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VIEWPOINT

Mammalian infections with highly pathogenic avian influenza viruses renew concerns of pandemic potential

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There is unprecedented spread of highly pathogenic avian influenza A H5N1 viruses in bird species on five continents, and many reports of infections in mammals most likely resulting from consumption of infected birds. As H5N1 viruses infect more species, their geographical range increases and more viral variants are produced that could have new biological properties including adaptation to mammals and potentially to humans. This highlights the need to continually monitor and assess mammalian-origin H5N1 clade 2.3.4.4b viruses for the presence of mutations that could potentially increase their pandemic risk for humans. Fortunately, to date there have been a limited number of human cases, but infection of mammals increases the opportunity for the virus to acquire mutations that enhance efficient infection, replication, and spread in mammals, properties that have not been seen in these viruses in the past.

There is unprecedented spread of H5N1 viruses in bird species on five of seven continents—only Australia and Antarctica are still spared (Wille and Klaassen, 2023). There are also many reports of infections in mammals, most likely from consuming infected birds. Fortunately, to date there have been a limited number of human cases, but infection of mammals increases the opportunity for the virus to acquire mutations that enhance efficient infection, replication, and spread in mammals, properties that have not been seen in these viruses in the past.

The pandemic potential of highly pathogenic avian influenza (HPAI) H5N1 "bird flu" viruses became a global public health concern with the first zoonotic outbreak in Hong Kong in 1997 (Subbarao et al., 1998). The virus re-emerged in humans in 2004 in Thailand and Vietnam and in the ensuing years, became enzootic in birds around the world, and has evolved into genetically and antigenically distinguishable clades and fourth-order subclades (Smith et al., 2015), all tracing back to influenza A/goose/Guangdong/1/96 (Xu et al., 1999). Avian influenza viruses are generally not efficient in infecting humans or other mammals. The preferential binding of avian influenza A viruses (IAVs) to α2,3-linked sialic acid (SA) receptors and the distribution of α2,3 and α2,6 receptors in the human respiratory tract are critical determinants of the host range restriction of IAVs. As a result of receptor specificity and additional host range restrictions, avian H5N1 viruses do not replicate efficiently in the human upper respiratory tract and do not spread efficiently from person to person. However, the 1918, 1957, and 1968 influenza pandemic IAVs were derived wholly or in part from avian IAVs.

IAVs must meet three criteria to cause a pandemic: (i) bear a hemagglutinin (HA) to which the human population is immunologically naive, (ii) cause disease in humans, and (iii) cause sustained chains of human-to-human transmission. For efficient transmission, the HA must recognize and bind receptors in the human respiratory tract. This notion is supported by the finding that the earliest isolates in the 1918 (Gamblin et al., 2004), 1957, and 1968 pandemics preferentially recognized α2,6-linked SA receptors (Matrosovich et al., 2000), even though their HAs were derived from avian IAVs. Sporadic human HPAI H5 cases have typically arisen by direct contact with infected birds but have not resulted in larger outbreaks. According to the World Health Organization (WHO), 874 sporadic human cases of H5N1 infection with 458 fatalities were recorded between January 2003 and April 24, 2023 (World Health Organization, 2023), representing a very high case fatality rate of 52%. However, this may be an overestimation, as many human cases of mild/asymptomatic or abortive infection may not have been recorded in areas where H5N1 viruses are widespread in poultry and there is increased human contact with the virus.

In 2014, HPAI "H5Nx" viruses that derived the HA from clade 2.3.4.4 paired with different neuraminidase (NA) subtypes started to spread. Clade 2.3.4.4 viruses first emerged in China between 2010 and 2011 and showed a propensity to reassort with other avian IAVs; this H5 HA has reassorted with at least four NA subtypes (N2, N5, N6,

