Perspective

The global H5N1 influenza panzootic in mammals

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Influenza A viruses have caused more documented global pandemics in human history than any other pathogen[1,2]. High pathogenicity avian influenza viruses belonging to the H5N1 subtype are a leading pandemic risk. Two decades after H5N1 'bird flu' became established in poultry in Southeast Asia, its descendants have resurged[3], setting off a H5N1 panzootic in wild birds that is fuelled by: (1) rapid intercontinental spread, reaching South America and Antarctica for the first time[4,5]; (2) fast evolution via genomic reassortment[6]; and (3) frequent spillover into terrestrial[7,8] and marine mammals[9]. The virus has sustained mammal-to-mammal transmission in multiple settings, including European fur farms[10,11], South American marine mammals[12–15] and US dairy cattle[16–19], raising questions about whether humans are next. Historically, swine are considered optimal intermediary hosts that help avian influenza viruses adapt to mammals before jumping to humans[20]. However, the altered ecology of H5N1 has opened the door to new evolutionary pathways. Dairy cattle, farmed mink or South American sea lions may have the potential to serve as new mammalian gateways for transmission of avian influenza viruses to humans. In this Perspective, we explore the molecular and ecological factors driving the sudden expansion in H5N1 host range and assess the likelihood of different zoonotic pathways leading to an H5N1 pandemic.

In recent years, H5N1, which was once mainly confined to Asia and poultry, has spread globally (Fig. 1) and into new species of mammals (Fig. 2), endangering wildlife, agricultural production and human health. This spread began in 2020, when a new genotype of H5N1 viruses belonging to clade 2.3.4.4b spread rapidly in wild birds[3] from Europe to Africa[21–23], North America[24,25], South America[5,12] and the Antarctic[4]. The arrival of H5N1 in North America seemed to be manageable at first. In 2014, when an earlier H5 virus was introduced to North America from Asia[26,27], US poultry farmers successfully eliminated the virus through intensive monitoring and culling of 50 million chickens and turkeys, ending the largest foreign animal disease outbreak in US history[28,29]. This time, despite the USA culling around 90 million domestic birds since 2022, poultry outbreaks continue to be reseeded from wild birds[30]. Wild birds also introduced H5N1 to dairy cattle and marine mammals. Images of seal carcasses on Argentine beaches and spoiled milk on H5N1-affected dairy farms emphasize that the 2.3.4.4b H5N1 panzootic is different from previous ones and indicate that the strategies used to control previous panzoonotics are not working.

The panzootic 2.3.4.4b H5N1 viruses that are circulating in wild birds are genetically different from previous strains owing to 'genomic reassortment', an evolutionary process that occurs in viruses with segmented genomes. When two or more viruses co-infect a single host, they can swap entire segments of their genomes during genome replication to create novel hybrids[31]. The reassortment event between 2.3.4.4b H5N8 and low-pathogenicity avian influenza (LPAI) viruses that generated the panzootic 2.3.4.4b H5N1 virus is believed to have occurred in Europe or central Asia around 2020[3,21]. The H5N8/LPAI reassortment event combined polymerase and surface proteins derived from different lineages (Fig. 3). Subsequent H5N1/LPAI reassortment events in Europe generated the AB and BB genotypes[21,32] (Fig. 3). Why Europe recently became a major source of new H5 reassortants, shifting the centre of H5 evolution west from Asia, is not clear. The westward shift continued when H5N1 arrived in the Americas and reassorted with LPAIs that circulate in the Western hemisphere[6,24], creating new reassortant genotypes such as B3.2 and B3.13 that infected South American marine mammals and US dairy cattle, respectively (Fig. 3). Understanding how this burst of new genotypes changes the capacity of H5N1 to switch to mammalian hosts, including humans, remains an active area of research.

In this Perspective, we review what has been learned about influenza A virus (IAV) spillover and H5N1 pandemic potential from three H5N1 case studies, in which evidence supports mammal-to-mammal transmission, including in fur farms in Europe, marine mammals in South America and dairy cattle in the USA. We examine how recent changes in the ecology and molecular evolution of H5N1 in wild and domestic birds increases opportunities for spillover to mammals. We evaluate the likelihood of

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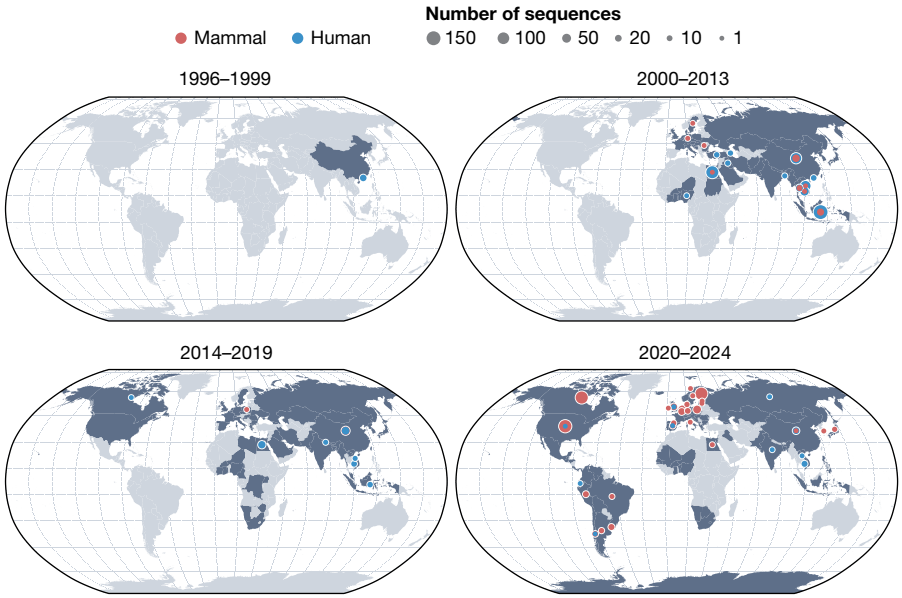

Fig. 1 | Geographical distribution of HPAI H5 viruses sampled in birds and mammals between 1996 and 2024. Dark grey shading indicates countries with HPAI H5 virus sequences that are available on the GISAID database, specifically from the A/goose/Guangdong/1/1996(H5N1) (Gs/Gd) lineage that emerged in China in 1996. Blue (human) and red (non-human mammals) circles are sized in proportion to the number of H5 GISAID sequences from that country and time period. Map made with Natural Earth.

various evolutionary pathways that could turn H5N1 into a pandemic virus. Finally, we identify research gaps that need to be addressed to design evidence-based control strategies for high pathogenicity avian influenza (HPAI) in domestic poultry, livestock and humans.

The current H5N1 panzootic in mammals

H5N1 often arrives silently in a new country or continent, brought by migrating aquatic wild birds that are the primary reservoir host for avian

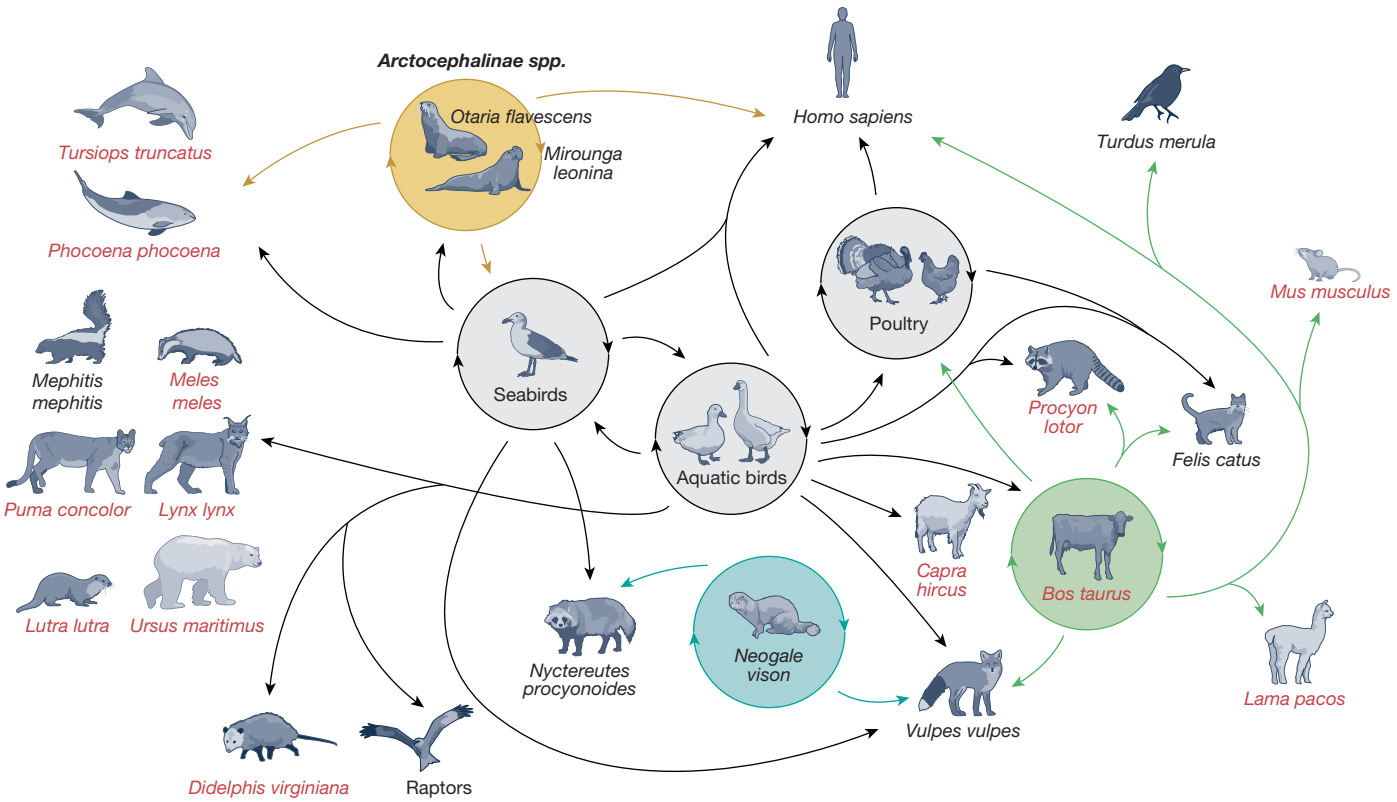

Fig. 2 | Multi-host ecology of H5N1 clade 2.3.4.4b since 2020. Wild aquatic birds (such as ducks, geese and swans) are the natural reservoir hosts for H5N1. Arrows indicate spillover into other host species. Cyclic arrows indicate sustained H5N1 transmission in that host species. New mammalian H5N1 hosts with sustained transmission are highlighted in yellow (South American marine mammals), green (US dairy cattle) and blue (European mink), with arrows in the same colours depicting spillovers from those mammalian outbreaks into additional species, possibly via unsampled intermediaries. Animals labelled in red are host species in which IAV has been detected for the first time during this outbreak (based on genetic sequence data, not serology).

