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H5N1 influenza A virus: lessons from past outbreaks and emerging threats

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SUMMARY

The first highly pathogenic H5N1 emerged in 1959 on a chicken farm in Scotland. The ancestor of the strains presently circulating was isolated in 1996 from a domestic goose in China. Since 1997, more than 900 severe human infections have been reported. However, in nearly thirty years, H5N1 has failed to adapt to human-to-human transmission. At present the abundant circulation in various animal species, including mammals, increases the possibility of reassortments of new pandemic strains. Particularly alarming was the recent report of H5N1 infection among U.S. dairy cattle. A strong international effort from a global health perspective addressed to limit the avian strains circulation and to improve the preparedness for a new pandemic is urgently needed.

Keywords: Influenza A virus, H5N1, avian influenza, prepandemic vaccines, global health.

INTRODUCTION

On May 19, 1997, an influenza A virus claimed the life of a three-year-old boy in a Hong Kong hospital. While tragic, the case itself might not have been considered newsworthy until it was revealed that the virus responsible was an avian H5N1 strain [1]. The first case of human infection with an avian influenza A virus (IAV) had been reported two years earlier, in 1995, in the United Kingdom. The strain involved was an H7N7 strain, which caused only mild symptoms [2]. However, in March 1997, few weeks before the occurrence of the Hong Kong case, Taubenberger and colleagues published in *Science* the first characterization of the Spanish flu virus sparking a debate about the origins of that pandemic and the role of avian IAV as a source of future pandemic viruses [3].

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The Hong Kong virus originated in chickens imported from China, where a highly pathogenic virus - A/goose/Guangdong/1/96 (H5N1), commonly referred to as Gs/GD - had been isolated in 1996 from a domestic goose and, notably, no human infections were reported [4]. In Hong Kong, H5N1 spread in markets and three farms, primarily affecting geese and ducks. During 1997, there were eighteen cases and six deaths [5-8]. All infections were acquired through contact with infected animals, with no evidence of human-to-human transmission. Serological studies revealed that asymptomatic infections were not particularly frequent and were mostly observed in individuals exposed for occupational reasons [9-11]. However, the number of such cases was sufficient to estimate the actual lethality of the infection at 1–2% [9].
In November 1997, H5N1 re-emerged among farmed birds in Hong Kong, prompting authorities to take the drastic step of culling all poultry [6]. Although this measure led to the definitive extinction of the "HK97" strain, a series of descendants of the Gs/GD strain continued to emerge, infecting domestic and wild birds, various mam-

malian species, and, occasionally, humans, without acquiring the capability for sustained human-to-human transmission [4, 6].
The aim of this review is to outline the spread and evolutionary history of H5N1 strains, summarize the epidemiological and clinical features of infection in humans, and report on the progress in therapies, vaccines, and vaccination strategies.

THE FIRST STEPS OF H5N1 STRAINS EVOLUTION

The first known highly pathogenic (HPAI) H5N1 is A/chicken/Scotland/59 (H5N1), emerged in 1959 in a chicken farm in Scotland [12]. Other epizootics caused by highly pathogenic H5 subtypes had been reported in subsequent times [13]. Between 1996 and 2002, reassortments between Gs/GD and Low Pathogenic Avian Influenza (LPAI) strains circulating in domestic and wild birds generated various H5N1 genotypes [14-17]. At the end of 2001, one of these strains emerged in Hong Kong and infected, between January and March 2002, twenty-two farms, causing the culling of 950,000 birds and, on the end of the year, various wild bird species housed in two aquatic parks [18, 19]. In 2003 - just the year of the SARS epidemic - a H5N1 genotype caused large outbreaks in poultry and some human cases in Vietnam, Thailand, and mainland China [20]. By February 2004, eight countries in East Asia were affected. The infection largely involved also village poultry, particularly free-range ducks. Live animal markets were a major source of the infection's spread and a priority target for containment measures, which were difficult to implement and unpopular in countries where poultry is a vital food resource. In Thailand, a major obstacle to containment efforts came from the refusal to cull fighting cocks and their breeding lines [21-23]. In mid-October 2004, an outbreak among zoo tigers in Thailand killed 45 animals. The strain involved was a Highly Pathogenic Avian Influenza (HPAI) virus, showing a lysine substitution at position 627 of the PB2 protein, which was absent in the original avian isolates [24].
In the meantime, the number of the human infections reported to the WHO was rising. In 2005, they were 98, three times the number reported the previous year. At the end of 2005 the human cases reported to WHO since 2003 were 148, with 79 deaths (53,4%) (Figure 1) reported only in East Asia countries (Vietnam, 93 cases; Thailand, 22 cases; Indonesia, 20 cases; Popular Republic of China, 9 cases; Cambodia, 4 cases).

