The global H5N1 influenza panzootic in mammals

Thomas Peacock[1,2], Louise Moncla[3], Gytis Dudas[4], David VanInsberghe[5.6], Ksenia Sukhova[2], James O. Lloyd-Smith[7,8], Michael Worobey[9], Anice C. Lowen[5.6], Martha I. Nelson[10*]

[1] The Pirbright Institute, Pirbright, Woking, United Kingdom.

[2] Department of Infectious Disease, St Mary's Medical School, Imperial College London, London, United Kingdom.

[3] Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce St., Philadelphia, PA 19104, USA.

[4] Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania.

[5] Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, USA.

[6] Emory Center of Excellence for Influenza Research and Response (Emory-CEIRR), Atlanta, GA, USA.

[7] Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, California, USA.

[8] Department of Computational Medicine, University of California Los Angeles, Los Angeles, California, USA.

[9] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, 85721, USA.

[10] National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health (NIH), Bethesda, MD, USA.

[*] Corresponding author: Email: nelsonma@mail.nih.gov.

Abstract

Influenza A viruses (IAV) have caused more documented global pandemics in human history than any other pathogen[1,2]. High pathogenicity avian influenza (HPAI) 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 an H5N1 panzootic in wild birds that is fueled by (a) rapid intercontinental spread, reaching South America and Antarctica for the first time[4,5]; (b) fast evolution via genomic reassortment[6]; and (c) 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 (AIV) adapt to mammals before jumping to humans[20]. However, the altered ecology of H5N1 has opened the door to new evolutionary pathways. Could dairy cattle, farmed mink, or South American sea lions serve as new mammalian gateways to humans? Here we explore the molecular and ecological factors driving H5N1’s sudden expansion in host range and assess the likelihood of different zoonotic pathways leading to an H5N1 pandemic.

Main

In recent years, an H5N1 problem that was once mainly confined to Asia and poultry has now spread globally (**Figure 1**), and into new species of mammals (**Figure 2**), endangering wildlife, agricultural production, and human health. The problem began in 2020, when a new genotype of H5N1 viruses belonging to clade 2.3.4.4b emerged that spread rapidly in wild birds[3] from Europe to Africa[21–23], North America[24,25], South America[5,12], and the Antarctic[4]. At first, H5N1's arrival in North America seemed manageable. Back 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 culling ~90 million US 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 decaying on Argentine beaches and yellow, curdled milk on H5N1-affected dairy farms show how the 2.3.4.4b H5N1 panzootic is different and previous control strategies are not working. The question is why.

The panzootic 2.3.4.4b H5N1 viruses circulating in wild birds are genetically different from prior strains due 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 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 (**Figure 3**). Subsequent H5N1/LPAI reassortment events in Europe generated the AB and BB genotypes[21,32] (**Figure 3**). Why Europe recently became a major source of new H5 reassortants, shifting the center 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 (**Figure 3**). Understanding how this burst of new genotypes changes H5N1's capacity to host-switch to mammals, including humans, remains an active area of research (see section below, *How could H5N1 become a pandemic?*).

In this Perspective, we review what has been learned about IAV spillover and H5N1 pandemic potential from three H5N1 case studies where evidence supports mammal-to-mammal transmission, including in (a) fur farms in Europe, (b) marine mammals in South America, and (c) dairy cattle in the United States. 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 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 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 AIV and often do not display symptoms[33] (**Figure 2**). An early sign of H5N1's arrival is dead poultry[25]. Mass die-offs can occur in social sea birds that congregate in large dense colonies, for example gannets in Europe[34] or penguins in Chile[35]. Birds of prey[36,37] (e.g., hawks, eagles, vultures) and terrestrial carnivores[7,8,38,39] (e.g., foxes, raccoons, bobcats) that scavenge dead H5N1-infected birds can die, often with neurological symptoms (**Figure 2)**. Most mammalian cases are "dead-end" infections, with very little evidence of onward transmission to additional hosts. Laboratory experiments proved that pre-

2.3.4.4b H5N1 viruses could transmit 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 existed in any real-world field settings remained unclear. Here we describe three field settings where 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 metastasized 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 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 - December 2023 on 71 fur farms in Finland that affected American mink (6 farms), arctic foxes (64 farms), and raccoon dogs (5 farms)[11,42]. Known mammalian adaptations in the polymerase were found in viruses collected from the farmed animals in both countries, including mutations 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 based on the close genetic relatedness of the viruses found on different farms. Experimental studies confirmed that the viruses could transmit efficiently between ferrets 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 a new reassortant H5N1 genotype “BB” (**Figure 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 who 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. While 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 an 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 circulating in Europe at the time, raising the possibility of cryptic transmission in mammals with mild symptoms.