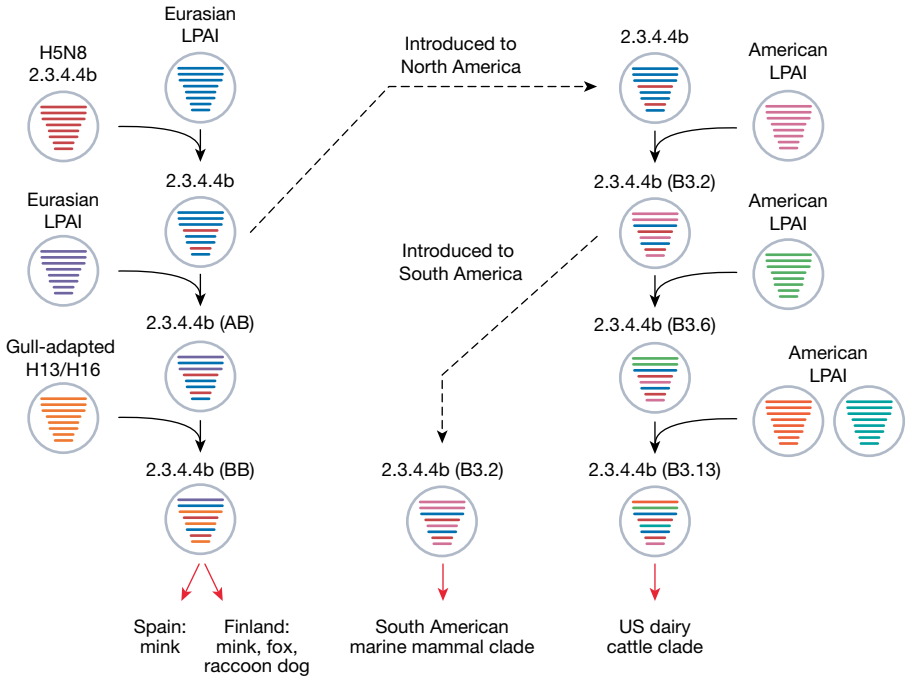

Fig. 3 | Genomic reassortment events in birds leading up to four H5N1 spillover events in mammals. Each circle represents a genotype, with eight bars representing the eight RNA segments of the IAV genome, ordered from longest to shortest, encoding PB2, PB1, PA, HA, nucleoprotein, neuraminidase, matrix protein and nonstructural protein, respectively. Each segment is shaded by lineage. Solid black arrows indicate donors during genomic reassortment events. Dashed black arrows indicate intercontinental migration events. Red arrows indicate spillover events into mammals.

influenza viruses (AIVs) and often do not display symptoms[33] (Fig. 2). An early sign of H5N1 arrival is dead poultry[25]. Mass die-offs can occur in social seabirds that congregate in large, dense colonies—for example, gannets in Europe[34] or penguins in Chile[35]. Birds of prey[36,37] (such as hawks, eagles and vultures) and terrestrial carnivores[7,8,38,39] (such as foxes, raccoons and bobcats) that scavenge dead H5N1-infected birds can die, often with neurological symptoms (Fig. 2). Most mammalian cases are 'dead-end' infections, with very little evidence of onward transmission to additional hosts. Laboratory experiments have shown that pre-2.3.4.4b H5N1 viruses could be transmitted from mammal to mammal by the respiratory route after serial passage in ferrets selected for mammalian-adapted mutations[40,41]. However, whether such strong selective pressures have existed in any real-world field settings remained unclear. Here we describe three field settings in which 2.3.4.4b viruses acquired key adaptive mutations that enabled the viruses to sustain mammal-to-mammal transmission. The 2022–2023 H5N1 outbreaks on European fur farms were successfully contained by culling, the 2023 South American marine mammal-adapted virus may still be percolating, and the 2024 US dairy cattle outbreak has developed into an ongoing problem for cattle, poultry and farm workers.

H5N1 transmission on fur farms in Europe

The first compelling evidence that H5N1 could spread from mammal to mammal in field settings came in October 2022 from a mink farm in Spain[10] (Table 1). A second larger H5N1 outbreak occurred from July to December 2023 on 71 fur farms in Finland that affected American mink (6 farms), arctic foxes (64 farms) and raccoon dogs[11,42] (5 farms). Known mammalian adaptations in the viral polymerase were found in viruses collected from farmed animals in both countries, including polymerase basic protein 2 (PB2) substitutions PB2(T271A)[43] on the Spanish mink farm and PB2(E627K)[44] in two phylogenetically distinct clusters in Finland[11]. Mammal-to-mammal transmission was suspected on the basis of the close genetic relatedness of the viruses found on different farms. Experimental studies confirmed that the viruses could transmit efficiently between ferrets that were in direct contact[45,46]. Farm-to-farm transmission was thought to have occurred through movement of contaminated equipment, clothing or infected carcasses fed to other mink[11]. Lingering gaps in surveillance and testing nevertheless obscure a complete picture of how much H5N1 transmission occurred within European mink farms, which were ultimately controlled by large-scale depopulation of tens of thousands of animals on infected farms[42].

Genetic sequencing revealed that the H5N1 viruses from the fur farm outbreaks in Spain and Finland both belong to the reassortant H5N1 genotype, BB (Fig. 3), that emerged in 2022 and caused mass die-offs in black-headed gulls throughout Europe[11,21]. The BB genotype contains five genome segments from H5N1 genotype AB and three segments from LPAI gull-adapted H13 and H16 lineages[47]. Gulls are opportunistic scavengers that visit farms, undeterred by the presence of other animals, and H5N1-infected gulls may have introduced the virus into fur farms while pilfering feed from animal sheds[42]. The emergence of a gull-adapted H5N1 BB reassortant warrants higher biosecurity and surveillance on European mink farms. Current H5N1 surveillance largely targets dead or severely ill animals, and serosurveys would be helpful to assess on how well mink, gulls and other species tolerate H5N1 infection and escape detection. Although there have been no reported H5N1 outbreaks in mink in Poland, Europe's largest mink producer, nor H5N1 testing, it was speculated that raw pet food sourced from mink farms could be a possible source of a H5N1 virus that killed more than 30 domestic cats in Poland in mid-2023, including some that lived entirely indoors[48]. The H5N1 viruses sequenced from the cats had identical mammalian adaptations[49] that were not seen in avian viruses that were circulating in Europe at the time, raising the possibility of cryptic transmission in mammals with mild symptoms.

Transmission in South American marine mammals

The arrival of a new North American reassortant H5N1 genotype (B3.2) into South America in late 2022 had a devastating impact on coastal birds and marine mammals[35,50]. The first H5N1 fatalities in South

Table 1 | H5N1 clade 2.3.4.4b outbreaks in mammals

Index species	Domestic or wild	Date	Duration	Location	Suspected source	H5N1 genotype	Reported animal deaths	Control strategy	PB2 mammalian adaptations	Mammal-to-mammal transmission	Spillover to other species	Zoonotic cases (detected)	References
Harbour (*Phoca vitulina*) and grey (*Halichoerus grypus*) seals	Wild	June 2022	< 1 month	ME, USA	Wild seabirds	Panzootic H5N1 2.3.4.4b	10	None	E627K (1 virus)	Unlikely	None	None	9
American mink (*Neovison vison*)	Domestic	October 2022	< 1 month	Galicia, Spain	Gulls	Gull reassortant genotype BB	>50,000 depopulated	Depopulation	T271A	Likely, within farm	None	None	10
South American sea lion (*Otaria flavescens*)	Wild	February to November 2023 (may be ongoing)	> 8 months (possibly ongoing)	South America (Argentina, Brazil, Chile, Peru and Uruguay)	Wild seabirds	American LPAI reassortant B3.2	>10,000	None	Q591K D701N	Likely, across 5 countries	Elephant seal, fur seal, Chilean dolphin, porpoise, human	1	12–15
Cat (*Felis catus*)	Domestic	June 2023	< 1 month	Poland	Raw pet food	Eurasian LPAI reassortant CH	<50	None	K526R E627K	Unlikely	None	None	48
American mink (*Neovison vison*)	Domestic	July to December 2023	6 months	Finland	Gulls	Gull reassortant genotype BB	70 farms depopulated	Depopulation	E627K	Likely, between farms	Arctic foxes, raccoon dogs	None	11,42
Dairy cattle (*Bos taurus*)	Domestic	February 2024 to present	>7 months (ongoing)	15 US states (CA, CO, IA, ID, KS, MI, MN, NC, NM, OH, OK, SD, TX, UT and WY)	Wild birds	American LPAI reassortant B3.13	Unknown (>50)	Test lactating cattle before interstate movement; quarantine infected cows	M631L	Extensive	Domestic cat, raccoon, fox, poultry, wild birds, alpaca, human	13	16,18,19,60

A summary of seven H5N1 clade 2.3.4.4b outbreaks in mammals that infected at least ten animals. The strength of evidence for mammal-to-mammal transmission is based on: (1) phylogenetic clustering of viruses collected from mammals together in a single clade, separate from avian viruses; (2) whether viruses from mammals have the same mammalian adaptations in PB2; and (3) the availability of well-sampled genetic sequence data. The primary control strategy is listed as of June 2024.