FROM DOMESTIC BACK TO WILD BIRDS: THE RISKS OF SPILLBACK

The infection of captive wild bird species in Hong Kong in 2002 had been considered a general re-

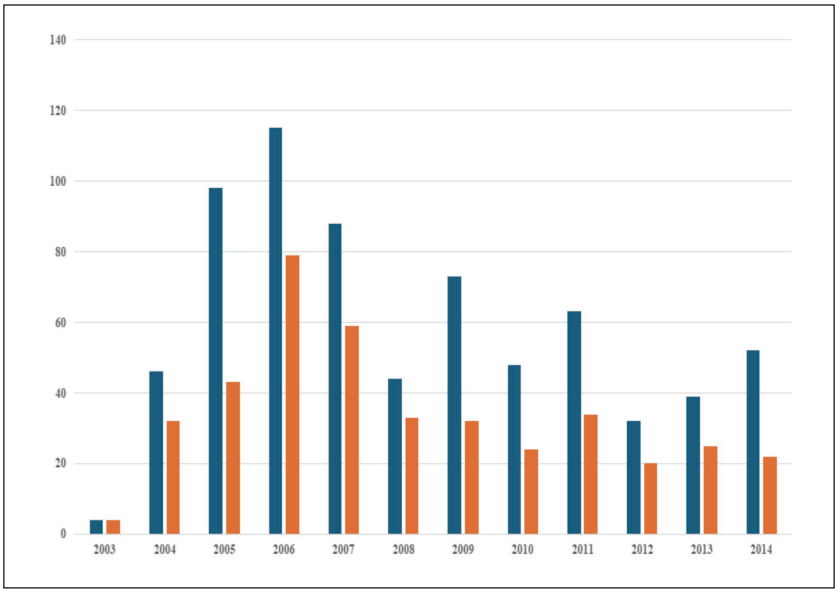

Figure 1
Numbers of human cases (blue bars) and deaths from H5N1 infection (orange bars) from 2003 to 2014.

hearsal for spillback, raising concerns about the "return" transfer of the infection from domestic birds to free-living migratory birds [19]. This scenario materialized in the spring of 2005, when a mass death involved various wild species at Lake Qinghai, where the virus had been brought by migratory birds infected through contact with domestic poultry in southern China [25, 26].
Subsequent studies demonstrated that migratory birds could carry both HPAI and LPAI strains over long distances without showing symptoms, and that avian influenza viruses can spread along migration routes [27-29]. During 2005, the virus spread to various species of birds, both wild and domestic, in Russia, Europe, Africa, and the Middle East.
In October 2005, the Global Influenza Program Surveillance Network published an investigation on the isolates obtained in humans and birds in 2004 and 2005, showing the existence of two clades of the virus, one widespread in China, Korea and Indonesia, and the other in Vietnam, Cambodia, Thailand and the Malay Peninsula, without geographical overlap. The isolates from patients were found to be entirely of avian origin, without reassortment with human influenza virus genes, and very similar to those from birds in the same geographic area, thus suggesting a direct passage from birds to humans [30].

2006-2008: OUT OF EAST ASIA BORDERS AND FURTHER SPLITS OF THE VIRUS

In February 2006, H5N1 was isolated in Sicily from sick swans. In the same year, it became clear to the international agencies that the virus had crossed the borders of East Asia. Epizootics and human infections were reported in Turkey, Azerbaijan, Iraq, Egypt, and Djibouti. The cases in humans reported to WHO during 2006 were 115, with 79 deaths (Figure 1). A family cluster, involving three people - a child, his mother, and his aunt who cared for him - was seen in Thailand and eight cases occurred in Sumatra within an extended family group [31].
During 2007, there were 88 cases, mainly contributed by Indonesia (42 cases) and Egypt (25 cases), with 59 deaths. In the same year, the H5N1 Evolution Working Group elaborated a unified nomenclature system based on a dataset of 859 H5 sequences. A subdivision into 10 clades (0-9) was proposed, of which clade 0 included the original Gs/GD strain, and Clade 2 was the most differentiated, being further classified into 5 subclades, named according to a hierarchical numbering system (2.1-2.5).
During 2008, H5N1 infections continued to occur both in animals and humans, even if the number of human cases appeared to decrease. The reported human cases were 44, just half of those of the previous year, with 33 deaths. On the contrary, in the first ten months of 2008, outbreaks in poultry arose in at least 24 countries across Europe, the Middle East, Asia, and Africa, and infections were reported in wild birds in China, Hong Kong, and the United Kingdom [32].
Clade 2.2, originated in 2005 from the outbreak of Lake Qinghai, was isolated in the same year in Europe, the Middle East and Africa, as well as in China and Mongolia. In the meantime, clade 2.3.4, which has been circulating in China since 2005, gave rise to the clade 2.3.4.4, destined to become the most widespread strain worldwide in 2014-2015 wave [33]. Other earlier HPAI H5 clades, such as 2.2, 2.3.2.1 showed a more restricted regional circulation primarily through domestic birds and human activity, with brief periods of dispersal through wild birds [34].