Long-range transmission of H5N1 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 American sea lions were reported in Peru[12,51] and Chile[13] in early 2023. H5N1 spread down South America's west coast 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 introduced independently from sea birds. 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 harbor seals in Denmark, 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 the phylogenetic tree, separate 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, oral-fecal) 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)) resided near a beach with H5N1-infected animals and his virus contains the same two PB2 mammalian adaptations 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], >450 kilometers off the coast of mainland South America, with no reversions seen in the mammalian-adapted PB2 mutations. It remains to be seen if wild birds will carry and potentially disperse mammalian-adapted B3.2 viruses long distance, 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 drops in milk production in lactating cattle and thick, yellow milk, which 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 (**Figure 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, skunk, **Figure 4**) that fall outside the cattle clade[16,18], suggesting 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 PA K497R[58,59] (**Table 1**).

The high genetic diversity of the H5N1 virus in Texas cattle suggests the bovine B3.13 outbreak originated in Texas and rapidly spread to additional states (13 total as of July 2024: Texas, New Mexico, Oklahoma, Colorado, Kansas, Idaho, Wyoming, South Dakota, Michigan, Iowa, Minnesota, Ohio, and North Carolina). 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 likely spread by transport of infected cattle or equipment (**Figure 4**)[16,60–62]. High viral titers in milk and the virus's mammary tissue tropism suggest a role for milk in transmission[60,61,63]. 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[61] (**Figure 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 (e.g., grackles, blackbirds), terrestrial mammals (e.g., foxes, raccoons, mice), and poultry[16,18,19,60] (**Figure 4**). Spillover from cattle to domestic barn cats likely occurs through ingestion of contaminated, unpasteurised 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 July 26, 2024, 13 documented human cases have been identified in association with the B3.13 bovine strain, including four dairy workers from Texas, Michigan, and Colorado and nine Colorado poultry workers infected by chickens carrying the bovine strain[64] (**Figure 4**). Human infections present primarily as conjunctivitis[65], similar to past H7 human infections in the Netherlands[66,67]. Less 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 backyard flocks were defeathered[69]. Accordingly, the CDC's Influenza Risk Assessment Tool (IRAT) and WHO's 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 H5N1's evolutionary potential going forward, including the range of directions H5N1 could mutate, host-switch, or reassort, based on decades of prior observations of IAV.

How could H5N1 become a pandemic?

For an influenza virus to start a pandemic it must fulfill two key criteria. First, the virus's main attachment glycoprotein, haemagglutinin (HA) (**Figure 5A**), must be antigenically novel and poorly recognized immunologically by a large fraction of the human population. All 17 HA subtypes[71] (**Figure 5B**) maintained in wild aquatic birds meet the first criterion. Antigenic novelty

is especially high for subtypes such as H5 that 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 exceeding 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 (URT). 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 also likely 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 allow the virus to use mammalian ANP32 proteins[78], histone chaperone proteins that helps synthesize viral RNA in the 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 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 mammals. The T271A PB2 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 (**Figure 5B**). The α2,3-linked form is abundant in avian tissues[81], the bovine mammary gland[82], the human lower respiratory tract (LRT),[83] and the human eye (conjunctiva).[84] While the documented human spillovers of cattle-derived H5N1 have mostly involved conjunctivitis, prior H5N1 cases in humans infected the LRT, which likely contributed to severe disease[85]. To transmit efficiently by the respiratory route, influenza viruses must replicate in the URT[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 URT[88].

Compared to 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 lab experiments: N224K[41,89], L226[41,89,90] 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] that have a high proportion of α2,6-linked receptors in the URT[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 H5N1's α2,3-linked binding breadth[94], and continued monitoring of molecular changes in receptor binding sites is warranted.

The third property of AIVs known to influence pandemic potential is HA stability. HA, like nearly all viral glycoproteins, is synthesized in a meta-stable form. Exposure to acidic pH triggers changes in HA 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 human-to-human, HA needs to be stable and triggered

only at more acidic pH so it survives the acidic microenvironment of airborne particles and mammalian respiratory secretions[41,42]. Mutations impacting HA stability occur throughout the protein[89], making this phenotype difficult to predict based on sequence alone. Thus, while 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 (**Figure 5B**). Fortunately, the mammalian species infected by 2.3.4.4b H5N1 viruses (e.g., mink, marine mammals, bovines, foxes, raccoons, domestic cats, **Figure 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 human and swine-origin viruses 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

299 present host range of H5N1 limits opportunities for reassortment with other mammalian adapted
300 viruses.
301 However, this could change. As autumn approaches in the Northern hemisphere, so
302 does the influenza season. A farm worker coinfected with H5N1 and a human seasonal virus
303 presents an opportunity for avian and human IAVs to reassort and combine many of the traits
304 needed to spread efficiently in humans, as occurred prior to the 1957 H2N2 and 1968 H3N2
305 pandemics[31]. H5N1 spillover into swine, which appear to be suitable hosts for H5N1 in
306 experimental studies[116,117], would present additional opportunities for reassortment[105,118], as
307 exemplified by the triple-reassortant swine-origin H1N1 pandemic virus from 2009[2]. Influenza
308 spillover from cattle to swine is a known possibility because it already occurs in this direction for
309 influenza D viruses, in the United States as well as other countries[119]. The continued absence of
310 H5N1 in US swine is highly fortunate.
311
312 ***Should the West vaccinate poultry for H5N1?***
313 The prospect of H5N1 becoming enzootic in Europe and the Americas is a turning point for
314 HPAI and new control strategies are needed, including vaccination. Currently, there is no oral
315 H5N1 vaccine that could be mass administered to wildlife, similar to the rabies vaccine[120].
316 Influenza vaccines are licensed for poultry that reduce disease burden, but do not prevent
317 infection and have varying degrees of success[121]. China's large-scale national vaccination
318 program in poultry has been credited with controlling H5 and H7 and reducing zoonosis[122,123].
319 However, vaccination campaigns have been less successful in controlling H6N2 in South Africa
320 or H5N2 in Mexico, which recently reported a zoonotic case[124]. One concern is that vaccines
321 could make HPAI harder to control by fostering silent spread and/or accelerating antigenic
322 evolution in poultry[125,126,127]. Major poultry exporters in Europe, Brazil, and the United States are
323 reluctant to use influenza vaccines in poultry or cattle because products from vaccinated
324 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 United States 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 (WOAH) 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 in humans[129]. There is hope that some day 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.