American sea lions were reported in Peru[12,51] and Chile[13] in early 2023. H5N1 spread down the west coast of South America from Peru and Chile to the southern tip of Patagonia and up the east coast through Argentina, Uruguay and Brazil (Table 1), leaving a trail of sea lion carcasses. The immediate question was whether the marine mammal die-offs were linked and represented sustained mammal-to-mammal transmission of H5N1 in marine mammals or were introduced independently from seabirds. Mammal-to-mammal transmission can be difficult to prove in the field, especially when there are few background available sequences from wild birds. The strongest prior evidence for mammal-to-mammal transmission of IAVs in marine mammals comes from the 2014–2015 outbreak of low-pathogenicity H10N7 viruses affecting harbour seals in Denmark, the Netherlands and Germany[52–54]. An outbreak of H5N1 occurred in New England seals in June 2022, but most sequenced viruses lacked mammalian adaptations and appeared to be independent spillovers from birds[9].

As more H5N1 viruses were sequenced from marine mammals in South America over the course of 2023, evidence accrued in support of mammal-to-mammal transmission. Five independent research groups collected H5N1 viruses from marine mammals in Peru[12], Chile[13], Argentina[14], Uruguay[15] and Brazil[55] with the same unusual combination of two mammalian adaptations in PB2, D701N and Q591K[56], plus other distinctive mutations that were not present in birds. Moreover, the marine mammal viruses all formed a single clade on a phylogenetic tree, separate from viruses from wild birds and poultry. The spatial-temporal pattern of wave-like spread down the west coast and up the east coast further supported mammal-to-mammal transmission in South America. Still, little is known about the mode of transmission between marine mammals (environmental, direct contact, respiratory or oral–faecal) or which pinniped species serves as the primary host. B3.2 viruses in the marine mammal clade have been identified in South American sea lions, common dolphin, Chilean dolphin, porpoise, sea otter, fur seal, elephant seal and one human[15]. The hospitalized man (A/Chile/25945/2023(H5N1)) lived near a beach with H5N1-infected animals and his virus contains the same two PB2 mammalian adaptations that were found in pinnipeds, consistent with environmental transmission[57]. Spillback of B3.2 viruses from marine mammals to wild birds was also reported in Chile[13], Argentina[14] and in the South Atlantic[14,15], more than 450 km off the coast of mainland South America, with no reversions seen in the mammalian-adapted PB2 mutations. It remains to be seen whether wild birds will carry and potentially disperse mammalian-adapted B3.2 viruses over long distances, possibly to the megafauna of Antarctica or to poultry and terrestrial mammals inland.

The 2024 H5N1 outbreak in US dairy cattle

Starting in February 2024[11], Texas dairy farmers noticed unexplained falls in milk production in lactating cattle and thick, yellow milk, which

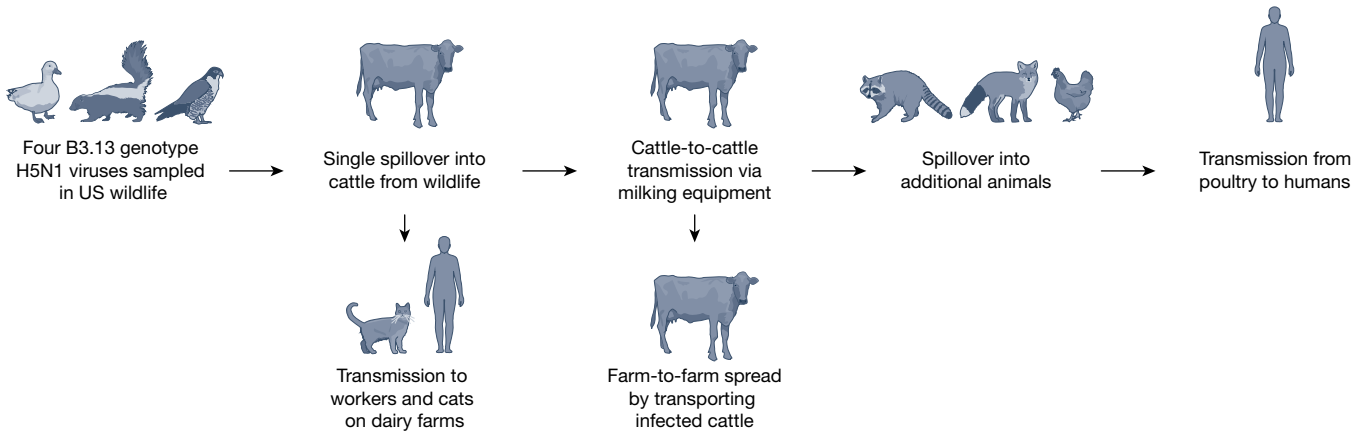

Fig. 4 | Leading hypotheses for the source and spread of the H5N1 outbreak in bovines. The most likely routes of H5N1 transmission between wildlife, domestic animals and humans are inferred from currently available genomic and epidemiological data.

was later accompanied by dead cats on several farms. Bovines were not considered permissive hosts for IAV, so hundreds of other potential agents were screened before H5N1 was identified as the cause of disease. All cattle viruses belong to the B3.13 genotype (Fig. 3) and are positioned in a single phylogenetic clade, which supports a single introduction from wild birds into cattle that is estimated to have occurred in late 2023 or early 2024[16,18]. Only four B3.13 genotype viruses have been identified in US wildlife (Canada goose, peregrine falcon and skunk; Fig. 4) that fall outside the cattle clade[16,18], suggesting that this genotype is rare in wild birds. It remains unclear why B3.13, as opposed to other genotypes that are more common in birds, made the jump to cattle. Two mammalian adaptations are found in the cattle clade, but not in the ancestral B3.13 viruses in wildlife, that improve virus replication in mammals: PB2(M631L) and polymerase acidic protein (PA) substitution PA(K497R)[58,59] (Table 1).

The high genetic diversity of the H5N1 virus in Texas cattle suggests that the bovine B3.13 outbreak originated in TX and rapidly spread to other states (15 in total as of November 2024: TX, NM, OK, CO, KS, ID, WY, SD, MI, IA, MN, OH, NC, CA and UT). In April–May 2024, more than one-third of retail pasteurized milk samples from 12 US states contained H5N1 genetic fragments that present no danger to humans, but indicate the widespread distribution of the virus in dairy cattle[17].

The virus is likely to have spread by transport of infected cattle or equipment[16,60,61] (Fig. 4). High viral titres in milk and the mammary tissue tropism of the virus suggest a role for milk in transmission[60,62,63]. Large numbers of infectious particles are generated when milk is expressed from the udder. Contaminated milking machinery is thought to be an important mode of H5N1 transmission between cattle from the same farm[62] (Fig. 4). However, respiratory tract infection has not been ruled out.

Bovine-origin H5N1 viruses have been detected in other species, including domestic cats, alpacas, wild birds that congregate in barns (such as grackles and blackbirds), terrestrial mammals (such as foxes, raccoons and mice) and poultry[16,18,19,60] (Fig. 4). Spillover from cattle to domestic barn cats probably occurs through ingestion of contaminated, unpasteurized milk[19]. Scavenging dead birds is also a way for cats to become infected, along with foxes, raccoons and other carnivores. It is less clear how wild birds, alpacas or poultry became infected, although fomite transmission, possibly involving workers' clothing and equipment, has been suggested. As of 26 July 2024, 13 documented human cases have been identified in association with the B3.13 bovine strain, including 4 dairy workers from TX, MI and CO and 9 poultry workers from CO who were infected by chickens carrying the bovine strain[64] (Fig. 4). Human infections present primarily as conjunctivitis[65], similar to past H7 human infections in the Netherlands[66,67]. Fewer than 20 human cases of 2.3.4.4b H5N1 viruses have been documented in Europe and the Americas since 2020[68], which is a low number compared to the 145 H5N1 human cases recorded in Asia and Egypt in 2015, where infections were often acquired from poultry in live animal markets or when domestic flocks were defeathered[69]. Accordingly, the US Centers for Disease Control and Prevention (CDC) Influenza Risk Assessment Tool (IRAT) and the World Health Organization Tool for Influenza Pandemic Risk Assessment (TIPRA) estimate a low pandemic risk for H5N1 2.3.4.4b viruses[70]. Note that these tools assess current risk and do not consider the future evolutionary potential of H5N1, including the range of directions in which H5N1 could mutate, switch host or reassort, based on decades of prior observations of IAV.

How H5N1 could become a pandemic

For an influenza virus to start a pandemic it must fulfil two key criteria. First, the main attachment glycoprotein, haemagglutinin (HA) (Fig. 5a), must be antigenically novel and poorly recognized immunologically by a large fraction of the human population. All 17 HA subtypes[71] (Fig. 5b) maintained in wild aquatic birds meet the first criterion. Antigenic novelty is especially high for subtypes such as H5, that have never circulated in humans and to which there is only limited evidence for cross-subtype immunity. Many AIVs can replicate and cause disease in mammalian hosts without prior adaptation, but few achieve the second criterion: efficient transmission between humans, with a reproductive number greater than one[72]. Experimental research shows that AIV must change in at least three ways to support transmission among mammals[73]. The first change is in the viral polymerase (PB2, PB1 and PA proteins) that helps the virus exploit mammalian host machinery to replicate. A second change must occur in HA to help the virus bind strongly to cell surface receptors abundant in the human upper respiratory tract. The third change must stabilize the HA protein to tolerate lower pH to prevent destruction of the virus when transiting between hosts through the air[74]. Several other virus adaptations have been described that are also likely to modulate pandemic potential[75–77].