THE EVOLUTION OF A NEW H5 STRAIN FROM A H5N8 VIRUS

Contrary to expectations, between 2010 and 2014 the number of cases observed in humans did not increase and the observed cases continued to occur only in people who had been in contact with infected poultry. There was a total of 233 reports, 120 of which reported in Egypt.
In February 2009, before the emergence of H1N1 pandemic, Solomon and Webster wrote on Cell that 'there is concern that if an H5N1 pandemic does not occur, scientists will lose public credibility, and pandemic planning will be supplanted by more pressing public health programs' [35].
After this period of apparent calm, epizootics have become increasingly frequent since 2014, sustained by strains carrying a H5 of the 2.3.4.4 clade associated with different subtypes of NA (N1, N2, N3, N5, N6, N8) [33] (Figure 2). In 2016-2017 the most widespread subtype was a H5N8 with the H5 of the subclade 2.3.4.4b, that originated in China, where was identified in poultry, and caused

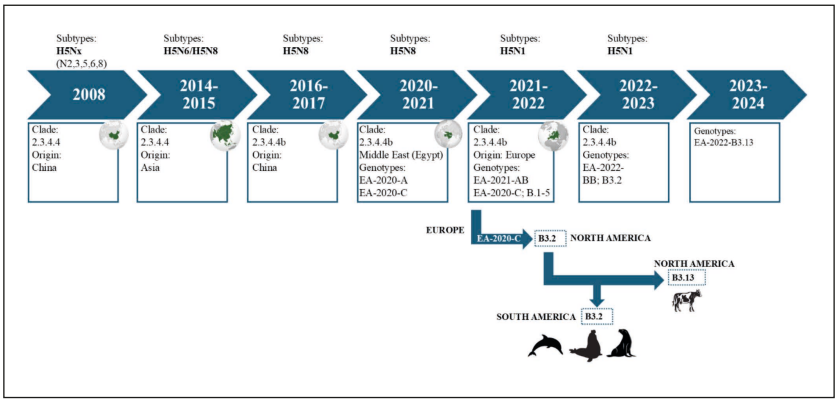

Figure 2
Timeline of the evolution and spread of H5 strains.

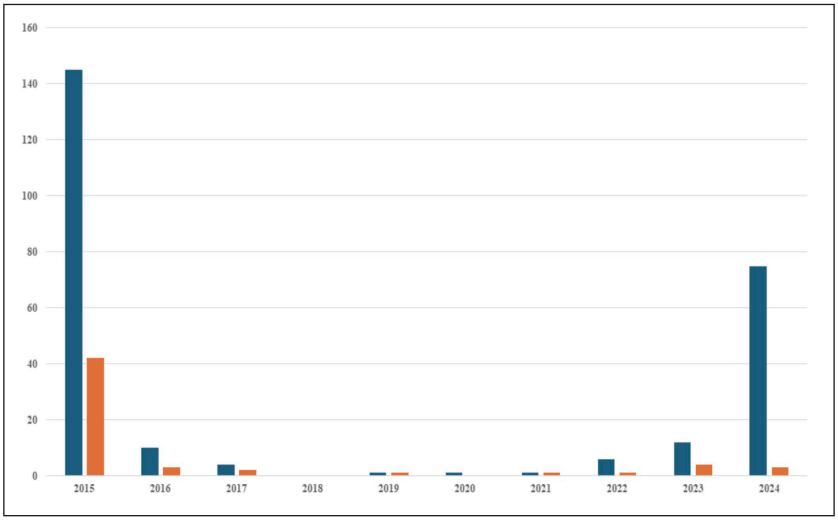

Figure 3
Numbers of human cases (blue bars) and deaths from H5N1 infection (orange bars) from 2015 to December 2024 (the reports of 2024 might be incomplete).

outbreaks in domestic and wild birds in South Corea [36, 37]. A 2.3.4.4b H5 strain prevailed also in the epizootics of 2021, even if all its eight genes derived from a virus endemically circulating in Egypt among poultry since 2016. The local relevance in Egypt is also witnessed by 136 human cases occurred in 2015 in that country on a total of 145 cases reported worldwide. Unexpectedly, however, in the following years, human cases almost disappeared (Figure 3).

