SL	1959	PQRETR/G	HPN1	LPAI	(Squires et al., 2012)
2.2.1	EG	2006	PQEKRRKKR/G	NA	Poultry and humans	(Arafa et al., 2016)
2.2.1.1	EG	2007	PQEKRRKKR/G	NA	Mostly poultry	(Arafa et al., 2016)
2.2.1.2	EG	2014	PQEKRRKKR/G	NA	Human cases	(Arafa et al., 2016)
2.3.2.1	HK, C	2007	PQRERRRKR/G	NA	HPAI	(Xing et al., 2024)
2.3.2.1a	SA/SEA	2009-2022	PQRERRRKR/G	NA	HPAI	(Xing et al., 2024)
2.3.2.1b	C	2007-2017	PQRERRRKR/G	NA	HPAI	(Xing et al., 2024)
2.3.2.1c	SEA	2011-2019	PQRERRRKR/G	NA	HPAI	(Xing et al., 2024)
2.3.2.1d	C	2007-2019	PQRERRRKR/G	NA	HPAI	(Xing et al., 2024)
2.3.2.1e	J, K	2009-2011	PQRERRRKR/G	NA	HPAI	(Xing et al., 2024)
2.3.2.1f	A, C	2013-2016	PQRERRRKR/G	NA	HPAI	(Xing et al., 2024)
2.3.4.4	EA	2014				(Kwon et al., 2023)
2.3.4.4a	G	2014	PLGEKRRKR/G	H5N8	Waterfowl, 80-90% M, 67% T	(Harder et al., 2015)
2.3.4.4b	C	2021	??RERRRKR/G	H5N8	Reassortant	(Zhang et al., 2021)
	R	2024	PLREKRRKR/G	H5N1	Consensus 98 birds	(Glazunova et al., 2024)
	C	2018	PLRERRRKKR/G	H5N6	Triple reassortant Gull reassortant, fur farm, T271A in PB2	(He et al., 2018) (Agüero et al., 2023)
	SP	2022	PEKQTR/G	H5N1 BB	LPAI reassortant, elephant seals, Q591K and D701N in PB2	(Uhart et al., 2024)
	AR	2023	PEKQTR/G	H5N1 B3.2	Coatis, E627K in PB2 LPAI reassortant, Texas dairy cows, E627K in PB2, M631L in PB2, and K497R in PA, replicate in udder of cows, zoonotic cases in humans	(Rodríguez et al., 2024) (Burrough et al., 2024; Halwe et al., 2024; Singh et al., 2024)
	SAM	2023	PEKQTR/G	H5N1 B 3.2		
	US	2024	PEKQTR/G	H5N1 B3.13		

[1] SL, Scotland; EG, Egypt; HK, Hongkong; C, China; SA, South Asia; SEA, Southeast Asia; J, Japan; K, Korea; A, Africa; EA, Eurasia; G, Germany; R, Russia; SP, Spain; AR, Argentina; SAM, South America; US, United States.

[2] Hemagglutinin (HA) cleavage sites were taken from OFFLU (OFFLU, 2022).

[3] After 2009, some of the H5N1 viruses resassorted with LPAI viruses. This resulted in new subtypes, *e.g.* H5N6. This was predominantly seen in the 2.3.4.4. H5N1 viruses.

[4] LPAI, low pathogenicity avian influenza; HPAI, high pathogenicity avian influenza; M, Mortality; T, transmission.

Table 2: Summary of anti-virals against H5N1 and other influenza viruses

Drug name	Effective against	Reference	AAS rendering reduced effectiveness	Reference	Approval
Neuraminidase inhibitors					
Ozeltamivir (Tamiflu)	H5	(Liem et al., 2009)	H275Y on NA I222M on NA	(de Jong et al., 2005) (Andreev et al., 2024)	US in 1999
Zanamavir (Relenzo)	H7 2.3.2.1/ 2.3.4.4	(Hu et al., 2013) (Andreev et al., 2024)	R292K on NA K423E/T438F on NA	(Kageyama et al., 2013) (Andreev et al., 2024)	US in 1999
Peramivir (Rapivab)	2.3.2.1/ 2.3.4.4	(Andreev et al., 2024)	K423E/T438F on NA	(Andreev et al., 2024)	US in 2014
Laninamavir (Inavir)	H5N1 H3N2	(Ikematsu & Kawai, 2011) (Higashiguchi et al., 2018)			Japan in 2010
CAP-dependent endonuclease inhibitor (N-terminal domain of PA)					
Baloxavir acid (Xofluza)	Influenza A/B H5N1, others	(Omoto et al., 2018) (Noshi et al., 2018)	I38 to T, F, or M on PA A36T on PA I38T	(Andreev et al., 2024) (Andreev et al., 2024) (Noshi et al., 2018)	US and Japan in 2018

Fig. 1. Genome insights into the H5N1 virus. The Figure shows the genomic architecture of the virus (A), indicates protein functions (B), and shows a small selection of animals that can be infected by H5N1 (C). The Figure was modified from (Charostad et al., 2023) and generated with Biorender.