Mammalian adaptations arise readily in the polymerase

All viruses must commandeer resources from host cells to copy their genomes. At least four mutations in the AIV polymerase PB2 protein enable the virus to use mammalian ANP32 proteins[78], histone chaperone proteins that helps synthesize viral RNA in the host cell's nucleus to produce new viruses: E627K[44,79], Q591K/R[56], D701N and M631L[56,58,80]. The evolutionary barrier to this AIV adaptation appears to be low, as these PB2 mutations have emerged rapidly and repeatedly following H5N1 spillover to mammals: M631L[16] in cattle, E627K[42] in several Finnish mink farms, and Q591K and D701N[12] in South American marine

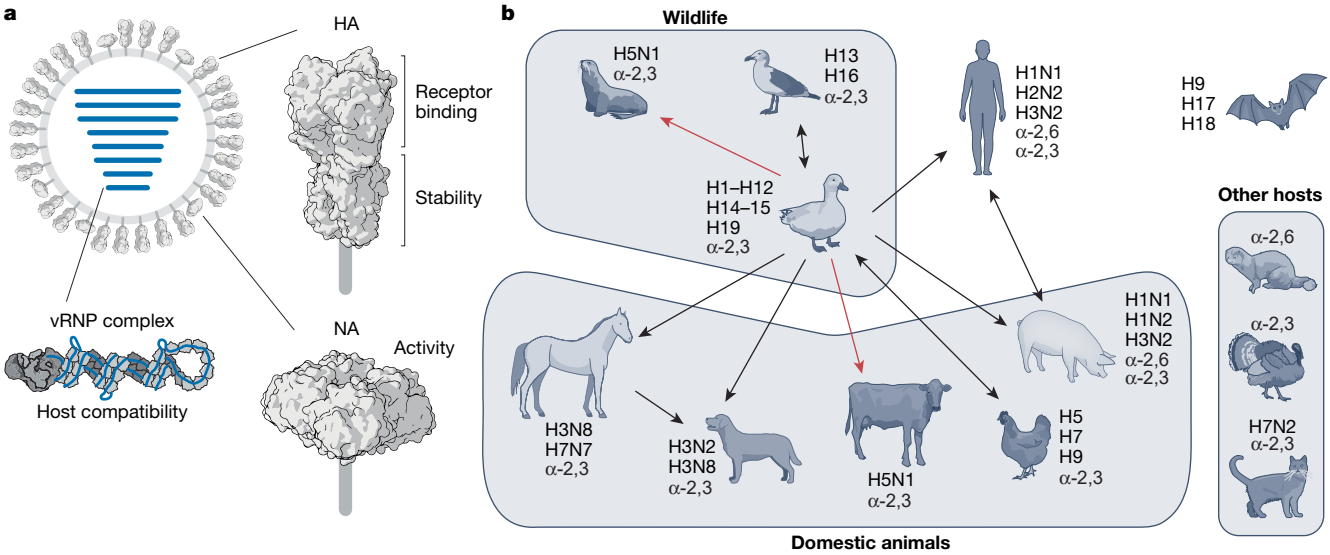

Fig. 5 | How IAVs adapt to new host species. a, Molecular features of IAV that are known to affect host range. The viral ribonucleoprotein (vRNP) complex includes the PB2, PB1 and PA polymerase proteins, nucleoprotein and viral RNA. NA, neuraminidase. **b**, Wild aquatic birds are the natural reservoir for IAV, maintaining 17 HA subtypes[71] that occasionally spillover into other species and can establish new host-specific lineages (black arrows). H7N7 and H3N8 represent subtypes that are extinct. Less than one year of data is available for recent H5N1 spillovers (red arrows). The main form of sialic acid receptor that HA binds in different hosts is indicated as α-2,3 or α-2,6. The full complexity of glycans that act as IAV receptors across species is not depicted, although differentiation between upper (α-2,6) and lower (α-2,3) respiratory tract receptors for swine and humans is shown. Other IAV hosts that experience sporadic outbreaks without long-term sustained transmission are listed on the right.

mammals. The PB2(T271A) mutation seen in Spanish mink is also suspected to be involved in mammalian adaptation, but its phenotype is less characterized.

Evolutionary constraints on HA

To gain entry into host cells, most influenza viruses attach via the HA protein to carbohydrates on the cell surface that are decorated with sialic acid receptors. These receptors come in different forms and have different distributions in birds, humans and other mammalian species (Fig. 5b). The α2,3-linked form is abundant in avian tissues[81], the bovine mammary gland[82], the human lower respiratory tract[83] and the human eye[84] (conjunctiva). Whereas the documented human spillovers of cattle-derived H5N1 have mostly involved conjunctivitis, previous H5N1 cases in humans infected the lower respiratory tract, which probably contributed to severe disease[85]. To transmit efficiently by the respiratory route, influenza viruses must replicate in the upper respiratory tract[86,87]. Therefore, a major evolutionary hurdle for AIVs to gain pandemic potential is the need to mutate the HA receptor-binding domain to switch receptor binding to glycans with α2,6-linked sialic acids, which are abundant in the human upper respiratory tract[88].

Compared with adaptation of the polymerase, change in the HA receptor-binding phenotype appears to be more constrained for H5N1 viruses. Mutations that allow binding to α2,6-linked receptors have been identified in laboratory experiments: N224K[41,89], Q226L[89,90] and G228S[90]. Combinations of these mutations are needed for efficient airborne transmission in ferrets, a model for humans[40,41]. Crucially, these mutations have not arisen widely during any H5N1 outbreak, even where we might expect there to be strong selective pressure[91], such as in farmed mink[10,42], which have a high proportion of α2,6-linked receptors in the upper respiratory tract[92]. Human-like α2,6-linked receptors also appear to be present in the bovine mammary gland[93], although possibly not in a form that can be utilized by H5N1[82], and there does not appear to be strong selective pressure for H5N1 in bovines to use human-like α2,6-linked sialic acids[94–96]. However, an HA substitution in bovine appears to expand α2,3-linked binding breadth of H5N1[94], and continued monitoring of molecular changes in receptor-binding sites is warranted.

The third property of AIVs that is known to influence pandemic potential is HA stability. HA, like nearly all viral fusion glycoproteins, is synthesized in a meta-stable form. Exposure to acidic pH triggers changes in HA that are needed to complete viral entry into cells by fusing host and viral membranes during endocytosis[97]. However, HA is easily triggered prematurely, which destroys viral infectivity. To efficiently transmit from human to human, HA needs to be stable and triggered only at more acidic pH, so that it survives the acidic microenvironment of airborne particles and mammalian respiratory secretions[41,42]. Mutations that affect HA stability occur throughout the protein[89], making this phenotype difficult to predict on the basis of sequence alone. Thus, although current evidence does not suggest the HA stability of panzootic H5N1 has changed[98], this phenotype requires close monitoring in clusters of mammalian cases that might be associated with airborne spread such as in sea lions[15], mink[45] and cattle[16].

Although the requirement for several mutations in the polymerase, HA and other genes to occur in tandem make the evolution of a pandemic virus less likely[99], genomic reassortment provides an evolutionary shortcut[100,101]. To retain antigenic novelty, the reassortant virus would need to retain the avian H5 while acquiring other genome segments. Therefore, a key constraint in the evolution of pandemic viruses is that HA receptor binding and stability must evolve through mutation alone.

Risk of H5N1 reassortment with mammal viruses

Horses[102], dogs[103,104], pigs[105], humans[106], poultry[107] and wild birds[33] are long-time reservoir hosts for IAV (Fig. 5b). However, the mammalian species that are infected by 2.3.4.4b H5N1 viruses (such as mink, marine mammals, bovines, foxes, raccoons and domestic cats; Fig. 2) are not. Influenza D viruses are enzootic in cattle, but this virus is too distinct from IAV for reassortment to occur[108]. There is some serological evidence of sporadic IAV infections in cattle over the years, but these appear to be rare and never sustained[109]. Turkeys[110] and farmed mink[111] have α2,6-linked sialic acids[112,113] that make them susceptible

to human and swine viruses[114], but viruses of human and swine origin are not maintained in turkeys or mink long term. Marine mammals are frequent spillover hosts for AIV[115], but these LPAIs also are generally not maintained long term. Mammalian wildlife tend to be incidental hosts, whereas intensive farming is more likely to promote viral amplification, endemicity and evolution. Thus, the present host range of H5N1 limits opportunities for reassortment with other mammalian-adapted viruses.

However, this could change. As autumn approaches in the Northern Hemisphere, so does the influenza season. A farm worker coinfected with H5N1 and a human seasonal virus presents an opportunity for avian and human IAVs to reassort and combine many of the traits needed to spread efficiently in humans, as occurred prior to the 1957 H2N2 and 1968 H3N2 pandemics[31]. H5N1 spillover into swine, which appear to be suitable hosts for H5N1 in experimental studies[116,117], would present additional opportunities for reassortment[105,118], as exemplified by the triple-reassortant swine-origin H1N1 pandemic virus from 2009[2]. Influenza spillover from cattle to swine is a known possibility because it already occurs in this direction for influenza D viruses, in the USA as well as other countries[119]. The continued absence of H5N1 in US swine is highly fortunate.