AN ACCELERATION AMONG WILD BIRDS, AN INCREASE OF EPIZOOTICS IN POULTRY

In autumn 2020 a strain emerged from the reassortment of H5N8 European virus with a subtype N1 and 5 genes (PB2, PB1, PA, NP and NS) of LPAIs circulating in Europe among wild birds since 2019, giving origin mainly to the genotype EA-2020-C. Both the epizootics waves of 2020-2021 and 2021-2022 caused by this new HPAI H5N1 strain involved also wild birds, in which, for the first time, there was an unprecedented high mortality [34,38]. According to data from the European Centre for Disease Prevention and Control (ECDC), from October 1, 2021, to March 15, 2022, Europe recorded 1,363 epizootic outbreaks, 311 of which occurred in Italy.

On January 5, 2022, an asymptomatic infection was reported in England in a man exposed to ducks that were found to be infected with the same strain [39]. In the spring, a further reassortment with a H13 subtype circulating primarily in sea gulls generated a new H5 genotype 2.3.4.4 b (EA-2022-BB)

which in Northern Europe, in the subsequent winter, was responsible of mass mortality among seabirds (especially gulls and terns) [38]. Since November 2021, this clade spread all over the world, investing Africa in 2021 and Asia in 2022 (Figure 2). In December 2021, the most prevalent genotypes circulating in Europe (EA-2020-C) was introduced in North America, where a reassortment with American LPAI virus gave origin to several genotypes (called B1-B5), the most prevalent of which became the B3.2 [40]. Since October 2022, this genotype has spread first to Mexico and then to the whole South America [38].

TIME OF MAMMALS

That mammals could be infected by avian H5N1 was known since the epizootic in tigers in zoos in Thailand in 2004 and subsequent reports in large felids, in raccoon dogs (*Nyctereutes procyonoides*) in China and in cats and dogs in Thailand [24, 41-43]. H5N1 was found also in farmed pigs, in which the infection is generally asymptomatic and infrequent, due to their low susceptibility to the virus [44, 45]. Between 2003 and 2019, the non-human mammal species in which the virus was isolated at least once were no less than 12. The infections in almost all the cases occurred in Asia, and involved mainly carnivores [46, 47].

Since 2020, after the emergence and the spread in poultry and wild birds of the new reassorted strains, the isolations of H5N1 in mammal species showed a marked acceleration, involving also marine mammals. In October 2022 a large outbreak, caused by a H5 virus of the European BB genotype, occurred on an intensive American mink (*Neogale vison*) farm in Galicia, northwestern Spain [48]. The strain that infected the minks showed mutations in the PB2 gene, such as T271A, E627K and D701N, that are associated with mammalian adaptation [38, 49, 50]. However, no infections occurred in farm workers. On the contrary, a mink-to-mink transmission was hypothesized. Additionally, several cases in seabirds, particularly in gannets (*Morus bassanus*), were reported in the area during the same period. In France, in December 2022, a domestic cat was infected with H5N1 from the BB clade, closely related to the virus that had affected a nearby duck farm [51].

The spread in South America of genotype B3.2 has caused the death of coastal birds and marine mammals with a wide mortality of sea lions (*Otaria flavescens*) starting from early 2023 in Peru and Chile and then spreading to Argentina, Uruguay and Brazil [50]. In Peru the 5% of sea lion population died during the epizootic [52]. The identification of mutations possibly associated to mammal-adaptation in PB2 (D701N and Q591K) and the grouping in phylogenetic trees of marine mammal isolates into a single clade separate from wild birds and poultry, suggests possible mammal-to-mammal transmission. Meanwhile, at the end of March, a case occurred in an inhabitant of the Antofagasta region of Chile. The virus infecting this patient belonged to the B3.2 clade and was almost identical to the virus circulating in birds in the same area, where infections were also reported in marine mammals, such as porpoises (*Phocoena spinipinnis*) [54] and dolphins (*Cephalorhynchus eutropia*) [46, 53] (Figure 2).

In April 2023, the Ministry of Health of Italy reported infections in two foxes (*Vulpes vulpes*), and in July, a BB strain was detected in 27 fur farms in Finland, involving foxes (*Vulpes lagopus* and *Vulpes vulpes*), American minks, and raccoon dogs [55]. In addition to fur animals, the virus was found in six black-headed gulls (*Chroicocephalus ridibundus*), one common gull (*Larus ganus*), and two wild mammals - a red fox (*Vulpes vulpes*) and an otter (*Lutra lutra*) - from the same region. The strain involved was the same responsible of the outbreak occurred in minks in Spain in 2022 [38, 49, 50]. On July 11, 2023, WHO reported an outbreak of H5N1 infection in cats in Poland. Twenty-nine out of 46 samples taken from cats, and one from a caracal kept in captivity, tested positive for H5N1 belonging to the BB clade and was very similar to viruses circulating in the area in both wild and domestic birds [56].