Fig. 2. Progression of H5N1 through clades, animals, and geographic locations. The timeline includes select examples of outbreaks during the indicated timeframe, including the species infected, as well as the country. References are explained in the text (Agüero et al., 2023; Arafa et al., 2016; Arafa et al., 2015; Baechlein et al., 2023; Burrough et al., 2024; Claas et al., 1998; Dharmayanti et al., 2014; El Romeh et al., 2017; Heo et al., 2024; Hu et al., 2016; Ke et al., 2022; Li et al., 2004; Murawski et al., 2024; Nooruzzaman et al., 2019; Pearce et al., 2017; Rodríguez et al., 2024; Sobolev et al., 2023; Soda et al., 2022; Tiensin et al., 2005; Uhart et al., 2024; Uno et al., 2020; Wade et al., 2018; Xu et al., 1999; Yang et al., 2023; Younan et al., 2013). The Illustration was generated with Biorender.

Fig. 3. H5N1 2.3.4.4b in Europe. The information about the reassortment events was taken from Peacock and coworkers (Peacock et al., 2024). Information on the HA cleavage sites and select hallmark amino acid substitutions were taken from (Agüero et al., 2023; Baechlein et al., 2023; Kwon et al., 2024) and integrated into the figure. Information on hallmark amino acid substitutions were taken from (Agüero et al., 2023; Baechlein et al., 2023; Kwon et al., 2022). The Illustration was generated with Biorender.

Fir. 4. H5N1 2.3.4.4b in the Americas. The information about the reassortment events was taken from Peacock and coworkers (Peacock et al., 2024). Information on the HA cleavage sites and select hallmark amino acid substitutions were taken from (Halwe et al., 2024; Rodríguez et al., 2024; Uhart et al., 2024) and integrated into the figure. Information on hallmark amino acid substitutions were taken from (Halwe et al., 2024; Peacock et al., 2024; Rodríguez et al., 2024). The Illustration was generated with Biorender.