Poultry vaccination for H5N1

The prospect of H5N1 becoming enzootic in Europe and the Americas is a turning point for HPAI and new control strategies are needed, including vaccination. Currently, there is no oral H5N1 vaccine that could be mass administered to wildlife, similar to the rabies vaccine[120]. Influenza vaccines are licensed for poultry that reduce disease burden, but do not prevent infection and have varying degrees of success[121]. The large-scale national vaccination programme in poultry in China has been credited with controlling H5 and H7 and reducing zoonosis[122,123]. However, vaccination campaigns have been less successful in controlling H6N2 in South Africa or H5N2 in Mexico, which recently reported a zoonotic case[124]. One concern is that vaccines could increase the difficulty of controlling HPAI by fostering silent spread and/or accelerating antigenic evolution in poultry[125–127]. Major poultry exporters in Europe, Brazil and the USA are reluctant to use influenza vaccines in poultry or cattle because products from vaccinated animals are subject to international trade restrictions. For example, when France became the first EU country to vaccinate domestic ducks for H5N1 in 2023, the USA banned duck products from France and all its trade partners, based on the perceived risk that vaccinated birds with subclinical infections could introduce H5N1 into the country.

As H5N1 becomes enzootic in wild birds globally, pressure is mounting to revisit trade restrictions designed for a different era. The World Organization of Animal Health issued a statement in 2023 that vaccinating poultry for influenza "should not be a barrier to safe trade"[128]. However, countries need to intensively monitor IAV populations in poultry and keep vaccine strains up to date, similar to what is done for IAV in humans[129]. There is hope that in the future, the NIH will succeed in its ambitious plan to develop new influenza vaccine platforms for humans that broadly protect against all genetically diverse IAV strains[130], providing more effective vaccine platforms for animal influenza vaccines as well. However, these products are still in early stages of research.

Possible elimination of H5N1 in US dairy cattle

Two features of the H5N1 outbreak in bovines make eradication feasible. First, most transmission appears to occur through a defined pathway via milking machinery[62] instead of the more diffuse respiratory route. Hygiene and biosecurity improvements could potentially break transmission. Second, spillover from wild birds into dairy cattle appears to be rare[16,18]. If US dairy farmers could manage to eliminate the current H5N1 outbreak through a combination of biosecurity, testing, quarantine, real-time genomic epidemiology and possibly vaccination and/or culling, the virus may not return from wild birds. However, six months into the outbreak, it may already be too late.

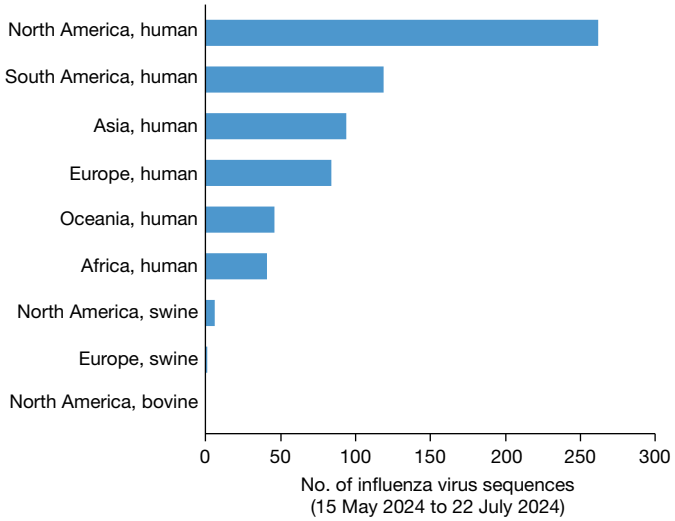

Fig. 6 | Number of published influenza virus genome sequences collected between 15 May 2024 to 22 July 2024. Bars indicate the number of influenza viruses collected from humans and animals in recent months (15 May 2024 to 22 July 2024) that are available in the GISAID database (downloaded on 22 July 2024). Numbers do not include viral sequences that have been submitted to the Sequence Read Archive for which the collection date is unknown.

US dairy farmers have not previously dealt with IAV or deadly bovine diseases such as rinderpest and bluetongue that shaped cattle biosecurity across other continents in recent decades[131]. Previous generations of US cattle producers eradicated foot-and-mouth disease by rapidly sharing epidemiological data[132]. During the 2024 H5N1 outbreak in bovines, months of missing data (Fig. 6) leave researchers, veterinarians and policy makers in the dark. Without data, it is not possible to identify the source of new outbreaks through phylodynamic analysis. H5N1 is a reportable disease in poultry, but not mammals, and the USDA requires H5N1 testing only in lactating cattle prior to interstate movement. Poultry farmers must depopulate the entire flock, sometimes millions of birds, each time B3.13 spills over from bovines, but there are no requirements for dairy farms to even test for the disease. In July 2024, CO became the first state to require weekly testing for H5N1 in bulk milk tanks on dairy farms[133].

Human H5N1 infection

US public health agencies have tested more than 200 people who were exposed to H5N1-infected animals between 24 March 2024 and 26 July 2024[134] and identified 13 confirmed cases. A small serosurvey for H5N1 antibodies in dairy and poultry workers in MI found no asymptomatic infections among the 35 people tested[135]. However, it is not clear how many exposed workers from the 171 H5N1-infected dairy herds have not been tested[134]. Veterinarians visiting H5N1-infected dairy farms anecdotally reported suspected human cases that never received testing, including workers with and without direct contact with cattle, raising questions about whether any limited human-to-human spread occurred. Limited human-to-human spread of earlier H5N1 strains occurred in Asia but reproductive numbers always remained less than one[136]. Even short chains of human-to-human transmission raise the risk of virus adaptation to humans, particularly when multiple mutations or co-infection with seasonal viruses are needed[99,137] Picking up rare transmission chains requires intensive contact tracing among workers, family members and other contacts. For example, a CDC investigation of a 2012 zoonotic outbreak of IAV in US children

involved in show pig competitions at agricultural fairs identified suspected human-to-human transmission in a child's daycare centre[138]. Agricultural fairs continued to take place in summer 2024 across the USA, bringing dairy cattle into the same environment in which zoonotic spillover of IAV routinely occurs from swine[139]. Some fairs are requiring lactating dairy cattle to be tested for H5N1 before arrival and/or are cancelling milking demonstrations. How much H5N1 testing is done in humans or wastewater at fairs remains to be seen.

Future prospects

Stocks of H5 vaccine that are antigenically related to circulating 2.3.4.4b viruses are available and could be produced at scale using mRNA platforms if H5N1 begins spreading in humans[140]. The potential severity of a future H5N1 pandemic remains unclear. Recent human infections with H5N1 2.3.4.4b viruses have had a substantially lower case fatality rate compared with previous H5N1 outbreaks in Asia, in which around half of people with reported infections died[141]. The milder symptoms seen in US farmers have been attributed to the route of infection through the eye[65] and absence of viral pneumonia in the lung. Whether B3.13 viruses cause less severe disease in humans or whether mild cases are simply under-detected in Asia is unclear owing to case ascertainment bias[142]. Older people appear to have partial immunity to H5N1 due to childhood exposure ('imprinting') to seasonal H1N1 and H2N2 viruses, whereas people born since the 1968 H3N2 pandemic may be more susceptible to severe disease in a H5N1 pandemic[143]. Some degree of cross-reactivity between H5N1 and the avian-origin N1 neuraminidase that has circulated in humans since the 2009 pandemic may also provide partial protection[144]. At the same time, symptoms and disease severity could change if B3.13 viruses further adapt to infect the respiratory tract[145].

Going forward, we know more about the global distribution (Fig. 1), non-human host range (Fig. 2) and genetic diversity (Fig. 3) of H5N1 than of almost any other zoonotic pathogen. Still, most H5N1 testing is conducted in dead or severely ill animals. One lesson from the COVID-19 pandemic is that symptomatic cases that result in severe disease are clinically important, but unobserved subclinical infections can be important in transmission and can fuel epidemics at a population level[146]. The H5N1 panzootic has been defined by powerful images of beaches littered with sea lion carcasses or barns of ill dairy cows wasting away after going off feed. But what keeps scientists up at night is the possibility of unseen chains of transmission silently spreading through farm worker barracks, swine barns or developing countries, evolving under the radar because testing criteria are narrow, government authorities are feared or resources are thin. A second lesson from the COVID-19 pandemic is not to underestimate the importance of human behaviour, culture and economic context. New technologies such as mRNA vaccines, next-generation sequencing and CRISPR–Cas diagnostics provide rapid, flexible tools for outbreak response, but are of little use when they are not allowed on the farm.