Can H5N1 be eliminated in US dairy cattle?

Two features of the H5N1 outbreak in bovine make eradication feasible. First, most transmission appears to occur through a defined pathway via milking machinery[61] 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, the proverbial cow may already be out of the barn.

US dairy farmers have not previously dealt with IAV or deadly bovine diseases like 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 (**Figure 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, Colorado became the first state to require weekly testing for H5N1 in bulk milk tanks on dairy farms[133].

Human H5N1 cases

US public health agencies have tested over 200 people who were exposed to H5N1 infected animals between March 24, 2024 - July 26, 2024[134] and identified 13 confirmed cases. A small serosurvey for H5N1 antibodies in dairy and poultry workers in Michigan 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 occurred. Limited human-to-human spread of earlier H5N1 strains occurred in Asia but reproductive numbers always remained below 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, CDC's investigation of a 2012 zoonotic outbreak of IAV in US children competing show pigs at agricultural fairs identified suspected human-to-human transmission in a child's daycare[138]. Agricultural fairs are already underway this summer across the US, bringing dairy cattle into the same environment where 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 canceling the milking demonstrations. How much H5N1 testing is done in humans or wastewater at fairs remains to be seen.

Prospects for the Future

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 severity of a future H5N1 pandemic remains unclear. Recent human infections with H5N1 2.3.4.4b viruses have a substantially lower case fatality rate compared to prior H5N1 outbreaks in Asia, where half of people with reported infections died[141]. The milder symptoms 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 due 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 younger 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 H5N1's global distribution (**Figure 1**), non-human host range (**Figure 2**), and genetic diversity (**Figure 3**) than virtually 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 fuel epidemics at a population level[146]. The H5N1 panzootic has been defined by powerful visuals of beaches littered with sea lion carcasses or barns of ill dairy cows wasting away after going off

403 feed. But what keeps scientists up at night is the possibility of unseen chains of transmission
404 silently spreading through farm worker barracks, swine barns, or developing countries, evolving
405 under the radar because testing criteria are narrow, government authorities are feared, or
406 resources are thin. A second lesson from the COVID-19 pandemic is not to underestimate the
407 importance of human behavior, culture, and economic context. New technologies like mRNA
408 vaccines, next-generation sequencing, and CRISPR-Cas diagnostics provide rapid, flexible
409 tools for outbreak response, but are of little use when they are not allowed on the farm.
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Author Contributions

TP, LM, JOL, MW, ACL, and MIN drafted and edited the manuscript. TP, GD, DV, KS, ACL, and MIN designed and generated figures.

Competing Interests

Authors declare that they have no competing interests.

Disclaimer

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Figure Legends

Figure 1. Geographical distribution of HPAI H5 viruses sampled in birds and mammals, 1996-2023. Red shading indicates countries with HPAI H5 virus sequences available on the GISAID database, specifically from the A/goose/Guangdong/1/1996(H5N1) ("Gs/Gd") lineage that emerged in China in 1996. Green (human) and yellow (non-human mammals) circles are sized in proportion to the number of H5 GISAID sequences from that country and time period. The source of the map is supplied by Natural Earth.

Figure 2. Multi-host ecology of H5N1 clade 2.3.4.4b since 2020. Wild aquatic birds (ducks, geese, 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 yellow (South American marine mammals), green (US dairy cattle), and blue (European mink), with arrows shaded the same colors depicting spillovers from those mammalian outbreaks into additional species, possibly via unsampled intermediaries. Animals with red names indicate host species for which IAV has been detected for the first time (based on genetic sequence data, not serology).

