

After SARS-CoV-2, will H5N6 and other influenza viruses follow the pandemic path?

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SUMMARY

While the world is focused on attending, controlling, and mitigating the current pandemic of COVID-19, caused by the SARS-CoV-2, other viral threats are possibly emerging and reemerging especially in Asia, posing a risk for the spread in that region and beyond. A predictable threat is the avian influenza virus, especially H5N6, which has recently led to significant outbreaks in China and the Philippines, deserving more attention and control. In the current review, we assess the histo-

ry of this highly pathogenic reemerging virus, as well as the contemporary implications of poultry outbreaks occurring in some Asian countries. We also look at outbreaks due to other strains not only in Asia but also across Europe and Africa, according to recent reports from the World Organization of Animal Health (OIE).

Keywords: Avian influenza, H5N6; SARS-CoV-2, emerging, pandemic, emergency.

INTRODUCTION

Avian Influenza (AI) is a highly contagious viral respiratory disease that affects all species of birds, including commercial, wild, and pet

birds [1-3]. Its causal agent, the Avian influenza virus (AIV) is a single-stranded segmented negative-sense RNA virus. Classification is based on the antigenic properties of their Hemagglutinin (HA) and Neuraminidase (NA) [4, 5]. According to the ability of the virus to cause significant disease, the different strains could be further divided into Non-Pathogenic AIV (NPAIV), Low-Pathogenic AIV (LPAIV), and Highly-pathogenic AIV

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(HPAIV) [6]. Spillover to human beings and other animal species may occur, leading to an infection that may be life-threatening [1]. Throughout history, most AIV did not infect humans. However, sporadic cross-species infections did occur, triggering mild outbreaks, and even pandemics (Spanish flu, 1918-1920 caused by H1N1) [7]. From 2010 to date, over 400 cases and more than 150 human deaths by an HPAI have been reported in Egypt, Ghana, Hong Kong, Indonesia, Nigeria, Bangladesh, Cambodia, Vietnam, and Canada [8, 9]. The threat of a new HPAI strain has been latent over the years, with occasional outbreaks in poultry associated with severe consequences on livelihoods, international trade, and also severely impacting public health systems [2, 10]. Recently, lineages of the H5Nx (H5N1, H5N2, H5N6, H5N8) have proven to pose a severe risk

to the veterinarian and human health due to the capacity for the zoonotic spread and the genomic interactions amongst this viral group (Figure 1) [11]. One example was the 2003 H5N1 epidemic, spreading from Asia to Europe and Africa, causing millions of animal deaths and hundreds of people infected, with reported case fatality rate close to 60% [2, 12]. The AIV H5N6 was an LPAIV first reported decades ago [11]. However, the first reported case of human infection with a novel HPAIV H5N6 dates from 2014 in China, with symptoms that included fever and sore throat [4, 12, 13]. The association with live bird markets (LBM) or wet markets was established, confirming once more the threat that these markets pose to public health as they provide a perfect scenario for the spread, but also the modification of the AIV genes due to the close