1. Kilbourne, E. D. Influenza pandemics of the 20th century. *Emerg. Infect. Dis.* **12**, 9–14 (2006).
2. Garten, R. J. et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* **325**, 197–201 (2009).
3. Xie, R. et al. The episodic resurgence of highly pathogenic avian influenza H5 virus. *Nature* **622**, 810–817 (2023).
   This analysis identifies key changes in H5 ecology and evolution in Eurasian wild birds leading up to 2020 H5N1 panzootic.
4. Banyard, A. C. et al. Detection and spread of high pathogenicity avian influenza virus H5N1 in the Antarctic Region. *Nat. Commun.* **15**, 7433 (2024).
5. Jimenez-Bluhm, P. et al. Detection and phylogenetic analysis of highly pathogenic A/H5N1 avian influenza clade 2.3.4.4b virus in Chile, 2022. *Emerg. Microbes Infect.* **12**, 2220569 (2023).
6. Kandeil, A. et al. Rapid evolution of A(H5N1) influenza viruses after intercontinental spread to North America. *Nat. Commun.* **14**, 3082 (2023).
   This epidemiological investigation of H5N1 outbreak on a mink farm in Spain provides evidence of sustained mammal-to-mammal transmission in the field.
7. Jakobek, B. T. et al. Influenza A(H5N1) virus infections in 2 free-ranging black bears (*Ursus americanus*), Quebec, Canada. *Emerg. Infect. Dis.* **29**, 2145–2149 (2023).
8. Elsmo, E. 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.* **29**, 2451–2460 (2023).
9. Puryear, W. et al. Highly pathogenic avian influenza A(H5N1) virus outbreak in New England Seals, United States. *Emerg. Infect. Dis.* **29**, 786–791 (2023).
10. Agüero, M. et al. Highly pathogenic avian influenza A(H5N1) virus infection in farmed minks, Spain, October 2022. *Eurosurveillance* **28**, 2300001 (2023).
11. Kareinen, L. et al. Highly pathogenic avian influenza A(H5N1) virus infections on fur farms connected to mass mortalities of black-headed gulls, Finland, July to October 2023. *Eurosurveillance* **29**, 2400063 (2024).
    This study identifies gulls as the source of a large H5N1 outbreak in Finnish fur farms housing mink, foxes and racoon dogs.
12. Leguia, M. et al. Highly pathogenic avian influenza A (H5N1) in marine mammals and seabirds in Peru. *Nat. Commun.* **14**, 5489 (2023).
13. Pardo-Roa, C. et al. Cross-species transmission and PB2 mammalian adaptations of highly pathogenic avian influenza A/H5N1 viruses in Chile. Preprint at *bioRxiv* https://doi.org/10.1101/2023.06.30.547205 (2023).
14. Uhart, M. et al. Epidemiological data of an influenza A/H5N1 outbreak in elephant seals in Argentina indicates mammal-to-mammal transmission. *Nat. Commun.* **15**, 9516 (2024).
15. Tomás, G. et al. Highly pathogenic avian influenza H5N1 virus infections in pinnipeds and seabirds in Uruguay: implications for bird-mammal transmission in South America. *Virus Evol.* **10**, veae031 (2024).
    This study identified Uruguay as the fifth South American country to detect the mammal-adapted H5N1 virus in pinnipeds, building a case for long-distance mammal-to-mammal spread.
16. Nguyen, T.-Q. et al. Emergence and interstate spread of highly pathogenic avian influenza A(H5N1) in dairy cattle. Preprint at *bioRxiv* https://doi.org/10.1101/2024.05.01.591751 (2024).
    This study found that early rapid growth of the H5N1 outbreak in US dairy cattle was mediated by long-range interstate animal movements.
17. Bowman, A. et al. Detection of A(H5N1) influenza virus nucleic acid in retail pasteurized milk. Preprint at *Research Square* https://doi.org/10.21203/rs.3.rs-4572362/v1 (2024).
    This study shows that a high rate of H5N1 detection by PCR in retail milk across 12 US states provided a useful proxy for how widespread the outbreak had become on dairy farms.
18. Worobey, M. et al. Preliminary Report on Genomic Epidemiology of the 2024 H5N1 Influenza A Virus Outbreak in U.S. Cattle (Part 1 of 2). *Virological.org* https://virological.org/t/preliminary-report-on-genomic-epidemiology-of-the-2024-h5n1-influenza-a-virus-outbreak-in-u-s-cattle-part-1-of-2/970 (2024).
19. Burrough, E. R. et al. Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus infection in domestic dairy cattle and cats, United States, 2024. *Emerg. Infect. Dis.* **30**, 1335–1343 (2024).
20. Scholtissek, C. Pigs as the 'mixing vessel' for the creation of new pandemic influenza A viruses. *Med. Princ. Pract.* **2**, 65–71 (1990).
21. Fusaro, A. et al. High pathogenic avian influenza A(H5) viruses of clade 2.3.4.4b in Europe-Why trends of virus evolution are more difficult to predict. *Virus Evol.* **10**, veae027 (2024).
22. Fusaro, A. et al. Disentangling the role of Africa in the global spread of H5 highly pathogenic avian influenza. *Nat. Commun.* **10**, 5310 (2019).
23. Olawuyi, K. et al. Detection of clade 2.3.4.4 highly pathogenic avian influenza H5 viruses in healthy wild birds in the Hadeji-Nguru wetland, Nigeria 2022. *Influenza Other Respir. Viruses* **18**, e13254 (2024).
24. Youk, S. 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* **587**, 109860 (2023).
25. Caliendo, V. et al. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. *Sci. Rep.* **12**, 11729 (2022).
26. Lee, D.-H. et al. Intercontinental spread of Asian-origin H5N8 to North America through Beringia by migratory birds. *J. Virol.* **89**, 6521–6524 (2015).
27. Lee, D.-H. et al. Transmission dynamics of highly pathogenic avian influenza virus A(H5Nx) clade 2.3.4.4, North America, 2014-2015. *Emerg. Infect. Dis.* **24**, 1840–1848 (2018).
28. *Final Report for the 2014–2015 Outbreak of Highly Pathogenic Avian Influenza (HPAI) in the United States* (US Department of Agriculture, 2016).
29. Hicks, J. T. et al. Agricultural and geographic factors shaped the North American 2015 highly pathogenic avian influenza H5N2 outbreak. *PLoS Pathog.* **16**, e1007857 (2020).
30. US Department of Agriculture. Detections of highly pathogenic avian influenza. *USDA Animal and Plant Health Inspection Service* https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections (2024).
31. Kawaoka, Y., Krauss, S. & Webster, R. G. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. *J. Virol.* **63**, 4603–4608 (1989).
32. Byrne, A. M. P. et al. Investigating the genetic diversity of H5 avian influenza viruses in the United Kingdom from 2020–2022. *Microbiol. Spectr.* **11**, e0477622 (2023).
33. Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M. & Kawaoka, Y. Evolution and ecology of influenza A viruses. *Microbiol. Rev.* **56**, 152–179 (1992).
34. Caliendo, V. et al. Effect of 2020–21 and 2021–22 highly pathogenic avian influenza H5 epidemics on wild birds, the Netherlands. *Emerg. Infect. Dis.* **30**, 50–57 (2024).
35. Muñoz, G. et al. Stranding and mass mortality in Humboldt penguins (*Spheniscus humboldti*), associated to HPAIV H5N1 outbreak in Chile. *Prev. Vet. Med.* **227**, 106206 (2024).
36. Nemeth, N. M. et al. Bald eagle mortality and nest failure due to clade 2.3.4.4 highly pathogenic H5N1 influenza a virus. *Sci. Rep.* **13**, 191 (2023).
37. Wünschmann, A. et al. Lesions and viral antigen distribution in bald eagles, red-tailed hawks, and great horned owls naturally infected with H5N1 clade 2.3.4.4b highly pathogenic avian influenza virus. *Vet. Pathol.* **61**, 410–420 (2024).
38. Stimmelmayr, R., Rotstein, D., Torchetti, M. K. & Gerlach, R. Highly pathogenic avian influenza virus A(H5N1) clade 2.3.4.4b infection in free-ranging polar bear, Alaska, USA. *Emerg. Infect. Dis.* **30**, 1660–1663 (2024).
39. Cronk, B. D. et al. Infection and tissue distribution of highly pathogenic avian influenza A type H5N1 (clade 2.3.4.4b) in red fox kits (*Vulpes vulpes*). *Emerg. Microbes Infect.* **12**, 2249554 (2023).