From 2021 to the end of 2023, at least 57 species of mammals were reported to become naturally infected with H5N1 (Table 1). Most species belong to the order Carnivora and some of them also are facultative scavengers. Thirteen species of marine mammals, including seven species of pinnipeds and six of dolphins, were also involved. The most plausible source of infection in all these animals is close contact with infected birds, and their predation and eating. Necropsied animals showed mainly lesions in lungs and brain [46, 50]. Regarding human infections, during 2023 the cases reported have been 12. In March, after nine years

Table 1 - List of mammal species naturally infected with H5N1 strains, per year of first isolation (colours indicate the continent in which the infection has been observed: yellow, Asia; green, Europe; orange, North America; light orange, South America; light blue, Africa).

Year	*Terrestrial carnivores*	*Other terrestrial mammals*	*Marine mammals*
2004	Panthera tigris*^	Sus scrofa domesticus*	
	Panthera pardus*		
	*Felis catus***		
	Canis lupus familiaris**		
2005	Chrotogale owstoni*		
2006	Nyctereutes procyonoides*^	Ochotona curzoniae***	
	Martes foina***		
	Neovison vison*		
2010		Equus africanus asinus*	
2016	Panthera leo*		
2021	Lutra lutra***		Tursiops truncatus***
	Vulpes vulpes***		
	Linx linx***		
2022	Nyctereutes viverrinus	Didelphis virginiana***	Phoca vitulina***^
	Felis catus**		Halichoerus grypus***^
	Neovison vison*^		
	Ursus thibetanus*		
	Meles meles***		
	Mustela putorius***		
	Vulpes vulpes***		Phocoena phocoena***
	Procyon lotor***		Pusa caspica***
	Lynx rufus***		Lagenorhynchus acutus***
	Canis latrans***		Cephalorhynchus eutropia***
	Urocyon cinereoargenteus ***		Delphinus delphis***
	Pekania pennanti***		Phocoena spinipinnis***
	Mephitis mephitis***		Arctocephalus australis***
	Ursus americanus***		
2023	Caracal caracal*	Ondatra zibethicus***	Callorhinus ursinus***
	Felis catus**^	Castor canadensis***	
	Nyctereutes procyonoides*^	Sciurus aberti ***	
	Vulpes vulpes*^	Sylvilagus audubonii***	
	Vulpes lagopus*^		
	Speotus venaticus venaticus*		
	Canis lupus familiaris**		
	Martes foina***		
	Martes martes***		
	Sus scrofa domesticus*		

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Year	*Terrestrial carnivores*	*Other terrestrial mammals*	*Marine mammals*
2023	Ursus maritimus***		Otaria flavescens***^
	Martes americana***		Mirunga leonina***^
	Lontra canadensis***		
	Panthera tigris*		
	Panthera pardus*		
	*Puma concolor****		
	Ursus arctos horribilis***		
	Ursus arctos middendorffi***		
	Lontra felina***		
	Lontra provocax***		
	Nasua nasua***		
	Panthera leo*		
2024		Bos taurus*^	
		Capra hircus*	
		Vicugna pacos*	
		Sus scrofa domesticus*	

*captive/farmed, **pet, ***wild, ^epizootic.

without human cases in that country, two persons, father and son, became infected in Cambodia with a virus belonging to 2.3.2.1c clade, circulating since several years in wild and domestic birds across Africa, Asia, Europe, and the Middle East [57]. In mid-May 2023, infections occurred in two workers on a poultry farm in UK. Both cases were asymptomatic and detected as part of an ongoing surveillance of workers exposed to poultry infected with avian influenza [58].

AND NOW THE COWS

In March 2024, juvenile goats with neurological symptoms tested positive for HPAI A(H5N1) and at the same time, cases of H5N1 infection have been reported in cows in the United States [59, 60]. It was involved a B3.13, genotype, relatively rare in U.S. avifauna, infecting dairy cows since February 2024 in Texas, and subsequently spread to 16 U.S. states, in a total of 891 dairy herds (data of December 25th). This genotype was identified in numerous other mammals (including cats, alpacas, foxes, raccoons), and workers in contact with cows or poultry.