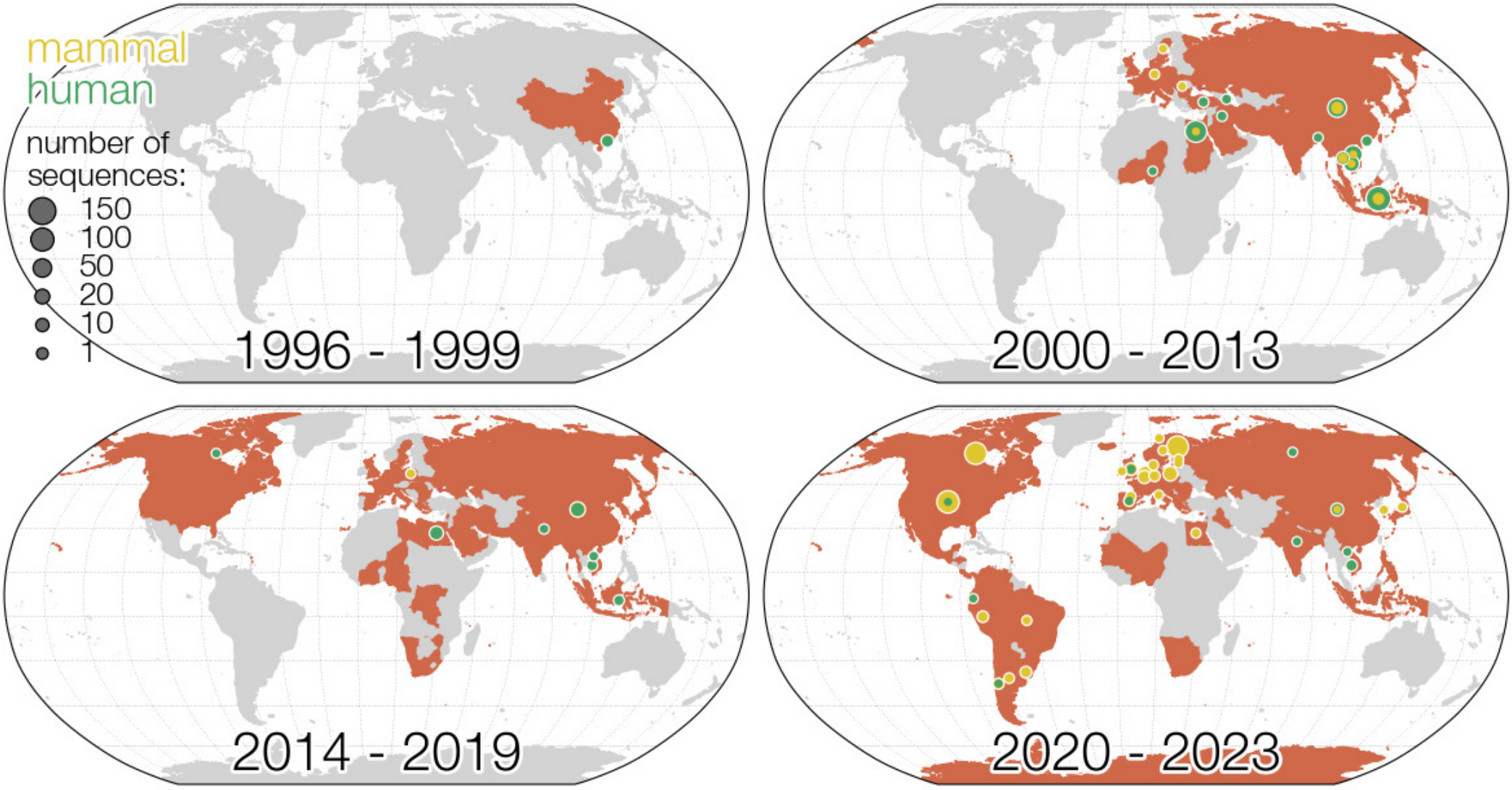
Figure 3. Genomic reassortment events in birds leading up to four H5N1 spillover events in mammals. Each oval represents a genotype, with eight bars representing the eight segments of the IAV genome, ordered from longest to shortest: PB2, PB1, PA, HA, NP, NA, MP, NS. Each segment is shaded by lineage. Solid black arrows indicate donors during genomic reassortment events. Broken black arrows indicate intercontinental migration events. Red arrows indicate spillover events into mammals.