List of Figures

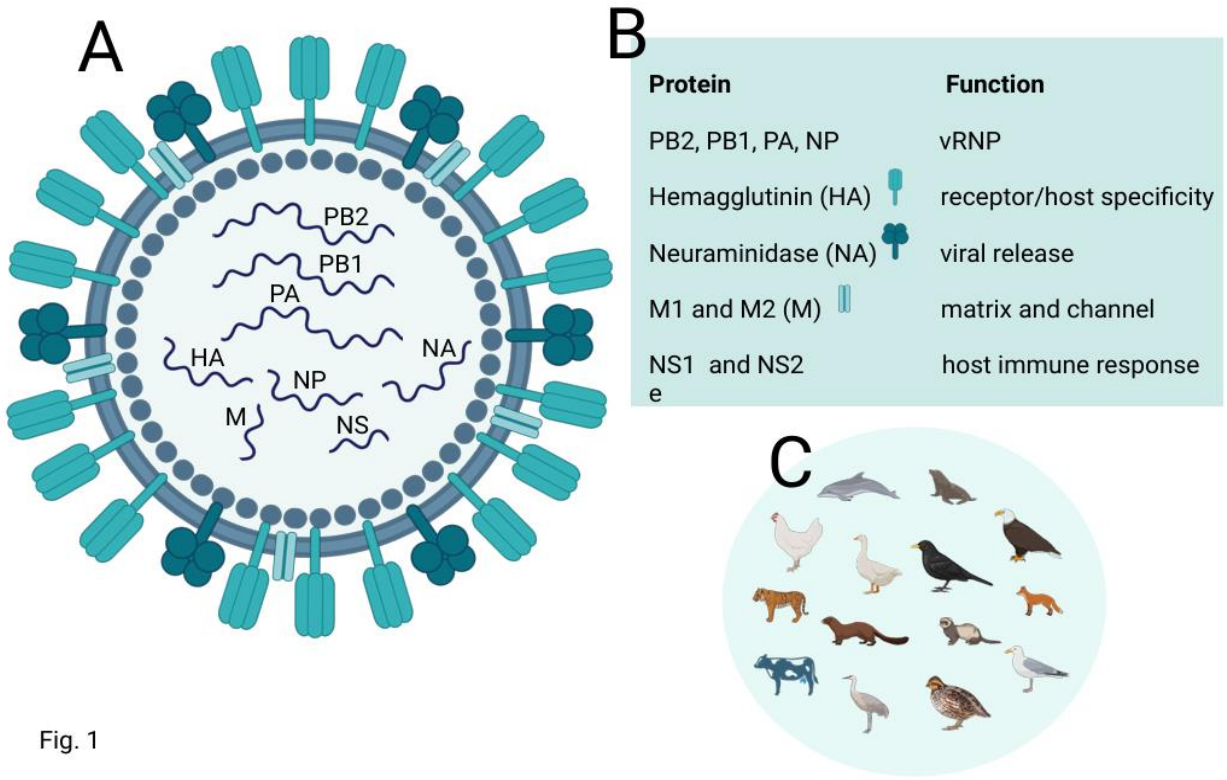

Fig. 1

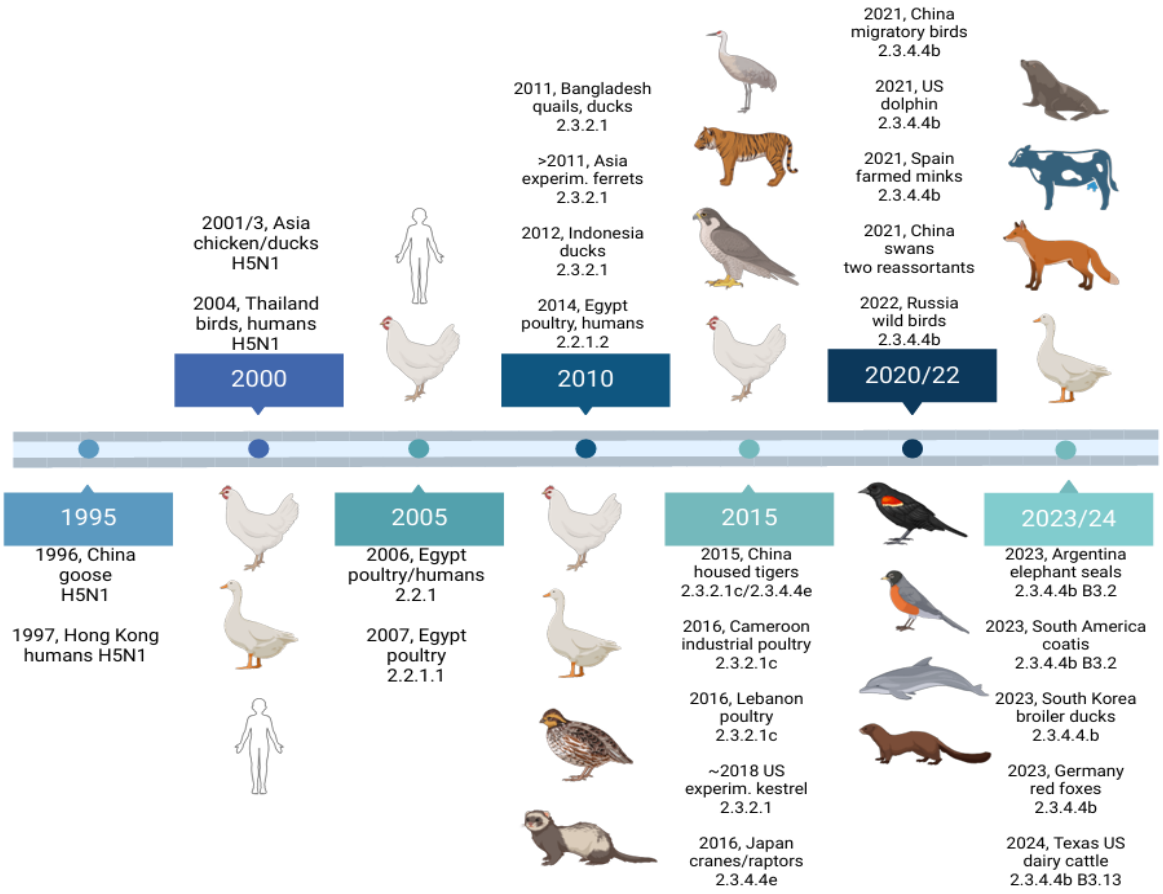
1995
1996, China goose H5N1
1997, Hong Kong humans H5N1
2000
2001/3, Asia chicken/ducks H5N1
2004, Thailand birds, humans H5N1
2005
2006, Egypt poultry/humans 2.2.1
2007, Egypt poultry 2.2.1.1
2010
2011, Bangladesh quails, ducks 2.3.2.1
>2011, Asia experim. ferrets 2.3.2.1
2012, Indonesia ducks 2.3.2.1
2014, Egypt poultry, humans 2.2.1.2
2015
2015, China housed tigers 2.3.2.1c/2.3.4.4e
2016, Cameroon industrial poultry 2.3.2.1c
2016, Lebanon poultry 2.3.2.1c
~2018 US experim. kestrel 2.3.2.1
2016, Japan cranes/raptors 2.3.4.4e
2020/22
2021, China migratory birds 2.3.4.4b
2021, US dolphin 2.3.4.4b
2021, Spain farmed minks 2.3.4.4b
2021, China swans two reassortants
2022, Russia wild birds 2.3.4.4b
2023/24
2023, Argentina elephant seals 2.3.4.4b B3.2
2023, South America coatis 2.3.4.4b B3.2
2023, South Korea broiler ducks 2.3.4.4.b
2023, Germany red foxes 2.3.4.4b
2024, Texas US dairy cattle 2.3.4.4b B3.13