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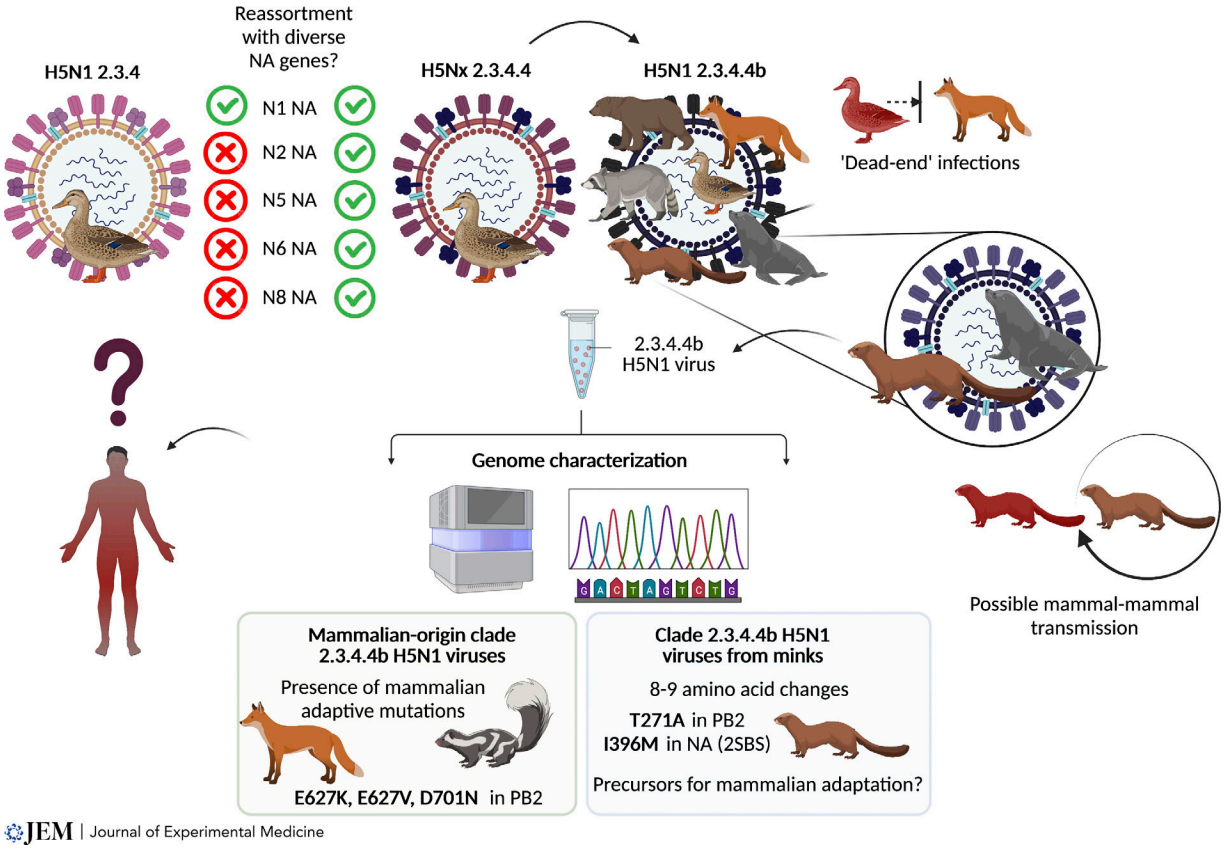

Evolution of HPAI H5N1 viruses. All circulating HPAI H5N1 viruses trace back to influenza A/goose/Guangdong/1/96. Over time, H5 viruses have diversified into different genetic clades. Clade 2.3.4.4 viruses originated from clade 2.3.4, and while both primarily only infect birds, clade 2.3.4.4 viruses have an increased capacity to reassort, giving rise to H5Nx viruses with novel NA pairings. Evolution of this lineage gave rise to clade 2.3.4.4b viruses where the H5 re-acquired an N1 NA pairing. Apart from birds, these viruses can now infect various small mammals, although these are largely dead-end infections acquired by direct contact with infected birds, without further spread between mammals. Two recent outbreaks in seals and minks mark the first reports of possible mammal-to-mammal transmission by these viruses. The mink viruses showed between eight and nine amino acid differences in comparison to closely related H5N1 viruses. Notably, all of the viruses from minks contained an alanine at position 271 of PB2 (T271A) and a methionine at position 396 located in the 2SBS of the NA (I396M; Agüero et al., 2023). A number of mammalian-origin clade 2.3.4.4b H5N1 viruses have also been found to contain mammalian adaptive mutations (E627K, E627V, and D701N) in the PB2 subunit of the RNA polymerase complex (Alkie et al., 2023) as well as mutations in other internal protein genes. Of concern, the rapid acquisition of adaptive mutations in mammalian-origin H5N1 2.3.4.4b viruses potentially increases their pandemic risk for humans.

and N8). Reassortment of the NA gene segment represents a glimpse into more widespread reassortment involving other gene segments in these viruses. Extensive reassortment was a concern, because a greater capacity to reassort suggested a fundamental change in virus biology that could increase pandemic potential by introducing new phenotypic properties, as was observed when the 2009 H1N1 pandemic virus acquired gene segments from a new source (Lakdawala et al., 2011). Since 2014, migrating birds have facilitated the spread of H5Nx viruses to several parts of the world (de Vries et al., 2015), causing widespread economic issues in the poultry industry and causing sporadic human infections (Pan et al., 2016).

During 2020, a subclade of 2.3.4.4 viruses paired with an N1 NA called 2.3.4.4b emerged and started to spread to many parts of the world including Africa, Asia, Europe, and North and South America. These viruses have devastated wild bird populations and caused outbreaks in domestic poultry. Notably, they have also caused infections in various small mammals, including badgers, black bears, bobcats, coyotes, ferrets, fisher cats, foxes, leopards, opossums, pigs, raccoons, skunks, sea lions, and wild otters. Most of these have been "dead end" infections and are attributed to direct contact, from animals preying on and ingesting infected birds. However, two recent reports of H5N1 outbreaks in New England seals (Puryear et al., 2023) and on a mink farm in Spain (Agüero et al., 2023) mark the first H5N1 infections potentially involving mammal-to-mammal transmission, renewing concerns that the virus could be poised for spillover into humans. If confirmed, this is particularly concerning as the capacity to transmit between mammals has not been associated with H5N1 viruses previously, and it would suggest that clade 2.3.4.4b viruses may also have an increased capacity to cause human infection. In the absence of population immunity in humans and ongoing evolution and spread of the virus, clade 2.3.4.4b H5 viruses could cause an influenza pandemic if they acquired the ability to transmit efficiently among humans. From 2020 to date, six detections or infections in humans by clade 2.3.4.4b H5N1 viruses have been reported to the WHO. Four were asymptomatic or mild, and two cases were associated with severe disease (World Health Organization, 2022).