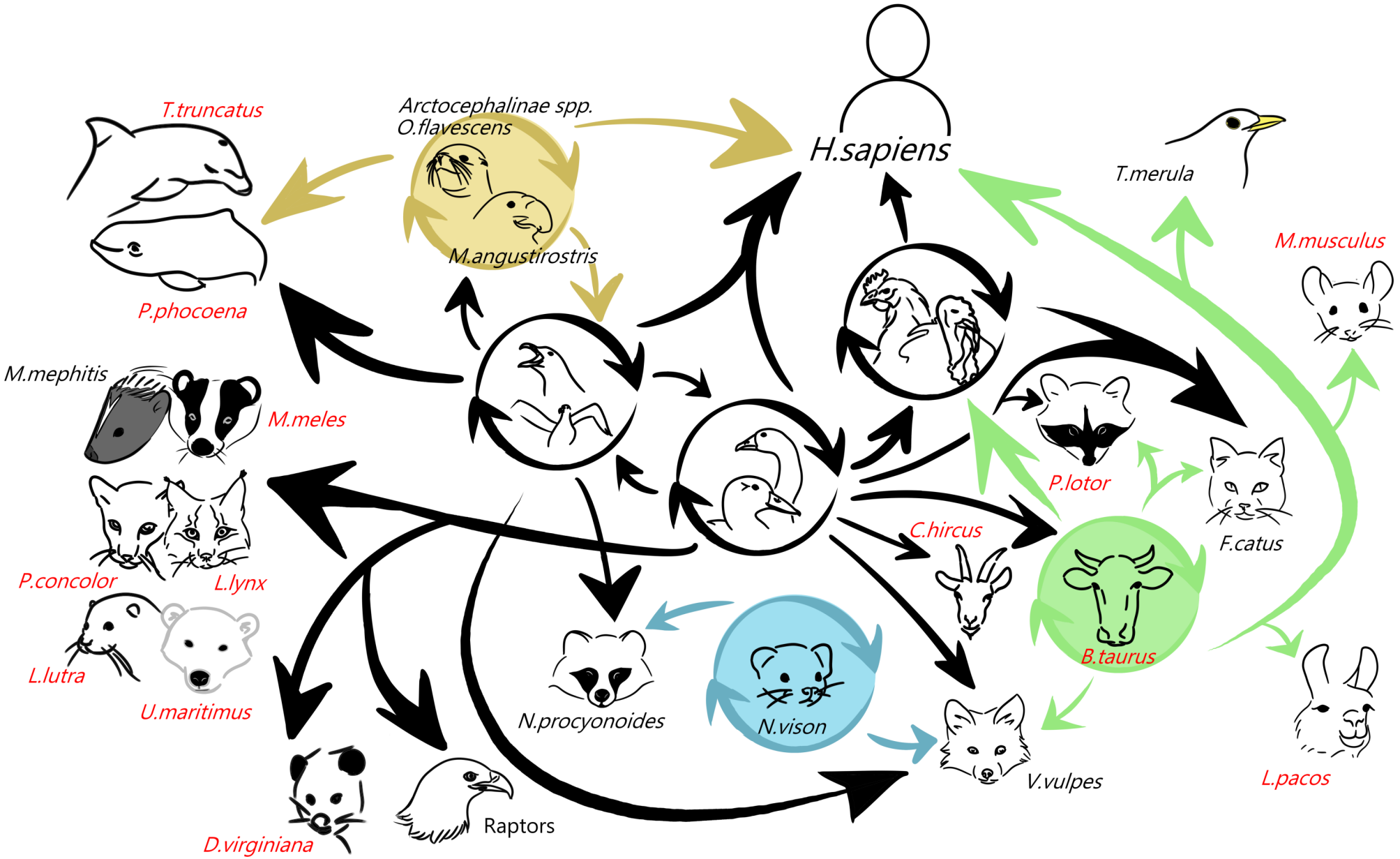
Figure 4. Leading hypotheses for the source and spread of the H5N1 outbreak in bovines. The most likely routes of H5N1 transmission between wildlife, domestic animals, and humans are inferred from currently available genomic and epidemiological data.