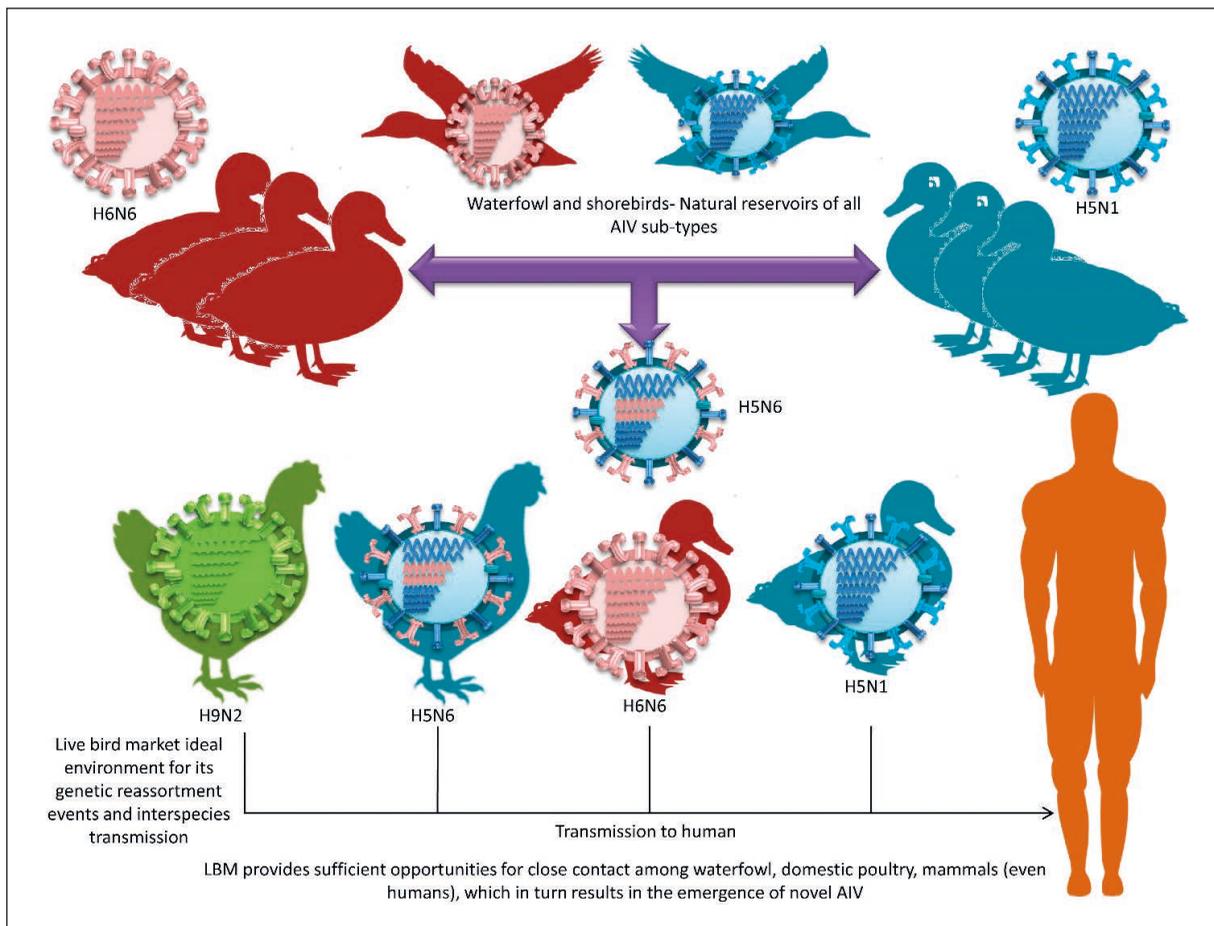


Figure 1 - Origin of H5N6 and other Avian Influenza viruses.

contact among birds and humans, and low or non-existent biosecurity measures [6, 14, 15]. By December 2018, the World Health Organization (WHO) reported a total of 23 human infections due to the H5N6 influenza virus, with 15 deaths [16]. This year, outbreaks have been recorded in the Philippines, Nigeria, Vietnam, and China, affecting only animals. Nevertheless, the risk for zoonotic spread and spillover has been well documented, with continuous evolution causing significant concern within the medical community due to the latent risk for a pandemic [17].

Under the current circumstances of public health emergency due to the COVID-19 pandemic, we must remain alert of the persistent threats that emerging infectious diseases signify to us daily. This article aims to perform a narrative review of the literature available about the H5N6 virus and raise awareness in the readership about the potential risk of H5N6 Influenza as the next probable public health emergency of international concern (PHEIC).

■ PREVIOUS SCENARIO

Currently, avian influenza outbreaks remain a global public health problem, with several subtypes of the H5Nx virus (H5N1, H5N2, H5N6, H5N8, among other) in circulation and continually threatening animal and even human health. Their zoonotic nature and the capacity for genomic interactions within this viral group gives rise to new variants whose pathogenicity in mammals and humans remains yet poorly understood [11]. Human-infection with the H5N1 subtype was first diagnosed in Hong Kong in 1997. Since then, it reemerged in 2003 and 2004, spreading from Asia to Europe and Africa, causing hundreds of human cases and deaths, as well as the slaughter of hundreds of millions of poultry who were implicated as host [18]. Now in 2020, a new outbreak of this virus has been reported in China, once again claiming thousands of animals' lives, for the time being without affecting humans.

Globally, since 2014 avian influenza has been identified in 77 countries, involving up to 13 different strains of AIV that have caused the death of millions of domestic and wild birds. During the first quarter of 2020, outbreaks of AIV H5N6 have been reported in countries such as the Philippines, Nigeria, Vietnam, and China, with available noti-

fied cases from the latter three to the World Organization for Animal Health (OIE) adding up to 8,781 affected birds, of which 8,331 died of illness [19]. An estimated 26,817 birds slaughtered to prevent the spread of the virus [2].

AIV H5N6 subtype represents a persistent threat due to its continuous evolution, evidenced in repetitive outbreaks in live birds' markets; spillover to wild bird populations that guarantees its spread during bird migration; and the latent risk of triggering an event of pandemic proportions in humans. An AIV H5N6 outbreaks would be of high impact. The handling and direct contact with infected birds, as in previous outbreaks, would BE the primary risk factor [6, 11, 17, 20]. Although it is impossible to continually monitor and establish the location of all subtypes of this Influenza, making it difficult to assess its threat to humans, it is necessary to define those groups of local and regional genotypes that circulate in each host as well as to carry out a genetic surveillance approach to better understand their transmissibility, pathogenicity and possible spill-overs between species [11]. That will make it possible to integrate a risk-based strategy that considers migratory patterns, the complex and dynamic nature of animals, and to understand the underlying socio-economic factors influencing human behavior [13].

■ THE VIRUS

Influenza viruses, as enveloped RNA viruses with a segmented genome, display great antigenic diversity. Influenza A and B viruses have two major antigenic surface glycoproteins embedded into the membrane, which are the HA (a surface glycoprotein that binds to sialic acid residues in the respiratory epithelium, necessary for the onset of infection) and the NA that induce antibody responses in humans. In contrast to subtypes B and C, type A virus can exhibit more significant variability in those glycoproteins allowing a full differential virulence amongst each other, thus affecting mainly different population groups. For example, subtype C has been reported to commonly cause respiratory illness in children, with subtype B being the one with a lesser propensity for antigenic changes [21]. Antigenic shift is the main feature of these viruses than can lead to reassortment among viruses co-infecting the same

cell based on the nature of its segmented genome, which may explain the pandemics of 1957, 1968, and 2009 [22]. Nevertheless, the segmented genome alone is not responsible for the antigenic shift, as the genome of Influenza B is also segmented, an antigenic shift occurs due to reassortment of genes between avian and human or other animal viruses like swine. The pandemics of 1957, 1968, and 2009 were caused by reassortment of genes leading to antigenic shift.