Many of the H5N1 viruses isolated from mammals have arisen from reassortment involving exchange of genome segments derived from North American and Eurasian IAVs. A number of these were found to contain mammalian adaptive mutations (E627K, E627V, and D701N) in the polymerase basic protein 2 (PB2) subunit of the RNA polymerase complex (Alkie et al., 2023). Other mutations that enhanced polymerase activity and/or virus replication in mammalian cells were also identified in other internal protein genes. The acquisition of adaptive mutations in a diverse range of mammals highlights the need to continually monitor and assess mammalian-origin H5N1 clade 2.3.4.4b viruses for the presence of mutations that could potentially increase their pandemic risk for humans.

Genetic characterization of the mink viruses revealed homology to the A/gull/France/22P015977/2022-like genotype within clade 2.3.4.4b. The viruses from infected minks also showed eight or nine unique amino acid differences in the PB2, PB1, PA, NA, NS2, M2, and PB1-F2 in comparison to closely related H5N1 viruses (Agüero et al., 2023). Of particular note, the viruses from minks were distinguished from other H5N1 viruses by the presence of an alanine at residue 271 of the PB2 protein (T271A), which, although rare, has been shown previously to enhance the polymerase activity of IAVs in mammalian cells and in mice (Bussey et al., 2010). The same mutation was identified in the avian-like PB2 gene of the 2009 swine-origin pandemic influenza virus (Zhang et al., 2012) and was associated with the acquisition of a mutation at position 226 of HA that confers recognition of an α2,6-linked SA receptor. Thus, this mutation in clade 2.3.4.4b H5N1 viruses has potential public health implications and warrants further investigation.

All four viruses characterized from minks also contained a methionine at position 396 located in the second SA binding site (2SBS) of the NA. H5N1 viruses typically encode an isoleucine at this position, and this residue has been shown to be critical for efficient binding of SA to the 2SBS (Du et al., 2021). The 2SBS preferentially binds α2,3-linked SAs and enhances activity of the neighboring catalytic site by bringing multivalent substrates in closer proximity. While the 2SBS is conserved in nearly all avian IAVs, it has been lost in all pandemic influenza viruses and in several other viruses adapted to mammalian host species. Conservation or loss of the 2SBS is thought to be associated with changes in host tropism. Disruption of the 2SBS in H5N1 viruses was reported to precede acquisition of mutations in HA that decreased binding to avian-type α2,3-linked SA receptors, while concomitantly increasing binding to human-type α2,6-linked receptors (Du et al., 2020). Similar to the acquisition of T271A in PB2, disruption of the 2SBS in H5N1 viruses may be a step toward human adaptation. Based on publicly available sequences, the other mutations identified in minks have rarely or never been identified in HPAI H5Nx viruses, and their biological significance is not known.

Recently, in a preprint, a number of circulating mammalian-origin clade 2.3.4.4b H5N1 viruses were shown to replicate in primary human airway epithelial cells and cause lethal infection in mice and ferrets (Kobasa et al., 2023). One isolate, A/Red Tailed Hawk/ON/FAV-0473-4/2022, also transmitted efficiently by direct contact between ferrets, resulting in severe disease. Although the RT.Hawk/ON/22 virus was of avian rather than mammalian origin, it was found to contain mammalian adaptive signatures, suggesting that it was acquired by the red-tailed hawk consuming infected mammalian carrion. Prior passage through infection of multiple animal species could also have contributed to the adaptation of the virus and its observed transmissibility between ferrets that were in direct contact. This is a concerning scenario that is likely to occur with increasing frequency as outbreaks in wild animal species and particularly mammals continue.

As H5N1 viruses infect more species, their geographical range increases and more viral variants are produced that could have new biological properties. This also increases the chances that the virus could adapt to mammals and potentially to humans. The unprecedented number of infected wild birds during the current H5N1 outbreak has led to strict measures to try to reduce the spread of the virus. These include culling of affected commercial flocks, the imposition of "protection zones" around affected premises, and an order to ensure that all domestic birds are housed indoors. Even with these measures, H5N1 viruses have continued to spread. For the moment, the WHO assesses the risk of H5N1 to humans as low (World Health Organization, 2022), but the risk will be reassessed as new data emerge. The importance of good monitoring and biosecurity and avoiding contact with sick or dead birds and avoiding live animal markets, which are a risk factor for contracting zoonoses, are all prudent public health measures. The COVID-19 pandemic and resulting "post-pandemic fatigue" have largely drawn attention away from other infectious disease threats, but we must remain vigilant to ensure an even more deadly pandemic does not eventuate.

Disclosures: The authors declare no competing financial interests.

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