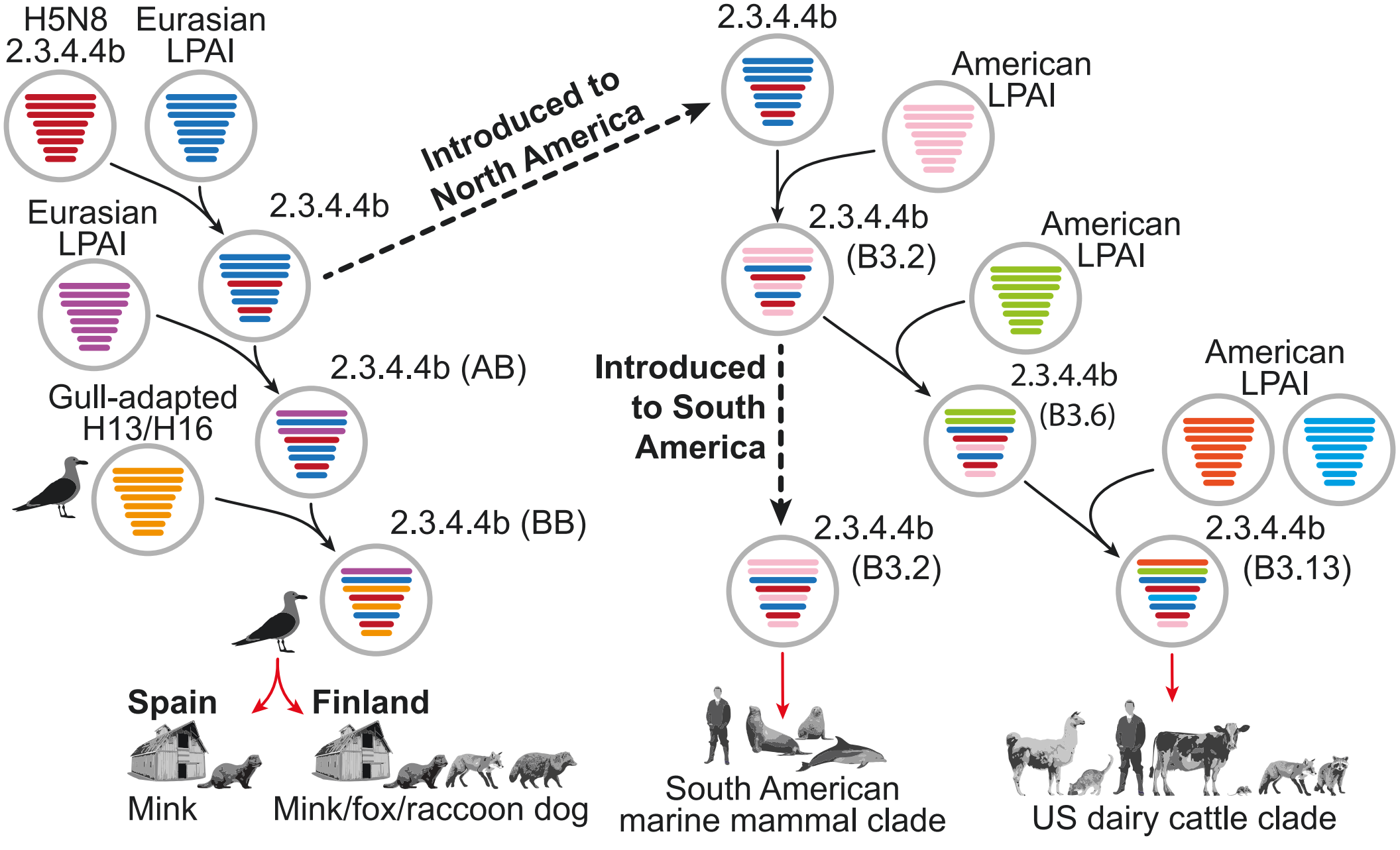
Figure 5. How IAVs adapt to new host species. (A) Molecular features of IAV that are known to impact host range. vRNP = viral ribonucleoprotein, which includes the PB2, PB1 and PA polymerase proteins, the nucleoprotein, and viral RNA. (B) Wild aquatic birds are the natural reservoir for IAV, maintaining 17 HA subtypes[71] that occasionally spill over into other species and can establish new host-specific lineages (black arrows). Lighter gray front indicates subtypes that have gone 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 alpha-2,3 or alpha-2,6. The full complexity of glycans that act as IAV receptors across species is not depicted, although differentiation between upper (a-2,6) and lower (a-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 side of panel B.

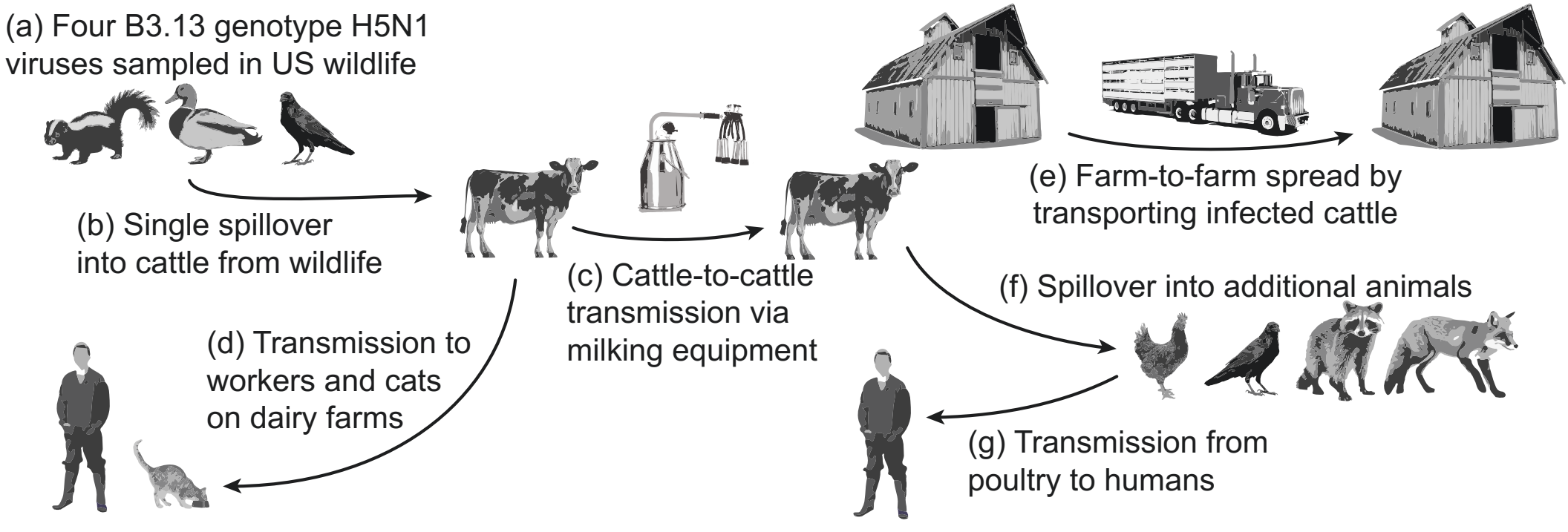
Figure 6. Number of published influenza virus genome sequences collected May 15, 2024 - July 22, 2024. Bars indicate the number of influenza viruses collected from humans and