Figure 2.

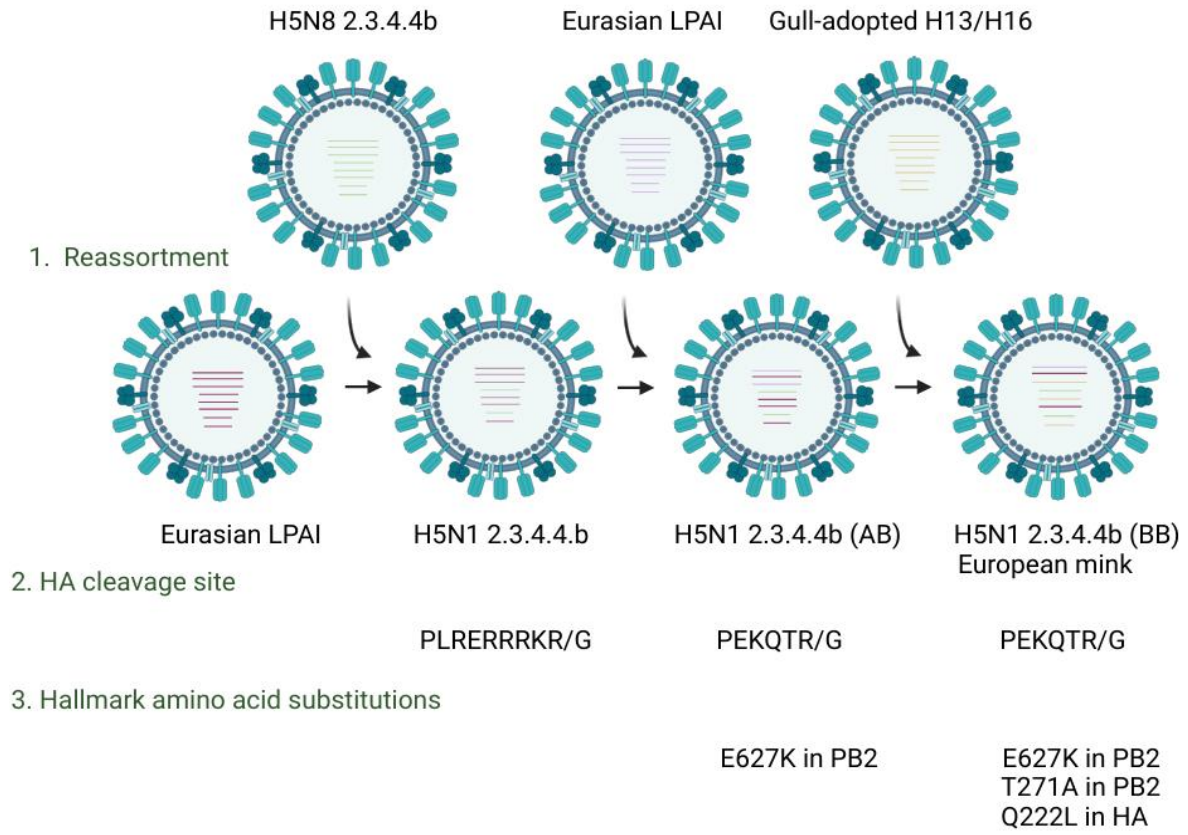
H5N8 2.3.4.4b
Eurasian LPAI
Gull-adopted H13/H16
1. Reassortment
Eurasian LPAI
H5N1 2.3.4.4.b
H5N1 2.3.4.4b (AB)
H5N1 2.3.4.4b (BB)
European mink
2. HA cleavage site
PLRERRRKR/G
PEKQTR/G
PEKQTR/G
3. Hallmark amino acid substitutions
E627K in PB2
E627K in PB2
T271A in PB2
Q222L in HA