This B3.13 genotype emerged in late 2023 from a reassortment event between panzootic Eurasian H5N1 genotype (EA-2020-C), and LPAI genotypes circulating in North America, in which PA, HA, NA, and MP are derived from the Eurasian genotype, while PB2, PB1, NP, and NS are derived from American genotypes. The dairy cattle virus forms a single clade separate from all the others in all gene segments, which suggests a single introduction of the virus into the population and possible cattle-to-cattle transmission [61, 62]. Several other genomes from wild and domestic birds, but also domestic cats and wild mammals, were included in the monophyletic group of the dairy cattle, suggesting a possible spread of the infection from cattle to other animals. In addition, H5N1 virus from cattle showed two mammalian adaptations in the polymerase (PB2 M631L and PA497R).

A single severe human case of H5N1 infection was also reported by the Public Health Agency of Canada that have occurred in British Columbia, involving a 2.3.4.4b H5N1 strain different from that causing the dairy cattle outbreak in US, corresponding to genotype D1.1 involved in an outgoing outbreak in poultry in the same Region [63]. A further severe case due to the D1.1 genotype occurred in Louisiana has been reported by the CDC on December 18th. On December 27th, a total of 66 human cases of H5N1 avian influenza occurred in

2024 have been notified to the Atlanta's CDC, 40 of which are related to dairy cows and 23 to poultry. However, significant changes in the HA which can modify the affinity for the human upper respiratory tract, have not been observed and this justifies the relatively low risk to date, of inter-human transmissibility of this virus [61, 64].

HOW LIKELY IS IT THAT AN H5 STRAIN WILL BE THE NEXT PANDEMIC IAV?

It is a fact that H5 strains, although widespread among birds since the end of the last century, have caused serious forms of disease in only a few hundred humans. Considering also the asymptomatic and mild symptomatic cases - that are more frequent, albeit occurring in people in contact with infected animals, and not, with very few exceptions, through human-to-human transmission - the estimate of the total of infections remains low. The question therefore arises whether H5 can adapt to human infection [65-67].
In IAVs, the protein that interacts with the receptors of the target cells and plays a crucial role in sustaining transmission is hemagglutinin (HA), which binds to sialic acids (SA) terminally attached to glycans, enabling viral endocytosis and membrane fusion. HAs of human adapted IAVs prefer SAs linked to galactose (Gal) in an α2,6 linkage, whereas avian IAVs prefer an α2,3 linkage [68, 69]. For this reason, the direct passage of an avian IAV to humans is difficult, although perhaps not impossible [70]. The recent widening of the range of infected species of mammals, including also pets, is a cause of concern. Felines, as well as canids, have sialic α-2,3 receptors in both upper and lower respiratory tracts, which facilitates the direct transmission of avian influenza subtypes [71, 72]. Pigs, on the other hand, have sialic receptors of both types. On December 2024, the first cases of H5N1 infection in swine in the US were reported. A high prevalence of seroconversion was also reported in 2023 in pigs raised on a farm in Ostia, Italy, where an epizootic caused by the BB clade had occurred in poultry [73]. The finding of asymptomatic infections in these animals raises renewed concern for their possible role as a mixing vessel for the reassortment of a future pandemic virus. Bovids were instead believed not to be hosts of IAVs, which made the recent isolation of 2.3.4.4b in these animals completely unexpected. Recent papers revealed that bovine mammary glands are rich of avian virus–specific SA α2,3-gal receptors, while their upper respiratory tract is devoid of receptors for IAV [74-76]. Cows could therefore spread the infection through the milk, while the ways in which they become infected and whether they transmit the infection to each other are currently being investigated. Preliminary data show that 2.3.4.4b H5s from cows bound to avian SA α2,3–gal, but not to SA α2,6–gal receptors of the human cells [77]. However, pasteurization was shown to inactivate H5N1 influenza virus in raw whole milk [78].

CLINICAL FEATURES OF HUMAN H5N1 INFECTIONS

In a study of 907 human cases reported between 1997 and 2015, the median age of patients was 19 years, with interquartile ranges of 5 to 32 years, confirming that the infection was more commonly observed in younger individuals. Over 90% of these cases required hospitalization, and the fatality rate was 53.3%. The median age of deceased patients was 30 years in North Africa and 19 years in East Asia. The median time from symptom onset to death or discharge was 10 days, with interquartile ranges of 7 to 15 days. Most patients (82.5%) reported contact with poultry, while only 5.4% knew exposure to infected persons [79].
The typical incubation period for human H5N1 infections was 2–5 days, though sporadic cases have been observed up to 7 days after presumed exposure. Fever was a common early symptom, often accompanied by cough, malaise, myalgia, headache, sore throat, abdominal pain, vomiting, and diarrhea [80-84]. Viral pneumonia leading to severe respiratory insufficiency was the most frequent cause of death. Neurological complications, including encephalopathy and seizures, were documented in some H5N1 patients, particularly in pediatric cases. These findings suggest the potential of the virus to cause direct or immune-mediated central nervous system damage, as observed in various mammalian species [46, 50, 59, 85].
Survivors of H5N1 infection often experienced long-term sequelae, such as pulmonary fibrosis, chronic fatigue, and psychological distress, highlighting the significant impact of severe infection on physical and mental health [86]. However, infections contracted through contact with infected