Avian influenza virus (AIV) is a single-stranded segmented negative-sense RNA virus, member of the Orthomyxoviridae family, Influenza virus A/Alpha-influenza-virus genus [4, 23]. Its external glycoproteins HA and NA define the subtypes: 18 HA (H1-H18), and 11 NA (N1-N11) are known [4]. The HA gene of AIV has changed, giving rise to reassorted subtypes, clades, and genes [23, 24]. According to different pathotypes, AIV leads to different infection outcomes and organic commitment, which allows to divide them into NPAIV, LPAIV, and HPAIV. In birds, LPAIVs can cause mild respiratory or gastrointestinal infection, and HPAIVs can induce multi-organ infection along with high morbidity and mortality [6]. Although the H5 HA genes of these viruses are closely related, the subtypes of their NA genes vary widely, making them the "H5Nx" viruses [11, 13, 25]. Since their emergence in China in 2010, subtypes H5N1, H5N2, H5N6, and H5N8 that are HPAIV have threatened global bird species as well as humans [23, 25-27]. They are classified into the clade 2.3.4.4, and phylogenetic analysis of available sequences suggests that they first appeared in Western Russia. However, the long-distance migratory pattern of wild birds later spread them into Western Europe and Asia [16, 17, 28].

The clade 2.3.4.4 is prevalent in Asia and South-East Asia, with the most pervasive NA subtypes being H5N6 AIV, the most harmful and the most widespread of all. This clade initially found in China has since spread to Laos and Vietnam in 2014-2015, as evidenced in recent outbreaks among poultry [6,26,29]. The H5N6 virus has a glutamine residue at position 226, and a glycine residue at position 228, suggestive of preferential binding to sialic acid receptors joined to sugar chains through an α -2,3 linkage; plus, an additional single amino acid deletion (133 sites of HA1) relative to the other clade 2.3.4.4 HA genes (ex, MDk/Korea/W452/14). The deletion at this po-

sition alters the 3D structure of the receptor-binding unit, causing an alteration of the HA receptor-binding specificity resulting in an increased affinity for the α -2,6 linkage [29]. The H5N6 possess multiple basic amino acids at the HA cleavage site resulting highly pathogenic in chickens and with distinct evolutionary characteristics like its presumed capacity to bind to the α 2, 6-linked receptor; and mutations in the HA protein such as the P128, A138, and A160 and an 11-aa deletion in the NA stalk, which may enhance their adaptation and potential infection in mammals, giving this virus a considerable zoonotic potential [6, 16, 17, 20, 29]. Also, the HPAIV harboring the H5 HA genes of clade 2.3.4.4 appeared to circulate more efficiently in migratory birds than the HPAIVs belonging to the other H5 genetic clades; which during 2016-2017 winter season H5N6 caused outbreaks in wild birds and poultry across Europe and East Asian countries, including China, Japan, South Korea, and Taiwan [17, 27, 30, 31]. There is a capability of AIVs heading to terrible population losses if a different AIV appear with the capacity of persistent transmission from human to human [1].

■ ASSOCIATED VIRUSES

HPAIV is a continual threat to animal and human health, causing infections and deaths not only in countless birds but also in humans. H5 subtypes like H5N1, which is derived from the H6N1 (an NPAIV), infected 18 people in 1997 in Hong Kong, six of whom died from the infection. Furthermore, since 2003, H5N1 has caused outbreaks both in birds and humans in more than 60 countries. Recently, subtypes H5N1, H5N2, H5N5, H5N6, and H5N8 have also been linked to poultry outbreaks in China, as well as in South Korea (subtype H5N8), Japan (subtype H5N8), Laos (subtype H5N8), Vietnam (subtypes H5N1 and H5N6), and USA (subtype H5N8). In all, adequate control of the epizootic was achieved by culling over millions of birds [6, 13].

H5N6 joined H5N1 as the only subtypes among the clade 2.3.4.4 viruses known to cause human infections [13]. It was first detected in Changsha, China; on February of 2014; and since then has continued to reassort with other AIV subtypes, deriving in 34 genotypes and four main genotypes: A (internal genes of H5N1-origin), B (PB2

of H6-origin, and others of H5N1-origin), C (internal genes of H9-origin), and D (PB2 of H6-origin and PB1 of H3-origin, and others of H5N1); being the latter the currently dominant gene type [4, 16, 26, 28, 32-34]. As of December 2018, the World Health Organization (WHO) has reported 23 human infections with the H5N6 virus, which resulted in 15 deaths [16]. Previous studies have shown that H5N6 viruses isolated from the patients had a very close genetic relationship (HA gene ~95% nucleotide sequence identity) with H5N6 viruses isolated from the live poultry market, suggesting an equivalent zoonotic infection risk [13, 20]. Hence, the H5N6 virus became a severe threat to the poultry industry as to human health [16].

Nevertheless, AIV H5N6 has not yet exhibited transmissibility from person to person due to a lack of mutations Q226L and G228S in the HA protein, which is known to increase affinity for the glycan-receptors in the lower respiratory tracts of humans [4]. Notwithstanding, the H5N6 virus was later detected in 2017-2018 in wild birds across several European countries, including the Netherlands, where there were three commercial poultry farms and two affected hobby holdings. This virus was a novel reassortant of the H5N8 clade 2.3.4.4 group B viruses, which were first detected at the Russia-Mongolia border in May 2016. This virus was also distinct from the zoonotic Asian H5N6 strains that caused infections in humans, and there are currently no reported human cases associated with this novel H5N6 reassortant [35]. One main concern for public health agencies, when confronted with a new AIV subtype, is to define the risk of a pandemic arrival. H5N6 and other zoonotic influenza viruses should be kept in mind in the immediate future [1].