Figure 3.

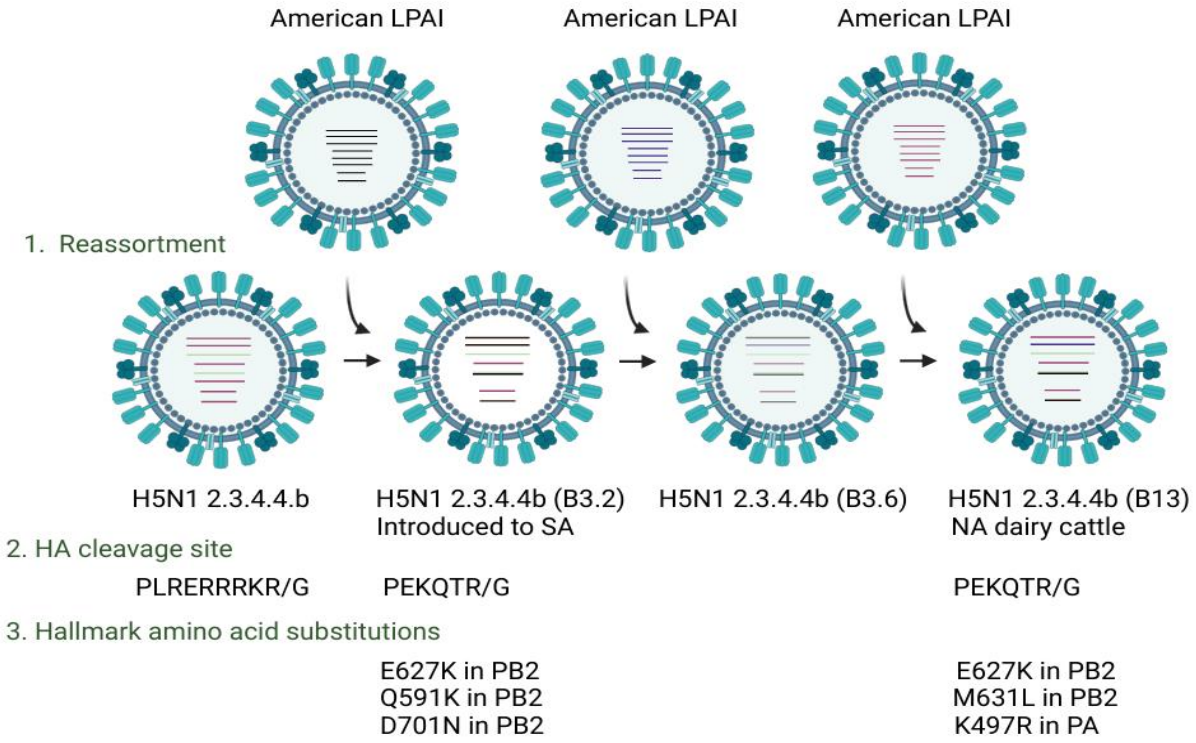
American LPAI
American LPAI
American LPAI
1. Reassortment
H5N1 2.3.4.4.b
H5N1 2.3.4.4b (B3.2)
Introduced to SA
H5N1 2.3.4.4b (B3.6)
H5N1 2.3.4.4b (B13)
NA dairy cattle
2. HA cleavage site
PLRERRRKR/G
PEKQTR/G
PEKQTR/G
3. Hallmark amino acid substitutions
E627K in PB2
Q591K in PB2
D701N in PB2
E627K in PB2
M631L in PB2
K497R in PA

Figure 4.

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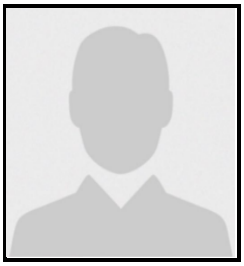
Biography of Author



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