Risk assessment: human health implications of the clade 2.3.4.4b highly pathogenic avian influenza (HPAI) H5Nx epizootic, 2021-2022

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Key Messages

- Highly pathogenic avian influenza (HPAI) H5N1 is currently widespread in Europe and North America, with an ongoing epizootic in 2021-2022 that is affecting domestic and wild birds, with spillover into other animals. Unprecedented involvement of other wild birds, such as raptors, and mammals, such as foxes, has been reported on both continents. Human cases have been reported following close exposure to infected poultry, including one asymptomatic infection in the United Kingdom in January 2022 and a case with mild illness in the United States in April 2022.
- The HPAI designation reflects avian clinical or virological features but does NOT predict severity in people. Of the 885 human infections due to HPAI H5N1 reported globally since 1997, about half have died but infections in people can range from asymptomatic to mild or severe. Although a serious disease, this tally represents a very low number of human cases globally, recognizing uncertainty in the number of people potentially exposed.
- The majority of human infections have followed poultry exposure. Limited person-to-person spread following close, prolonged, unprotected contact has occurred but sustained human-to-human transmission of these avian influenza viruses has not been established. However, influenza viruses are highly changeable. Exposure to novel subtypes is concerning for the potential for human adaptation and associated pandemic risk. Such risk may be viewed as a "low probability, high impact" event.
- Prudence requires that exposures to potentially infected animals be minimized, that the risk of acquiring infection be mitigated, and that monitoring be undertaken to ensure timely identification and isolation of human cases and the collection of critical information to inform real-time risk assessment.
- Clinicians should be vigilant and have a low threshold for seasonal and avian influenza virus testing of individuals who report sick bird or other exposures of concern within the ten days prior to illness onset, even if their symptoms are not severe. See Executive Summary for clinical and exposure indications.
- During assessment, testing and care, clinicians should implement appropriate protective measures. For novel influenza viruses, these include airborne, droplet and contact precautions unless otherwise advised. If there are questions or concerns, consult the appropriate infection control, clinical or public health authority.
- For testing of any suspect avian influenza in humans, clinicians are asked to consult the BCCDC Public Health Laboratory (PHL) Medical Microbiologist in advance (604-661-7033; 24/7). Specimens should be collected as close as possible to illness onset. Inform the local public health authority of human cases under investigation (24/7 contact information for clinicians in Supplementary Material 4). Complete the Virology Requisition and clearly flag "Suspect Human AI" on the form. For other testing details refer to the BCCDC PHL test menu on eLab and search "avian influenza".
- The antiviral drugs oseltamivir or zanamivir can reduce the duration and severity of illness if they are given within 48 hours of illness onset, and preferably as soon as possible.
- Visit the Public Health Agency of Canada website for general information on avian influenza and for periodic human emerging respiratory pathogen updates. Stay alert for updated Canadian and BC guidelines for monitoring and management. The website of the US CDC is a useful resource. For a map of where infected birds in North America have been detected see the US National Wildlife Health Center and here for infected Canadian domestic and Canadian wild bird populations. The list of areas with affected premises in British Columbia is available at the website of the BC Ministry of Agriculture and Food.

Executive Summary

In the 25 years since its first emergence in 1996, the Goose/Guangdong (Gs/GD) ("Asian") lineage of highly pathogenic avian influenza (HPAI) H5N1 viruses has caused 885 human infections in 20 countries, among whom about half have died. Cases typically occurred following exposure to infected poultry. Limited person-to-person spread following close, prolonged, unprotected contact has been documented but sustained human-to-human transmission has not been established. Although a serious disease, this represents a very low number of human cases globally, recognizing uncertainty in the number of people potentially exposed.