■ OUTBREAK OF AVIAN INFLUENZA - 2003

During 2003, avian Influenza played a threatening role for public health again. At the end of the year, several countries notified the simultaneous epidemics of HPAIV H5N1 with devastating results for animal health. In that year, 204,682 cases of AIV were reported to OIE throughout this year from countries such as Germany, Belgium, Korea, Hong Kong, Holland, and Vietnam, which led to the death of approximately 18,648 birds and the slaughter of over 30 million animals for the control of the disease [2]. Unfortunately, throughout

this period, human health was affected simultaneously by an HPAIV H7N7 outbreak that caused 89 human cases in the Netherlands, mostly mild symptomatic and with one reported death, a veterinarian with confirmed close contact to infected birds, highlighting the zoonotic potential of this virus [36].

■ THE DISEASE

HPAIV produces one of the most devastating infectious diseases in the world. Its infections usually cause a highly contagious systemic disease with significant morbidity and mortality amongst susceptible populations. The disease presentation encompasses a broad spectrum of clinical signs and symptoms, including diarrhea, sneezing, unexplained emaciation, discharge from the mouth, nose, ears or vent; extensive swelling, abnormal feathers, behavioral abnormalities, locomotion abnormalities, and mass mortality (unexpected mortality considering the natural history of the species) [37].

Waterfowl and shorebirds are considered to be the natural reservoirs for all AIV sub-types, which most of them are NPAIV or LPAIV; however, AIV underwent a further combination of genetic drifts and shifts that resulted in the production of HPAIV as the H5 clade 2.3.4.4 [37]. An example of this is the enzootic H5N1 that reemerged in 2003 and remained to date as a cause of outbreaks in poultry throughout Africa, Europe, and the Middle East, after being disseminated along flyways by migratory birds [13, 28]. Since 2010 AIV with different NA subtypes also have been detected, including H5N2, H5N3, H5N6, and H5N8 [13]. Wild birds' migration acts out as a risk factor for the first introduction, the genetic information obtained out of virus outbreaks in Korea, Europe, Canada, and the USA, suggested the involvement of them in the spread of H5 clade 2.3.4.4 over long distances. However, they may not be the hosts to support secondary spread along with poultry [13]. The worldwide co-circulation of LPAIV and HPAIV, comprised a fixed "gene pool," before 2010 with no evidence of reassortment of the H5N1 viruses with NA subtypes other than N1. However, since 2010 influenza A viruses with different NA subtypes have been detected in wild birds and poultry in China, including H5N2, H5N3, H5N6, and H5N8 [13]. Although not yet reaching pandemic

proportions, these recurring AIV outbreaks pose a persistent threat throughout the world [11].

Live birds' markets pose a real threat to public health since AIV is more frequently detected in them than in farms, making an ideal environment for genetic reassortment events and interspecies transmission [38]. As an example, Vietnam has an approximately 308 million population of poultry; almost 80% of them are raised under conditions without biosecurity applications [15]. Case-control studies have established risk factors for an outbreak, such as "in-house" poultry slaughtering, having a greater variety of species (*i.e.*, selling ducks alongside other species), visitors, and open water sources [15, 39]. That means that genetically diverse populations of the virus are distributed in discrete parts of the poultry trade network, so even intermediaries and more than one retailer can play an essential role as risk factors in the spread and modification of AIV genes [15]. Live birds' markets provide sufficient opportunities for close contact among waterfowl, domestic poultry, mammals (even humans), which in turn results in the emergence of novel AIV [6, 14].

H5N6 ended up as an HPAIV after being around in live birds' markets [40]. This subtype was an LPAIV noted in the past in Wisconsin, USA (1975), Germany (1984), and California, USA (2013), but had little impact until now [11]. Since 2014 it joined as the latest addition to emerging zoonotic avian influenza threats in East and Southeast Asia [40]. As another enzootic HPAIV has noticeable clinical signs during its outbreaks, as seen in several swans in the Fleet Lagoon in December 2017 in Japan with compulsively spinning on the water, lethargic or very poor coordinated, and in the following months some of them even presented abnormally twisted necks. During this epidemic, 61 swans died [41]. Also, dead-bird surveillance has enabled us to learn more about pathologic manifestations of wild birds affected by HPAIV H5N6, which showed hepatic and pancreatic necrosis, intestinal hemorrhage, and although not apparent, gross lesions in the brain, kidney, and spleen in some subjects [26].

High levels of AIV endemicity exposes the naïve immunological system of humans to its antigens, turning out to sporadic cases reported each year and sustaining the ongoing threat of pandemic emergence [15]. Despite the absence of data of when and how the AIV leaped to humans, the

whole gene sequences of cases show the consistency with the evolution of H5N6 isolated from live birds' markets, suggesting the infection might originate in this environment [6, 42]. Likewise, it is known that the direct contact with feces of the sick animal or its corpses, favors the spread of the human-infecting virus [29]. Like live birds' markets act as deemed potential hotbeds for infection, the first human infection with AIV H5N6 happened in Changsha, China, in February 2014. A girl aged five and a half years was exposed to the market trading site for about 5 minutes a day for four days and after an incubation period of 2-5 days sought medical attention due to influenza-like illness including fever (38.9°C) and sore throat, her blood showed $23.12 \times 10^9/L$ white blood cells, twice the upper bound of the normal range; neutrophils higher than the normal range, and lymphocytes lower than the normal range, after a diagnosis of suppurative tonsillitis she was sent home with a combination of amoxicillin and clavulanate potassium, ibuprofen suspension and herb medicine. After three days, her symptoms did not improve. Chills appeared, so throat swab samples were collected on day seven and day 11 respectively which tested positive for H5 but negative for N1 using real-time PCR, the phylogenetic analyses revealed that the HA gene of A/Changsha/1/2014(H5N6) was closely related to A/duck/Sichuan/NCX-N11/2014(H5N1) virus (98% identity). The NA gene was closely associated with A/chicken/Jiangxi/12782/2014(H10N6) virus (99% identity), demonstrating the infection with the triple reassortant subtype H5N6; then, she recovered quickly after Tamiflu therapy [4, 13].