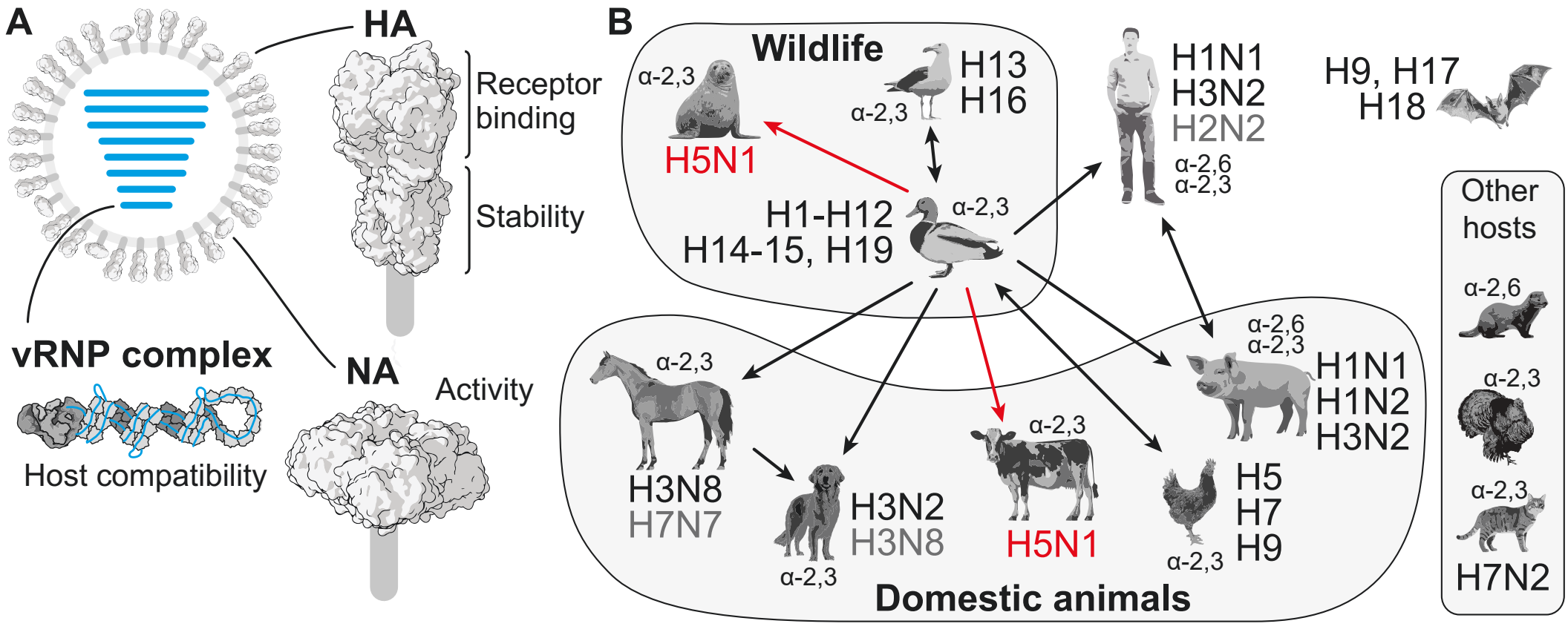
877 animals in recent months (May 15, 2024 - July 22, 2024) that are available in the GISAID
878 database (downloaded July 22, 2024). The number does not include viral sequences submitted
879 to SRA for which the collection date is unknown.
880
881 **Table 1. H5N1 clade 2.3.4.4b outbreaks in mammals.** A summary of six H5N1 clade 2.3.4.4b
882 outbreaks in mammals that infected at least 10 animals and occurred during 2022 - 2024,
883 ordered by time. The strength of evidence for mammal-to-mammal transmission is based on (a)
884 phylogenetic clustering of viruses collected from mammals together in a single clade, separate
885 from avian viruses; (b) whether viruses from mammals have the same mammalian adaptations
886 in PB2; and (c) the availability of well-sampled genetic sequence data. The primary control
887 strategy is listed as of June 2024.
888
889