In 2008, the Gs/GD lineage acquired the capacity to reassort its neuraminidase (N) gene, generating multiple H5Nx viruses called clade 2.3.4.4 that have spread inter-continentally since 2014 through the flyways of migratory birds. Once introduced, HPAI H5Nx viruses can reassort with local avian influenza viruses, causing regional epizootics. Since 2016, a sub-group of HPAI H5Nx called clade 2.3.4.4b has been most concerning because of its widespread distribution and diversification over a broad geographic range, causing epizootics in both Europe and North America in 2021-22. This includes infections among wild and domestic birds, as well as backyard flocks, with poultry outbreaks unexpectedly detected during the summer 2021 in Europe. Spillover infection of animals, including raptors, and mammals, such as foxes, has been reported on both continents. During poultry outbreaks clade 2.3.4.4b viruses can transmit from infected birds to humans with about one dozen human clade 2.3.4.4b H5N8 or H5N1 cases, all mild or asymptomatic, from Russia, Nigeria, the United Kingdom and United States since December 2020. Additionally, 37 human HPAI H5N6 cases (all but one in China) had onset in 2021 including at least 18 known to be clade 2.3.4.4b (others pending clade assignment). Unexpected for human and avian influenza viruses which have a strong winter predilection, human H5N6 cases showed sharp increase in summer 2021 with elevated activity continuing in 2022. Of 49 human H5N6 cases reported in 2021 and 2022 (to date), at least 12 (24%) have died. Note that HPAI designation reflects avian virological/clinical features, but does not predict severity in humans.

While risk to the general population remains very low overall, influenza viruses are highly changeable. The associated pandemic risk constitutes what may be viewed as a "low probability, high impact" event. Prudence requires exposures to potentially infected animals be minimized, the risk of acquiring infection be mitigated, and that monitoring be undertaken to ensure timely identification and isolation of human cases and the collection of critical information to inform real-time risk assessment.

Clinicians should have a low threshold for seasonal and avian influenza virus testing of individuals with clinically compatible symptoms[1] who report sick bird or other exposures of concern[2] within the ten days prior to onset. Appropriate protective measures should be implemented which for novel influenza viruses include airborne, droplet and contact precautions unless otherwise advised. If there are questions or concerns, consult the appropriate infection control, clinical or public health authority. For testing of any suspect avian influenza in humans the BCCDC Public Health Laboratory Medical Microbiologist should be consulted (604-661-7033; 24/7). Separate nasopharyngeal and oropharyngeal specimens may improve detection and should be collected as close as possible to the onset of illness. The local public health authority should be informed of human cases under investigation (24/7 contact information for health care professionals, see: Supplementary Material 4). The antiviral drugs oseltamivir or zanamivir can reduce the duration and severity of illness if given within 48 hours of illness onset, preferably as soon as possible. Visit the Public Health Agency of Canada website for general information on avian influenza and for periodic human emerging respiratory pathogen updates. Stay alert for updated Canadian guidelines for monitoring and management. The website of the US CDC is also a useful resource. For information on the geographic distribution of affected domestic and wild birds in North America, see the US National Wildlife Health Center and the following links for infected Canadian domestic and Canadian wild bird populations. Areas in British Columbia with affected premises are available at the website of the BC Ministry of Agriculture and Food.

[1] **Clinical signs/symptoms:** conjunctivitis (red eye, discharge from eye) or acute respiratory or influenza-like illness with one or more of cough, sore throat, fever or feverishness, rhinorrhea, fatigue, myalgia, arthralgia, headache. May include mild, moderate (e.g. shortness of breath, difficulty breathing, altered mental status, seizures) or severe manifestations (e.g. pneumonia, respiratory failure, acute respiratory distress syndrome, multi-organ failure, meningo-encephalitis). Gastro-intestinal symptoms may also be present.

[2] **Exposures of concern:** Close exposure (within 2 meters) to a bird, animal or other human with confirmed avian influenza A virus infection. Exposures can include, but are not limited to: being in the same close airspace, touching or handling; OR consuming under- or uncooked poultry or egg products; OR direct contact with contaminated surfaces; OR being exposed to manure or litter containing high concentration of virus or being in a contaminated air space or environment; OR visiting a live poultry market with confirmed bird infections or associated with a case of human infection. Where avian influenza test results are not available but there is a high index of suspicion and other exposure criteria are met, also consider testing. Unprotected laboratory exposure also qualifies as testing indication.