After the first reported human case in 2014, another three cases of AIV H5N6 presented in adults during that same year, one of them died; in all of them, previous exposure to poultry was confirmed or likely [4, 13]. A later series of ten cases emerged after December 2015, a sharp increase in frequency [42]. Throughout the next three years, by December 2018, there was an overtime total of 23 human infections reported by the WHO, with 15 of them resulting in death, with no evidence of human-to-human transmission of AIV H5N6 among the close contacts [13, 16]. Until March 16, 2020, the WHO had not received reports of new cases of human infection with H5N6 to date [43]. Even though from the H5 clade 2.3.4.4, H5N6 and

H5N1 are the only ones with human infections reported nowadays, there is evidence from sera of people exposed to infected or dead birds during outbreaks in Russia that tested positive for antibodies to AIV H5N8 [44].

This disease has gone from being an exclusive avian disease to one that affects other mammals with potential zoonotic spread, and sadly it remains outside of epidemiological statistics worldwide [45]. There is a need to base a One Health approach that works effectively so the human health sector, with qualified program staff; will control and prevent this zoonosis through programs that include active and passive surveillance, early reporting and response, the slaughter of poultry in infected farms and use of mass or targeted vaccination [13, 46].

■ CURRENT SCENARIO - OCCURRENCE OF H5N6 AVIAN INFLUENZA DURING THE COVID-19 PANDEMIC TIME

It is essential to mention that to date, multiple outbreaks of H5N6 avian Influenza have been reported so far in 2020. The care protocols for these outbreaks have caused poultry to be sacrificed to contain the disease, which so far has resulted in more than 15,000 poultry being culled or dying from the epidemic in countries such as the Philippines [47].

The epidemiology of AI is complex. AI viruses constantly evolve by mutation and reassortment with the emergence of new subtypes, causing a significant impact on animal health and production. Some AI subtypes can be zoonotic, as occurs with H5N6, and therefore pose a considerable threat to human health [47].

If we look into the last four reports of the World Organization for Animal Health (OIE), from January 1 to March 26, 2020, the situation would be considering worrying [47]. Over this period, when the pandemic of COVID-19 originated and increased, in three continents, Asia, Europe, Africa, there have been 18 countries affected with 153 outbreaks of H5N6, H5N1, H5N2, H5N8, in poultry and non-poultry, 98 (64%) in Asia, leading to the losses of 1,589,203 animals, notified in the ongoing and new outbreaks (Table 1). According to the OIE, the impact of the disease is measured in terms of losses, which are calculated by the sum of dead and culled animals from the infected farm

or backyard premises of the reported outbreak. In the case of non-poultry, the losses correspond to the dead animals reported [48, 49].

Since 2014 (as of February 15, 2020), there were 24 human cases of avian influenza A (H5N6) reported globally, and all occurred in mainland China. The latest case was reported [on August 19, 2019] [50,51]. Recently, in three countries of Asia, and one of Africa, five human cases of avian Influenza AH9N2 infections reported in the past six months, in the provinces of Fujian and Anhui, China; at the Melghat District of Maharashtra State, India; Hong Kong; and Senegal, all occurring in people <18 years old [52].

Slaughtering must be done according to strict protocols provided by entities such as the OIE in their efforts to contain the virus locally and that there is no spread to other contiguous farms and, subsequently, globally, with all the implications in terms of economy and public health that this entails [47].

In recent decades, multiple subtypes (*i.e.*, H9N2, H5N1, and H7N9) of AIV have become widespread in China, causing enormous economic losses and posing considerable threats to the public health. This represents a challenge and the need for global insights into control strategies and guidelines for the prevention of AIV spread in China and globally in the immediate future [53]. Multiple reasons may explain why AIV has persisted in China, such as the socio-economic features, including poultry biosecurity, live bird markets, live bird transportation, wild birds, poultry waterfowl, poultry density, poultry population, and infected birds. Given this complex scenario, more research, enhanced surveillance, including genomic epidemiology, is necessary on AIV subtypes H9, H5 and H7 in China and neighbor countries, to elucidate the effectiveness of the strategies currently employed in China (*i.e.*, culling, mass vaccination and biosecurity improvement) to control the disease, as well to provide a close vision on the future epidemiological scenario. Recently, an outbreak report of H7N3 on April 2020 in the United States was informed by the OIE, at a commercial meat-type turkey flock in a farm in Chesterfield County, South Carolina, including 34,160 susceptible animals, with 1,583 deaths (4.63%), with 32,577 killed and disposal. Clinical signs included respiratory signs, snicking, and increased mortality

ty. State officials have quarantined the affected premises, and depopulation of birds has been completed. Movement controls have also been

implemented. This was the first confirmed case of HPAI in commercial poultry in the United States in 2020 [54].

Table 1 - HPAI outbreaks reported through early warning system, from January 1 to March 26, 2020, summarized from the OIE reports [47].