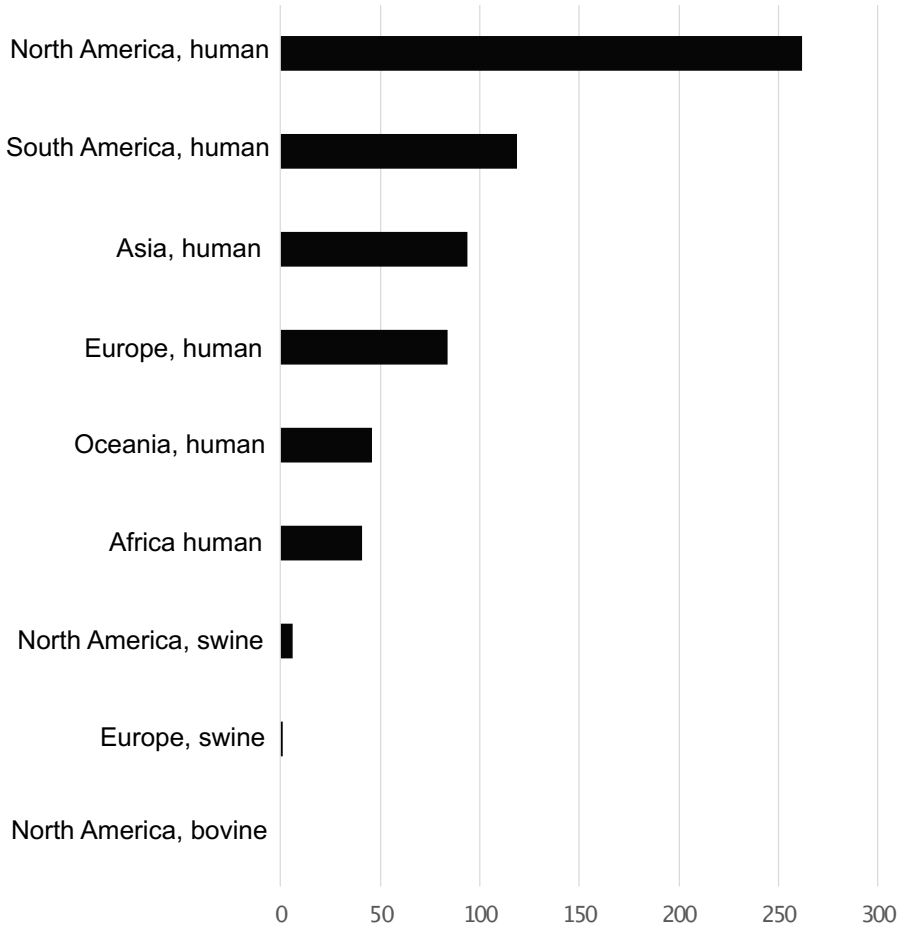

mammal
human
number of sequences:
150
100
50
20
10
1
1996 - 1999
2000 - 2013
2014 - 2019
2020 - 2023


T.truncatus
P.phocoena
Arctocephalinae spp.
O.flavescens
M.angustirostris
H.sapiens
T.merula
M.musculus
M.mephitis
M.meles
P.concolor
L.lynx
L.lutra
U.maritimus
D.virginiana
Raptors
N.procyonoides
N.vison
V.vulpes
C.hircus
P.lotor
F.catus
B.taurus
L.pacos


H5N8
2.3.4.4b
Eurasian
LPAI
Eurasian
LPAI
2.3.4.4b
Introduced to
North America
Gull-adapted
H13/H16
2.3.4.4b (AB)
2.3.4.4b (BB)
Spain
Finland
Mink
Mink/fox/raccoon dog
2.3.4.4b
American
LPAI
2.3.4.4b
(B3.2)
Introduced
to South
America
2.3.4.4b
(B3.2)
South American
marine mammal clade
American
LPAI
2.3.4.4b
(B3.6)
American
LPAI
2.3.4.4b
(B3.13)
US dairy cattle clade


(a) Four B3.13 genotype H5N1 viruses sampled in US wildlife
(b) Single spillover into cattle from wildlife
(c) Cattle-to-cattle transmission via milking equipment
(d) Transmission to workers and cats on dairy farms
(e) Farm-to-farm spread by transporting infected cattle
(f) Spillover into additional animals
(g) Transmission from poultry to humans


A
HA
Receptor binding
Stability
vRNP complex
NA
Activity
Host compatibility
B
Wildlife
α-2,3
H5N1
α-2,3
H13
H16
H1-H12
H14-15, H19
α-2,3
H1N1
H3N2
H2N2
α-2,6
α-2,3
H9, H17
H18
Other hosts
α-2,6
α-2,3
α-2,3
H7N2
α-2,6
α-2,3
H1N1
H1N2
H3N2
α-2,3
H3N8
H7N7
α-2,3
H3N2
H3N8
α-2,3
H5N1
α-2,3
H5
H7
H9
Domestic animals



No. genome sequences, influenza viruses, collected May 15, 2024 - July 22, 2024

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)	Ref
Harbor (Phoca vitulina) and gray (Halichoerus grypus) seals	wild	June 2022	< 1 month	Maine, 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 - November 2023 (may be ongoing)	> 8 months (possibly ongoing)	South America (Argentina, Brazil, Chile, Peru, 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 - 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 - present	>7 months (ongoing)	13 US states (CO, IA, ID, KS, MI, MN, NC, NM, OH, OK, SD, TX, 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

Table 1. H5N1 clade 2.3.4.4b outbreaks in mammals. A summary of seven H5N1 clade 2.3.4.4b outbreaks in mammals that infected at least 10 animals. The strength of evidence for mammal-to-mammal transmission is based on (a) phylogenetic clustering of viruses collected from mammals together in a single clade, separate from avian viruses; (b) whether viruses from mammals have the same mammalian adaptations in PB2; and (c) the availability of well-sampled genetic sequence data. The primary control strategy is listed as of June 2024.