40. Herfst, S. et al. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* **336**, 1534–1541 (2012).
41. Imai, M. et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* **486**, 420–428 (2012).
Together with Herfst et al. (2012), this article describes studies investigating the ability of H5N1 viruses to adapt to transmit by the airborne route between ferrets.
42. Lindh, E. et al. Highly pathogenic avian influenza A(H5N1) virus infection on multiple fur farms in the South and Central Ostrobothnia regions of Finland, July 2023. *Eurosurveillance* **28**, 2300400 (2023).
43. Bussey, K. A., Bousse, T. L., Desmet, E. A., Kim, B. & Takimoto, T. PB2 residue 271 plays a key role in enhanced polymerase activity of influenza A viruses in mammalian host cells. *J. Virol.* **84**, 4395–4406 (2010).
44. Subbarao, E. K., London, W. & Murphy, B. R. A single amino acid in the *PB2* gene of influenza A virus is a determinant of host range. *J. Virol.* **67**, 1761–1764 (1993).
This study identifies an important mammalian adaptation in the IAV polymerase.
45. Restori, K. H. et al. Risk assessment of a highly pathogenic H5N1 influenza virus from mink. *Nat. Commun.* **15**, 4112 (2024).
46. Maemura, T. et al. Characterization of highly pathogenic clade 2.3.4.4b H5N1 mink influenza viruses. *EBioMedicine* **97**, 104827 (2023).
47. Verhagen, J. et al. Phylogeography and antigenic diversity of low-pathogenic avian influenza H13 and H16 viruses. *J. Virol.* **94**, e00537-20 (2020).
48. Domańska-Blicharz, K. et al. Outbreak of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus in cats, Poland, June to July 2023. *Eurosurveillance* **28**, 2300366 (2023).
This study demonstrated that domestic cats in Poland died from a H5N1 from an unknown source with mammalian adaptations.
49. Song, W. et al. The K526R substitution in viral protein PB2 enhances the effects of E627K on influenza virus replication. *Nat. Commun.* **5**, 5509 (2014).
50. Rimondi, A. et al. Highly pathogenic avian influenza A(H5N1) viruses from multispecies outbreak, Argentina, August 2023. *Emerg. Infect. Dis.* **30**, 812–814 (2024).
51. Gamarra-Toledo, V. et al. Mass mortality of sea lions caused by highly pathogenic avian influenza A(H5N1) virus. *Emerg. Infect. Dis.* **29**, 2553–2556 (2023).
This report describes a mass mortality event in which H5N1 killed more than 5,000 South American sea lions in Peru in the opening months of 2023.
52. Krog, J. S. et al. Influenza A(H10N7) virus in dead harbor seals, Denmark. *Emerg. Infect. Dis.* **21**, 684–687 (2015).
53. Bodewes, R. et al. Spatiotemporal analysis of the genetic diversity of seal influenza A(H10N7) virus, northwestern Europe. *J. Virol.* **90**, 4269–4277 (2016).
54. Herfst, S. et al. Hemagglutinin traits determine transmission of avian A/H10N7 influenza virus between mammals. *Cell Host Microbe* **28**, 602–613.e7 (2020).
55. de Araújo, A. C. et al. Incursion of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus, Brazil, 2023. *Emerg. Infect. Dis.* **30**, 619–621 (2024).
56. Peacock, T. P. et al. Mammalian ANP32A and ANP32B proteins drive differential polymerase adaptations in avian influenza virus. *J. Virol.* **97**, e0021323 (2023).
57. Castillo, A. et al. The first case of human infection with H5N1 avian Influenza A virus in Chile. *J. Travel Med.* **30**, taad083 (2023).
58. Sheppard, C. M. et al. An influenza A virus can evolve to use human ANP32E through altering polymerase dimerization. *Nat. Commun.* **14**, 6135 (2023).
59. Yamayoshi, S. et al. Enhanced replication of highly pathogenic influenza A(H7N9) virus in humans. *Emerg. Infect. Dis.* **24**, 746–750 (2018).
60. Caserta, L. C. et al. Spillover of highly pathogenic avian influenza H5N1 virus to dairy cattle. *Nature* **634**, 669–676 (2024).
Detailed investigation into the avian-origin dairy cattle H5N1 outbreak in the USA.
61. Schwabenlander, S. et al. *2024 Highly Pathogenic Avian Influenza (H5N1) - Michigan Dairy Herd and Poultry Flock Summary* (US Department of Agriculture, 2024).
62. Le Sage, V., Campbell, A. J., Reed, D. S., Duprex, W. P. & Lakdawala, S. S. Persistence of influenza H5N1 and H1N1 viruses in unpasteurized milk on milking unit surfaces. *Emerg. Infect. Dis.* **30**, 1721–1723 (2024).
63. Franziska, K. et al. Inactivation of avian influenza A(H5N1) virus in raw milk at 63 °C and 72 °C. *N. Engl. J. Med.* **391**, 90–92 (2024).
64. Centers for Disease Control and Prevention. CDC A(H5N1) bird flu response update July 26, 2024. *CDC Avian Influenza (Bird Flu)* https://www.cdc.gov/bird-flu/spotlights/h5n1-response-07262024.html (2024).
65. Uyeki Timothy, M. et al. Highly pathogenic avian influenza A(H5N1) virus infection in a dairy farm worker. *N. Engl. J. Med.* **390**, 2028–2029 (2024).
66. Fouchier, R. A. M. et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc. Natl Acad. Sci. USA* **101**, 1356–1361 (2004).
67. Nguyen-Van-Tam, J. S. et al. Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. *Eurosurveillance* **11**, e060504.2 (2006).
68. World Health Organization. *Cumulative Number of Confirmed Human Cases for Avian Influenza A(H5N1) Reported to WHO, 2003–2024, 26 February 2024* (WHO, 2024).
69. Zhou, J. et al. Isolation of H5N6, H7N9 and H9N2 avian influenza A viruses from air sampled at live poultry markets in China, 2014 and 2015. *Eurosurveillance* **21**, 30331 (2016).
70. Cox, N. J., Trock, S. C. & Burke, S. A. Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Curr. Top. Microbiol. Immunol.* **385**, 119–136 (2014).
71. Karakus, U. et al. H19 influenza A virus exhibits species-specific MHC class II receptor usage. *Cell Host Microbe* **32**, 1089–1102.e10 (2024).
This study showed that AIV uses an alternative receptor, a protein, rather than canonical sialylated glycans.
72. Lloyd-Smith, J. O. et al. Epidemic dynamics at the human-animal interface. *Science* **326**, 1362–1367 (2009).
73. Lipsitch, M. et al. Viral factors in influenza pandemic risk assessment. *eLife* **5**, e18491 (2016).
74. Singanayagam, A. et al. Characterising viable virus from air exhaled by H1N1 influenza-infected ferrets reveals the importance of haemagglutinin stability for airborne infectivity. *PLoS Pathog.* **16**, e1008362 (2020).
75. Mänz, B. et al. Pandemic influenza A viruses escape from restriction by human MxA through adaptive mutations in the nucleoprotein. *PLoS Pathog.* **9**, e1003279 (2013).
76. Pinto, R. M. et al. BTN3A3 evasion promotes the zoonotic potential of influenza A viruses. *Nature* **619**, 338–347 (2023).
This work describes a restriction factor in humans that limits AIV zoonotic potential.
77. Du, W., de Vries, E., van Kuppeveld, F. J. M., Matrosovich, M. & de Haan, C. A. M. Second sialic acid-binding site of influenza A virus neuraminidase: binding receptors for efficient release. *FEBS J.* **288**, 5598–5612 (2021).
78. Sugiyama, K., Kawaguchi, A., Okuwaki, M. & Nagata, K. pp32 and APRIL are host cell-derived regulators of influenza virus RNA synthesis from cRNA. *eLife* **4**, e08939 (2015).
79. Long, J. S. et al. Species difference in ANP32A underlies influenza A virus polymerase host restriction. *Nature* **529**, 101–104 (2016).
This study investigates the molecular basis of one of the most common mammalian adaptations seen in AIVs.
80. Staller, E. et al. Structures of H5N1 influenza polymerase with ANP32B reveal mechanisms of genome replication and host adaptation. *Nat. Commun.* **15**, 4123 (2024).
81. Kuchipudi, S. V. et al. Differences in influenza virus receptors in chickens and ducks: Implications for interspecies transmission. *J. Mol. Genet. Med.* **3**, 143–151 (2009).
82. Carrasco, M. R., Gröne, A., van den Brand, J. M. A. & de Vries, R. P. The mammary glands of cows abundantly display receptors for circulating avian H5 viruses. *J. Virol.* **0**, e01052-24 (2024).
This study used recombinant influenza HA to probe influenza receptor distribution in various cattle tissues.
83. Walther, T. et al. Glycomic analysis of human respiratory tract tissues and correlation with influenza virus infection. *PLoS Pathog.* **9**, e1003223 (2013).
84. Chan, M. C. W. et al. Tropism and innate host responses of the 2009 pandemic H1N1 influenza virus in ex vivo and in vitro cultures of human conjunctiva and respiratory tract. *Am. J. Pathol.* **176**, 1828–1840 (2010).
85. van Riel, D. et al. H5N1 virus attachment to lower respiratory tract. *Science* **312**, 399 (2006).
86. Lakdawala, S. S. et al. The soft palate is an important site of adaptation for transmissible influenza viruses. *Nature* **526**, 122–125 (2015).
87. Richard, M. et al. Influenza A viruses are transmitted via the air from the nasal respiratory epithelium of ferrets. *Nat. Commun.* **11**, 766 (2020).
88. Matrosovich, M. et al. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J. Virol.* **74**, 8502–8512 (2000).
89. Dadonaite, B. et al. Deep mutational scanning of H5 hemagglutinin to inform influenza virus surveillance. *PLoS Biol.* **22**, e3002916 (2024).
A deep mutagenesis scanning approach identifies mutations in H5N1 associated with human airborne transmissibility.
90. Linster, M. et al. Identification, characterization, and natural selection of mutations driving airborne transmission of A/H5N1 virus. *Cell* **157**, 329–339 (2014).
91. Wu, N. C. et al. Major antigenic site B of human influenza H3N2 viruses has an evolving local fitness landscape. *Nat. Commun.* **11**, 1233 (2020).
92. Sun, H. et al. Mink is a highly susceptible host species to circulating human and avian influenza viruses. *Emerg. Microbes Infect.* **10**, 472–480 (2021).
93. Kristensen, C., Jensen, H. E., Trebbien, R., Webby, R. J. & Larsen, L. E. Avian and human influenza A virus receptors in bovine mammary gland. *Emerg. Infect. Dis.* **30**, 1907–1911 (2024).
94. Good, M. R., Ji, W., Fernández-Quintero, M. L., Ward, A. B. & Guthmiller, J. J. A single mutation in dairy cow-associated H5N1 viruses increases receptor binding breadth. Preprint at *bioRxiv* https://doi.org/10.1101/2024.06.22.600211 (2024).
95. Chopra, P. et al. Receptor binding specificity of a bovine A(H5N1) influenza virus. Preprint at *bioRxiv* https://doi.org/10.1101/2024.07.30.605893 (2024).
This study describes in depth characterization of bovine H5N1 receptor binding shows a lack of binding to human-like receptor analogues.
96. Eisfeld, A. J. et al. Pathogenicity and transmissibility of bovine H5N1 influenza virus. *Nature* **633**, 426–432 (2024).
97. Benton, D. J., Gamblin, S. J., Rosenthal, P. B. & Skehel, J. J. Structural transitions in influenza haemagglutinin at membrane fusion pH. *Nature* **583**, 150–153 (2020).
This structural study captures fusion intermediates of the influenza HA protein.
98. Yang, J. et al. The haemagglutinin genes of the UK clade 2.3.4.4b H5N1 avian influenza viruses from 2020 to 2022 retain strong avian phenotype. Preprint at *bioRxiv* https://doi.org/10.1101/2024.07.09.602706 (2024).
99. Antia, R., Regoes, R. R., Koella, J. C. & Bergstrom, C. T. The role of evolution in the emergence of infectious diseases. *Nature* **426**, 658–661 (2003).
100. Lowen, A. C. Constraints, drivers, and implications of influenza A virus reassortment. *Annu. Rev. Virol.* **4**, 105–121 (2017).
101. Ma, E. J., Hill, N. J., Zabilansky, J., Yuan, K. & Runstadler, J. A. Reticulate evolution is favored in influenza niche switching. *Proc. Natl Acad. Sci. USA* **113**, 5335–5339 (2016).
102. Lewis, N. S. et al. Antigenic and genetic evolution of equine influenza A (H3N8) virus from 1968 to 2007. *J. Virol.* **85**, 12742–12749 (2011).
103. Dalziel, B. D. et al. Contact heterogeneity, rather than transmission efficiency, limits the emergence and spread of canine influenza virus. *PLoS Pathog.* **10**, e1004455 (2014).
104. Martinez-Sobrido, L. et al. Characterizing Emerging Canine H3 Influenza Viruses. *PLoS Pathog.* **16**, e1008409 (2020).
105. Lewis, N. S. The global antigenic diversity of swine influenza A viruses. *eLife* **5**, e12217 (2016).
106. Bedford, T. et al. Global circulation patterns of seasonal influenza viruses vary with antigenic drift. *Nature* **523**, 217 (2015).
107. Carnaccini, S. & Perez, D. R. H9 influenza viruses: an emerging challenge. *Cold Spring Harb. Perspect. Med.* **10**, a038588 (2020).
108. Horimoto, T. et al. Generation of influenza A viruses with chimeric (type A/B) hemagglutinins. *J. Virol.* **77**, 8031–8038 (2003).
109. Sreenivasan, C. C., Thomas, M., Kaushik, R. S., Wang, D. & Li, F. Influenza A in bovine species: a narrative literature review. *Viruses* **11**, 561 (2019).