Period	Region	Country	#	Administrative divisions affected	Subtype(s)		Number of Outbreaks		Global animal losses
					Poultry	Non-poultry	Poultry	Non-poultry	
March 6 to 26, 2020	Asia	Chinese Taipei, Philippines	2	3	H5, H5N2, H5N5, H5N6	NA	6	NA	143,125
	Africa	South Africa	1	1	H5N8	NA	1	NA	
	Europe	Germany, Hungary, Poland	3	7	H5N8	H5N8	8	2	
February 14 to March 5, 2020	Asia	Chinese Taipei, India, Vietnam	3	16	H5N1, H5N2, H5N5, H5N6	NA	37	NA	440,095
	Europe	Bulgaria, Czech Republic, Poland	3	7	H5N8	NA	13	NA	
January 25 to February 14, 2020	Asia	China (People's Rep. of), Chinese Taipei, Israel, Saudi Arabia, Vietnam	5	13	H5N1, H5N6, H5N2, H5N5, H5N8	H5N8	30	1	587,029
	Europe	Germany, Poland, Slovakia	3	4	H5N8	NA	7	NA	
January 1 to 24, 2020	Africa	South Africa	1	1	H5N8	NA	2	0	418,954
	Asia	China (People's Rep. of), Chinese Taipei, India	3	10	H5N1, H5N2, H5N5	H5N6	25	3	
	Europe	Czech Republic, Germany, Hungary, Poland, Romania, Slovakia, Ukraine	7	12	H5N8	H5N8	24	2	
January 1 to March 26, 2020									
Subtotals	Africa	South Africa	1				3	0	
	Asia	Chinese Taipei, Philippines, India, Vietnam, China (People's Rep. of), Israel, Saudi Arabia, Vietnam, India	9				98	4	
	Europe	Germany, Hungary, Poland, Bulgaria, Czech Republic, Slovakia, Romania, Ukraine	8				52	4	
Total			18				153	8	1,589,203

■ CONCLUSIONS

The panzootic caused by A/goose/Guangdong/1/96-lineage highly pathogenic avian influenza (HPAI) A(H5) viruses have occurred in multiple waves since 1996. From 2013 onwards, clade 2.3.4.4 viruses of subtypes A(H5N2), A(H5N6), and A(H5N8) emerged to cause panzootic waves of unprecedented magnitude among avian species accompanied by severe losses to the poultry industry around the world. Clade 2.3.4.4 A(H5) viruses have expanded in distinct geographical and evolutionary pathways likely via long-distance migratory bird dispersal onto several continents and by poultry trade among neighboring countries. Coupled with regional circulation, the viruses have evolved further by reassorting with local viruses. As of February 2019, there have been 23 cases of humans infected with clade 2.3.4.4 H5N6 viruses, 16 (70%) of whom had fatal outcomes. To date, no HPAI A(H5) virus has caused sustainable human-to-human transmission. However, due to the lack of population immunity in humans and the ongoing evolution of the virus, there is a continuing risk that clade 2.3.4.4 A(H5) viruses could cause an influenza pandemic if these viruses should transmit efficiently among humans. Therefore, multisectoral collaborations among the veterinary, environmental, and public health sectors are essential to conduct risk assessments and develop countermeasures to prevent disease and to control spread [55]. The increased human interaction, the lack of adequate controls, and the significant international travel rate may pose a risk with AIV H5N6 for the next global health emergency.

Conflict of interests

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Authors' contributions Credit

DKBA, Conceptualization; Data curation; Formal analysis; Methodology; Software; Writing - original draft; Writing - review & editing. AJRM, Data curation; Formal analysis; Methodology; Writing - review & editing. MAF, Writing - review & editing. RVP, Writing - review & editing. EGO, Writing - review & editing. JFHM, Writing - review & editing. ACV, Writing - review & editing. RS,

Writing - review & editing. AAR, Writing - review & editing. KD, Writing - review & editing. PS, Writing - review & editing. APM, Writing - review & editing.

■ REFERENCES

- [1] Philippon DAM, Wu P, Cowling BJ, and Lau EHY. Avian Influenza Human Infections at the Human-Animal Interface. *J Infect Dis*. 2020.
- [2] World Organization for Animal Health. "Avian Influenza Portal." OIE, <https://www.oie.int/en/animal-health-in-the-world/avian-influenza-portal/>.
- [3] Kapoor S, and Dhama K. *Insight into Influenza Viruses of Animals and Humans*: Springer, 2014.
- [4] Zhang R, Chen T, Ou X, et al. Clinical, epidemiological and virological characteristics of the first detected human case of avian influenza A(H5N6) Virus. *Infect Genet Evol*. 2016; 40, 236-42.
- [5] Dhama K, Chauhan R, Kataria J, et al Avian influenza: The current perspectives *J Immunol Immunopathol*. 2005; 7 (2), 1-33.
- [6] Yuan R, Wang Z, Kang Y, et al. Continuing Reassortant of H5n6 Subtype Highly Pathogenic Avian Influenza Virus in Guangdong. *Front Microbiol* 2016; 7, 520.
- [7] Thomas JK, and Noppenberger J. Avian Influenza: A Review. *Am J Health Syst Pharm*. 2007; 64 (2), 149-65.
- [8] World Health Organization. "Influenza". WHO, https://www.who.int/influenza/human_animal_interface/en/.
- [9] World Organization for Animal Health. "Zoonotic Diseases in Humans". OIE, https://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Zoonoses.
- [10] Dhama K. *Avian/Bird Flu Virus: Poultry Pathogen Having*, J. Med. Sci: researchgate.net, 2013.
- [11] Li M, Zhao N, Luo J, et al. Genetic Characterization of Continually Evolving Highly Pathogenic H5n6 Influenza Viruses in China, 2012-2016. *Front Microbiol*. 2017; 8, 260.
- [12] Pan M, Gao R, Lv Q, et al. Human infection with a novel, highly pathogenic Avian Influenza a (H5N6) Virus: virological and clinical findings. *J Infect*. 2016; 72 (1), 52-9.
- [13] Claes F, Morzaria SP, and Donis RO. Emergence and dissemination of Clade 2.3.4.4 H5nx Influenza Viruses-How is the Asian Hpai H5 lineage maintained. *Curr Opin Virol*. 2016; 16, 158-63.
- [14] Yu Z, Gao X, Wang T, et al. Fatal H5N6 Avian Influenza Virus Infection in a Domestic Cat and Wild Birds in China. *Sci Rep*. 2015; 5, 10704.
- [15] Sealy JE, Fournie G, Trang PH, et al. Poultry trading behaviours in Vietnamese live bird markets as risk factors for Avian Influenza infection in chickens. *Transbound Emerg Dis*. 2019; 66 (6), 2507-2516.