cows presented a markedly milder clinical course. Of 45 reported cases, none required hospitalization, and no fatalities were observed. Among these patients, 42 (93%) had conjunctivitis, 22 (49%) reported fever, 16 (36%) exhibited respiratory symptoms, and 15 (33%) presented with conjunctivitis only [87].

THE TREATMENT OF H5N1 INFECTION

The neuraminidase inhibitor oseltamivir is the recommended treatment for suspected, probable, or confirmed H5N1 cases [88]. Treatment should begin as soon as possible, ideally within the first 48 hours of illness onset, even before confirmatory test results are available. However, due to the lack of clinical trials, the current evidence is derived solely from observational studies, which lack adequate controls. This limitation is exacerbated by the challenges of early diagnosis, resulting in many patients receiving antiviral treatment outside the optimal window for maximum efficacy.
Additionally, the early development of resistance to oseltamivir has raised concerns about appropriate dosing [89]. Resistance to antivirals has been documented both prior to treatment initiation and in individuals receiving neuraminidase inhibitors as prophylaxis [90]. Gastrointestinal symptoms, such as diarrhea, may further complicate treatment by impairing drug absorption. Combination antiviral therapies, such as oseltamivir and zanamivir, have been investigated in animal models and may provide benefits in severe or resistant H5N1 infections [91].
Adjunctive therapies, including corticosteroids and immunomodulatory agents like intravenous immunoglobulin (IVIG), have been used in severe cases of H5N1 infection. However, their benefits remain controversial, with some evidence suggesting potential harm due to immune suppression [92]. For patients with severe disease, supportive care, particularly ensuring adequate oxygenation, is essential.
Post-exposure prophylaxis with oseltamivir for 5 to 10 days is recommended for individuals in close contact with confirmed cases [93].

H5N1 HUMAN VACCINES

The potential for the avian influenza virus to mutate drastically upon adapting to humans has raised questions about the effectiveness of pre-pandemic vaccines derived from circulating avian strains. Nonetheless, three H5N1 influenza vaccines based on H5N1 strains that circulated in the early 2000s - A/Vietnam/1194/2004 (clade 1) and A/Indonesia/5/2005 (clade 2.1) - have been licensed in the United States. These vaccines, available either unadjuvanted or adjuvanted with oil-in-water adjuvants such as MF59 or AS03, are stored in the U.S. stockpile for pandemic preparedness.
A recent study demonstrated that these vaccines generate cross-neutralizing antibodies against the highly pathogenic H5N1 clade 2.3.4.4b influenza virus [94]. In late July 2024, the World Health Organization (WHO) announced plans to develop a mRNA vaccine to protect people in low- and middle-income countries from the highly pathogenic avian influenza A(H5N1) virus [95]. This initiative is part of WHO's mRNA Technology Transfer Program, launched in 2021, which aims to empower lower-resource countries to develop and produce mRNA vaccines for more equitable responses to future pandemics.

DIAGNOSIS OF H5N1 INFECTION

Diagnostic workup algorithm of suspected H5N1 is depicted in Figure 4. Hematological and biochemical investigations are generally unhelpful, as only transient lymphopenia is typically observed. The diagnostic process primarily relies on molecular methods, particularly real-time RT-PCR, which can detect even a single viral particle in a sample [96]. Repeated testing is necessary to rule out false-positive results and confirm productive avian influenza infection in symptomatic or asymptomatic individuals. Confirmed positive samples for H5 virus should be sent to a WHO Collaborating Centre for Reference and Research on Influenza (WHO-CCRRI).
Positive samples are further subjected to whole genome sequencing (WGS) to obtain detailed information about the viral genotype and identify specific mutations, particularly those associated with antiviral resistance [97]. Genomic surveillance has become an indispensable tool for identifying emerging zoonotic influenza viruses [96].
Rapid antigenic diagnostic tests developed for avian influenza diagnosis show sensitivities of 50–80% and specificities of 90% [98]. Additionally,