110. Mathieu, C. et al. Pandemic (H1N1) 2009 in breeding turkeys, Valparaiso, Chile. *Emerg. Infect. Dis.* **16**, 709–711 (2010).
111. Peacock, T. P. & Barclay, W. S. Mink farming poses risks for future viral pandemics. *Proc. Natl. Acad Sci. USA* **120**, e2303408120 (2023).
112. Peng, L. et al. Molecular characterization of H9N2 influenza virus isolated from mink and its pathogenesis in mink. *Vet. Microbiol.* **176**, 88–96 (2015).
113. Jia, N. et al. Glycomic characterization of respiratory tract tissues of ferrets: implications for its use in influenza virus infection studies. *J. Biol. Chem.* **289**, 28489–28504 (2014).
114. Kuchinski, K. S. et al. Detection of a reassortant swine- and human-origin H3N2 influenza A virus in farmed mink in British Columbia, Canada. Preprint at *bioRxiv* https://doi.org/10.1101/2024.05.27.596080 (2024).
115. Puryear, W. B. et al. Prevalence of influenza A virus in live-captured North Atlantic gray seals: a possible wild reservoir. *Emerg. Microbes Infect.* **5**, e81 (2016).
116. Kwon, T. et al. Pigs are highly susceptible to but do not transmit mink-derived highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b. *Emerg. Microbes Infect.* **13**, 2353292 (2024).
117. Arruda, B. et al. Divergent pathogenesis and transmission of highly pathogenic avian influenza A(H5N1) in swine. *Emerg. Infect. Dis.* **30**, 738–751 (2024).
118. Vijaykrishna, D. et al. Long-term evolution and transmission dynamics of swine influenza A virus. *Nature* **473**, 519–522 (2011).
119. Liu, R., Sheng, Z., Huang, C., Wang, D. & Li, F. Influenza D virus. *Curr. Opin. Virol.* **44**, 154–161 (2020).
120. Fooks, A. R. et al. Current status of rabies and prospects for elimination. *Lancet* **384**, 1389–1399 (2014).
121. Swayne, D. E., Spackman, E. & Pantin-Jackwood, M. Success factors for avian influenza vaccine use in poultry and potential impact at the wild bird-agricultural interface. *Ecohealth* **11**, 94–108 (2014).
122. Hou, Y. et al. Evolution of H7N9 highly pathogenic avian influenza virus in the context of vaccination. *Emerg. Microbes Infect.* **13**, 2343912 (2024).
123. Zeng, X. et al. Vaccination of poultry successfully eliminated human infection with H7N9 virus in China. *Sci. China Life Sci.* **61**, 1465–1473 (2018).
124. World Health Organization. Disease Outbreak News; Avian Influenza A (H5N2) – Mexico. *WHO* https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON520 (2024).
125. Cattoli, G. et al. Evidence for differing evolutionary dynamics of A/H5N1 viruses among countries applying or not applying avian influenza vaccination in poultry. *Vaccine* **29**, 9368–9375 (2011).
126. Read, A. F. et al. Imperfect vaccination can enhance the transmission of highly virulent pathogens. *PLoS Biol.* **13**, e1002198 (2015).
This study provides experimental evidence that vaccinating chickens against Marek's disease virus can promote more pathogenic strains.
127. Li, B. et al. Association of poultry vaccination with the interspecies transmission and molecular evolution of H5 subtype avian influenza virus. Preprint at *bioRxiv* https://doi.org/10.1101/2023.12.20.572711 (2023).
128. World Organization for Animal Health. Avian influenza vaccination: why it should not be a barrier to safe trade. *WOAH* https://www.woah.org/en/avian-influenza-vaccination-why-it-should-not-be-a-barrier-to-safe-trade/ (2023).
129. Ziegler, T., Mamahit, A. & Cox, N. J. 65 years of influenza surveillance by a World Health Organization-coordinated global network. *Influenza Other Respir. Viruses* **12**, 558–565 (2018).
130. Erbelding, E. J. et al. A universal influenza vaccine: the strategic plan for the National Institute of Allergy and Infectious Diseases. *J. Infect. Dis.* **218**, 347–354 (2018).
131. Jacquot, M., Nomikou, K., Palmarini, M., Mertens, P. & Biek, R. Bluetongue virus spread in Europe is a consequence of climatic, landscape and vertebrate host factors as revealed by phylogeographic inference. *Proc. Biol. Sci.* **284**, 20170919 (2017).
132. Spear, D. P. California besieged: the foot-and-mouth epidemic of 1924. *Agric. Hist.* **56**, 528–541 (1982).
133. Colorado Department of Agriculture. HPAI in dairy cattle. *Colorado Department of Agriculture* https://ag.colorado.gov/animal-health/reportable-diseases/avian-influenza/hpai-in-dairy-cattle (2024).
134. Centers for Disease Control and Prevention. H5 bird flu: current situation. *CDC Avian Influenza (Bird Flu)* https://www.cdc.gov/bird-flu/situation-summary/index.html (2024).
135. Centers for Disease Control and Prevention. CDC A(H5N1) bird flu response update, July 19, 2024. *CDC Avian Influenza (Bird Flu)* https://www.cdc.gov/bird-flu/spotlights/h5n1-response-07192024.html (2024).
136. Kucharski, A. J. & Edmunds, W. J. Characterizing the transmission potential of zoonotic infections from minor outbreaks. *PLoS Comput. Biol.* **11**, e1004154 (2015).
137. Park, M., Loverdo, C., Schreiber, S. J. & Lloyd-Smith, J. O. Multiple scales of selection influence the evolutionary emergence of novel pathogens. *Philos. Trans. R. Soc. B* **368**, 20120333 (2013).
138. Epperson, S. et al. Human infections with influenza A(H3N2) variant virus in the United States, 2011–2012. *Clin. Infect. Dis.* **57**, S4–S11 (2013).
This paper describes a detailed epidemiological investigation of a zoonotic outbreak of swine influenza in US children in 2012 by the CDC, which identifies limited human-to-human transmission.
139. Jhung, M. A. et al. Outbreak of variant influenza A(H3N2) virus in the United States. *Clin. Infect. Dis.* **57**, 1703–1712 (2013).
140. Furey, C. et al. Development of a nucleoside-modified mRNA vaccine against clade 2.3.4.4b H5 highly pathogenic avian influenza virus. *Nat. Commun.* **15**, 4350 (2024).
141. World Health Organization. Cumulative number of confirmed human cases for avian influenza A (H5N1) reported to WHO, 2003–2023, 5 January 2023. *WHO* https://www.who.int/publications/m/item/cumulative-number-of-confirmed-human-cases-for-avian-influenza-a(h5n1)-reported-to-who-2003-2022-5-jan-2023 (2023).
142. Chen, X. et al. Serological evidence of human infections with highly pathogenic avian influenza A(H5N1) virus: a systematic review and meta-analysis. *BMC Med.* **18**, 377 (2020).
143. Gostic, K. M., Ambrose, M., Worobey, M. & Lloyd-Smith, J. O. Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science* **354**, 722–726 (2016).
This study provides epidemiological evidence that older people retain partial immunity to their first IAV infection, explaining differences in age patterns seen for H5N1 and H7N9.
144. Daulagala, P. et al. Avian influenza A(H5N1) neuraminidase inhibition antibodies in healthy adults after exposure to influenza A(H1N1)pdm09. *Emerg. Infect. Dis.* **30**, 168–171 (2024).
145. Granata, G., Simonsen, L., Petrosillo, N. & Petersen, E. Mortality of H5N1 human infections might be due to H5N1 virus pneumonia and could decrease by switching receptor. *Lancet Infect. Dis.* **24**, e544–e545 (2024).
146. Ma, Q. et al. Global percentage of asymptomatic SARS-CoV-2 infections among the tested population and individuals with confirmed COVID-19 diagnosis: a systematic review and meta-analysis. *JAMA Netw. Open* **4**, e2137257 (2021).

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