- [16] Sun J, Zhao L, Li X, et al. Novel H5N6 Avian Influenza Virus reassortants with European H5N8 isolated in migratory birds, China. *Transbound Emerg Dis.* 2020; 67 (2), 648-660.
- [17] He G, Zhou L, Zhu C, et al. Identification of two novel avian influenza A (H5N6) Viruses in wild birds, Shanghai, in 2016. *Vet Microbiol.* 2017; 208, 53-7.
- [18] World Health Organization. "Avian Influenza H5N1 Infection in Humans: Urgent Need to Eliminate the Animal Reservoir - Update 5." WHO, https://www.who.int/csr/don/2004_01_22/en/.
- [19] Reuters-Redacción. "Brote De Gripe Aviar H5N6 Se Detectó En Granja De Filipinas." <https://www.el-universo.com/noticias/2020/03/17/nota/7785837/brote-gripe-aviar-h5n6-se-detecto-granja-filipinas>.
- [20] Zhao T, Qian YH, Chen SH, et al. Novel H7N2 and H5N6 avian influenza A viruses in sentinel chickens: a sentinel chicken surveillance study. *Front Microbiol.* 2016; 7, 1766.
- [21] Nesmith N, Williams JV, Johnson M, Zhu Y, Griffin M, and Talbot HK. Sensitive diagnostics confirm that influenza C is an uncommon cause of medically attended respiratory illness in adults. *Clin Infect Dis.* 2017; 65 (6), 1037-9.
- [22] Webster RG, Wright SM, Castrucci MR, Bean WJ, Kawaoka Y. Influenza - a model of an emerging virus disease. *Intervirology.* 1993; 35 (1-4), 16-25.
- [23] Zhang T, Li R, Zhu G, Chang J, and Xu B. first detection of a novel reassortant avian influenza A(H5N6) Clade 2.3.2.1c Virus, isolated from a wild bird in China. *Microbiol Resour Announc.* 2019; 8 (36).
- [24] Mok CK, Da Guan W, Liu XQ, et al. Genetic characterization of highly pathogenic avian influenza A(H5N6) Virus, Guangdong, China. *Emerg Infect Dis.* 2015; 21 (12), 2268-71.
- [25] Okamatsu M, Ozawa M, Soda K, et al. Characterization of highly pathogenic avian influenza virus A(H5N6), Japan, November 2016. *Emerg Infect Dis.* 2017; 23 (4), 691-5.
- [26] Tsunekuni R, Yaguchi Y, Kashima Y, et al. Spatial transmission of H5N6 highly pathogenic avian influenza viruses among wild birds in Ibaraki prefecture, Japan, 2016-2017. *Arch Virol.* 2018; 163 (5), 1195-1207.
- [27] Ozawa M, Matsuu A, Khalil AM, et al. Phylogenetic variations of highly pathogenic H5N6 avian influenza viruses isolated from wild birds in the Izumi Plain, Japan, during the 2016-17 winter season. *Transbound Emerg Dis.* 2019; 66 (2), 797-806.
- [28] Kang Y, Liu L, Feng M, et al. Highly pathogenic H5n6 influenza A viruses recovered from wild birds in Guangdong, Southern China, 2014-2015. *Sci Rep.* 2017; 7, 44410.
- [29] Si YJ, Lee IW, Kim EH, et al. Genetic Characterisation of novel, highly pathogenic avian influenza (HPAI) H5N6 viruses isolated in birds, South Korea, November 2016. *Euro Surveill.* 2017; 22 (1).
- [30] Hiono T, Okamatsu M, Matsuno K, et al. Characterization of H5N6 highly pathogenic avian influenza viruses isolated from wild and captive birds in the winter season of 2016-2017 in Northern Japan. *Microbiol Immunol.* 2017; 61 (9), 387-97.
- [31] Mine J, Uchida Y, Nakayama M, et al. Genetics and pathogenicity of H5N6 Highly pathogenic avian influenza viruses isolated from wild birds and a chicken in Japan During Winter 2017-2018. *Virology.* 2019; 533, 1-11.
- [32] Lu S, Zhao Z, Zhang J, et al. Genetics, pathogenicity and transmissibility of novel reassortant H5N6 highly pathogenic avian influenza viruses first isolated from migratory birds in Western China. *Emerg Microbes Infect.* 2018; 7 (1), 6.
- [33] Chen LJ, Lin XD, Guo WP, et al. Diversity and evolution of avian influenza viruses in live poultry markets, free-range poultry and wild wetland birds in China. *J Gen Virol.* 2016; 97 (4), 844-54.
- [34] Chen LJ, Lin XD, Tian JH, et al. Diversity, evolution and population dynamics of avian influenza viruses circulating in the live poultry markets in China. *Virology.* 2017; 505, 33-41.
- [35] Beerens N, Heutink R, Pritz-Verschuren S, et al. Genetic relationship between poultry and wild bird viruses during the highly pathogenic avian influenza H5N6 epidemic in the Netherlands, 2017-2018. *Transbound Emerg Dis.* 2019; 66 (3), 1370-8.
- [36] World Organization for Animal Health. "Animal HealthData (Prior to 2005)". OIE, <https://www.oie.int/en/animal-health-in-the-world/the-world-animal-health-information-system/data-before-2005-handistatus/>.
- [37] Food and Agriculture Organization of the United Nations. "Wild Bird Highly Pathogenic Avian Influenza Surveillance." FAO, <http://www.fao.org/3/a0960e/a0960e00.htm>.
- [38] Vergne T, Meyer A, Long PT, et al. Optimising the detectability of H5N1 and H5N6 highly pathogenic avian influenza viruses in Vietnamese live-bird markets. *Sci Rep.* 2019; 9 (1), 1031.
- [39] Kim HK, Kim HJ, Noh JY, et al. Serological evidence of H5-Subtype influenza A virus infection in indigenous avian and mammalian species in Korea. *Arch Virol.* 2018; 163 (3), 649-57.
- [40] Chu DH, Okamatsu M, Matsuno K, et al. Genetic and antigenic characterization of H5, H6 and H9 avian influenza viruses circulating in live bird markets with intervention in the center part of Vietnam. *Vet Microbiol.* 2016; 192, 194-203.
- [41] Hill SC, Hansen R, Watson S, et al. Comparative micro-epidemiology of pathogenic avian influenza virus outbreaks in a wild bird population. *Philos Trans R Soc Lond B Biol Sci.* 2019; 374 (1775), 20180259.
- [42] Bi Y, Chen Q, Wang Q, et al. Genesis, evolution and prevalence of H5N6 Avian Influenza Viruses in China. *Cell Host Microbe.* 2016; 20 (6), 810-21.
- [43] World Health Organization. "Influenza Up-