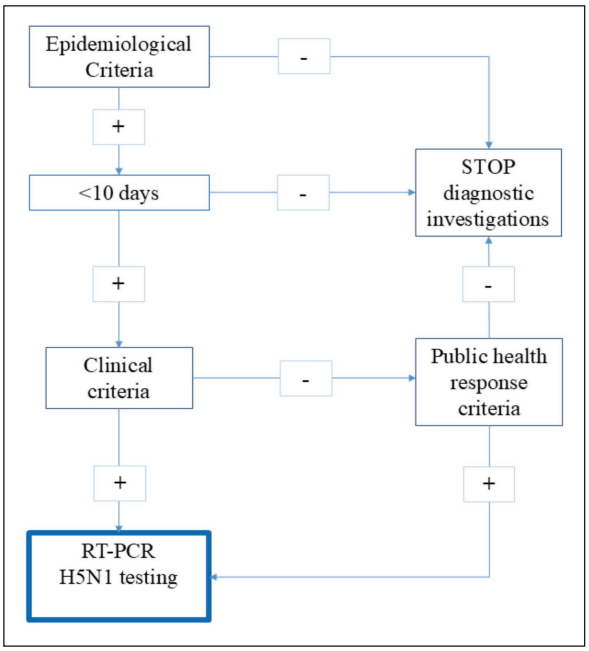

Figure 4 - Diagnostic workup of avian flu. Epidemiological criteria: Exposure to HPAI A(H5N1) virus-infected birds or other animals, OR exposure to an infected person, OR laboratory exposure. *Clinical criteria:* Persons with signs and symptoms consistent with acute upper or lower respiratory tract infections, conjunctivitis, or complications of acute respiratory illness of unknown origin. Additionally, gastrointestinal symptoms, such as diarrhea, are often reported with HPAI A(H5N1) virus infection. *Public health response:* Asymptomatic individuals should be monitored and tested in accordance with public health measure.

serological methods for detecting antibodies against the virus are used retrospectively in epidemiological investigations [99].

GLOBAL PREPAREDNESS FOR AVIAN INFLUENZA

Preparedness for avian influenza presents significant regional and national challenges. Even countries with robust public health infrastructures and comprehensive surveillance systems face difficulties in tracking morbidity, mortality, and potential avian influenza cases due to resource limitations [100]. Despite advancements in molecular testing during the COVID-19 pandemic, even in countries with established surveillance systems specialized testing for A (H5N1) in suspected human and animal cases is often restricted to national reference laboratories or WHO Collaborating Centres [101]. Additionally, not all countries have the necessary resources - such as expertise and specialized facilities (e.g., BSL3 labs) - to conduct virus characterization or identify mutations that could enhance transmissibility, reduce antiviral effectiveness, or alter diagnostic test accuracy. The limited availability of diagnostic tools in many countries, particularly for testing the clade 2.3.4.4b of the H5N1 influenza virus, remains a major concern among scientists [102]. Besides strengthening surveillance, and the fostering of international cooperation, investing in research is essential to prevent the H5N1 virus from igniting the next global health crisis [103]. Technological tools, such as artificial intelligence and machine learning, could offer significant support to forecasting, detection, early warning and management of avian influenza outbreaks [104].

CONCLUSIONS

Over the course of nearly thirty years, H5N1 has failed to adapt to human-to-human transmission, and we do not know whether a H5 strain will ever be able to do so. Nevertheless, the acquisition of the interhuman transmissibility of a such a strain, that would be completely new to our species, predicts a scenario of billions of infected people. All past influenza pandemics, including the 2009 flu pandemic, which was generally mild enough to be labelled a flop by some, have taken a heavy toll in human lives. It must also be remembered that the drug therapies against IAVs are far from being completely satisfactory and that, in the event of a pandemic, the time needed for a fully active vaccine to be available and administered worldwide would not be short. However, envisaging apocalyptic scenarios, without finding ways to address the problem, more than useless, would perhaps be harmful, as demonstrated by at least two cases in the past, the alarm raised by the H1N1 at Fort Dix in 1976 and that concerning the same H5N1 in 2005. Moreover, the emergence of a new pandemic H1N1 virus in 2009 led to increased scepticism in the media about H5N1 as a pandemic agent candidate [105]. It is a matter of fact, however, that at present the abundant circulation in various animal species of IAV having genes, especially PB2, carrying mutations favouring the replication in mam-

mals, increases the possibility of reassortments of strains potentially dangerous for humans, even other than H5. It is therefore clear that to prevent the emergence of a new pandemic IAV is an issue that should be addressed urgently, from a global health perspective [106] and with a strong international effort.

Conflict of interest
None to declare

Funding
None to declare

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