date-363". WHO, https://www.who.int/influenza/surveillance_monitoring/updates/latest_update_GIP_surveillance/en/.

[44] Adlhoch C, Kuiken T, Monne I, et al. Avian influenza overview November 2018 - February 2019. *EFSA Journal*. 2019; 17 (3).

[45] Kim YI, Si YJ, Kwon HI, et al. Pathogenicity and genetic characterisation of a novel reassortant, highly pathogenic avian influenza (HPAI) H5N6 virus isolated in Korea, 2017. *Euro Surveill*. 2018; 23 (7).

[46] Nguyen DT, Jang Y, Nguyen TD, et al. Shifting clade distribution, reassortment, and emergence of new subtypes of highly pathogenic avian influenza A(H5) viruses collected from Vietnamese poultry from 2012 to 2015. *J Virol*. 2017; 91 (5).

[47] OIE. "Highly Pathogenic Avian Influenza(Hpai) Report N°6: March 6 to March 26, 2020 - World Animal Health Information and Analysis Department. https://www.oie.int/fileadmin/home/eng/Animal_Health_in_the_World/Docs/Pdf/Oie_Ai_Situation_Report/Hpai_Asof26032020_Final.Pdf.

[48] Hassan KE, Saad N, Abozeid HH, et al. Genotyping and reassortment analysis of highly pathogenic avian influenza viruses H5N8 and H5N2 from Egypt reveals successive annual replacement of genotypes. *Infect Genet Evol*. 2020, 104375.

[49] Bisset AT, and Hoyne GF. Evolution and Adapta-

tion of the Avian H7N9 Virus into the Human Host. *Microorganisms*. 2020; 8 (5).

[50] Centre for Health Protection of Hong Kong. "Avian Influenza Report. Volume 16, Number 7. https://www.chp.gov.hk/files/pdf/2020_Avian_Influenza_Report_Vol16_Wk07.Pdf.

[51] ProMED-mail. Avian Influenza (32): Philippines (Ne) Backyard Poultry, Hpai H5N6, Oie *ProMED-mail* 2020; 20200318.7103916 (<https://promedmail.org/promed-post/?id=7103916>).

[52] Centre for Health Protection of Hong Kong. "Avian Influenza Report. Volume 16, Number 13. https://www.chp.gov.hk/files/pdf/2020_Avian_Influenza_Report_Vol16_Wk13.Pdf.

[53] Liu S, Zhuang Q, Wang S, et al. Control of Avian Influenza in China: Strategies and Lessons. *Transbound Emerg Dis*. 2020; 67 (4), 1463-1471.

[54] OIE. Information Received on 09/04/2020 from Dr Mark Davidson, Associate Administrator, USDA-Aphis, United States Department of Agriculture, Washington, United States of America. https://www.oie.int/wahis_2/public/wahid.php/reviewreport/Review?Page_Refer=Mapfulleventreport&Reportid=33951&Newlang=En.

[55] Yamaji R, Saad MD, Davis CT, et al. Pandemic Potential of Highly Pathogenic Avian Influenza Clade 2.3.4.4 a(H5) Viruses. *Rev Med Virol*. 2020, e2099.