A. Essential influenza A virology concepts

Influenza A viruses are subtyped based on their surface hemagglutinin (H) and neuraminidase (N) proteins. Hemagglutinin facilitates cell entry while neuraminidase enables progeny viruses to exit and infect other cells. Both proteins are targets of the antibody response and evolve to escape immunity. Seventeen H and 9 N subtypes are known in nature [1]. The main natural reservoir of influenza A is ducks with multiple subtypes circulating in wild aquatic and shore birds [2-4]. Because influenza viruses are highly changeable, multiple subtypes in birds pose an ongoing pandemic threat. Sero-archeology shows prior H (H1, H2, H3) and N (N1, N2, N8) subtype combinations that have adapted for sustained transmission in humans, including contemporary H3N2 and H1N1 viruses [5].

In wild bird reservoirs, avian influenza (AI) is maintained in a low pathogenic (LPAI) form associated with asymptomatic infection or mild illness. Replication is intestinal with cloacal shedding for variable but potentially prolonged periods [2,3,6,7]. Transition from LPAI to highly pathogenic avian influenza (HPAI) typically occurs in domestic poultry with the majority of HPAI outbreaks involving chickens and turkeys [4]. In poultry, HPAI is associated with severe illness, including systemic, respiratory and/or neurological signs, with high flock mortality, often within a few days [6,8]. In wild birds, HPAI H5N1 is clinically more heterogeneous ranging asymptomatic to severe. The incubation period for HPAI is highly variable, ranging a few hours in individual birds to 2 weeks in the flock [6,8]. A 21-day period, taking into account transmission dynamics, is used for disease control [6]. Unlike LPAI, HPAI replication is typically (even predominantly) respiratory with shedding that can also be prolonged up to several weeks [2,3,6,7].

LPAI and HPAI designations are based on molecular characteristics of avian influenza virus and pathogenicity in birds but neither designation is predictive of severity in humans. Both have transmitted from poultry to humans, causing human infections ranging from asymptomatic or mild to severe. Examples of accumulating AI events in humans (infections, deaths) include: HPAI H5N1 (885, 462) (**Supplementary Material 1**); HPAI H5N6 (79, 32) (**Supplementary Material 2**); HPAI H5N8 (7, 0); LPAI/HPAI H7N9 (1568, 616); HPAI H7N7 (89, 1); LPAI H9N2 (99, 2) etc [9-11]. In British Columbia (BC), two locally-acquired H7N3 infections, one LPAI the other HPAI, were reported during a 2004 poultry epidemic [12-14] and two H7N9 infections were acquired among BC travelers to China in 2014 [10,15], all with mild illness. Another Canadian resident of Alberta acquired HPAI H5N1 during a brief trip to China in 2013, with fatal outcome [16]. Clinical features of infection in humans include conjunctival, respiratory, gastrointestinal and neurological manifestations. Limited data suggest the incubation period for H5N1 and H7N9 viruses in humans is typically 2-5 days, but up to 10 days, with RT-PCR detection extending up to 21 days [17-19]. However, the lack of information on viable virus and limited secondary transmission events make it difficult to interpret infectivity. Shedding at least as long as seasonal human influenza (1 day before up to 7 days after onset), but possibly longer given lack of immunity (as for seasonal influenza in children or the immunocompromised), may be assumed [20]. Limited spread following close, prolonged, unprotected contact has been reported but sustained human-to-human transmission has not been established [21]. The goal in preventing human infections is not only to avoid potentially severe disease in those exposed, but also to reduce the risk of novel influenza viruses becoming well-adapted to humans.

Immunological priming by the first influenza virus exposure of childhood is influential upon subsequent influenza risk and can modulate the morbidity and mortality associated with emerging or pandemic influenza viruses [5,22,23]. The H-based subtypes of influenza A are broadly sub-divided into two groups with the greatest immunological interactions being among subtypes within the same group. Group 1 subtypes include H1, H2, H5, H6, H9 etc while Group 2 includes H3, H7, H10 etc [24]. Group 1 vs. Group 2 priming is hypothesized to influence novel influenza virus risk [25]. For example, H1 or H2 priming in childhood (birth before the 1968 H3N2 pandemic) may lower the risk of other Group 1 viruses (e.g. H5) [25]. Differential Group-specific imprinting may also partially explain the younger age of H5N1 (26 years) vs. H7N9 (62 years) reported in humans [17]. While speculative, these birth cohort effects may be relevant to AI risk assessment. Prior neuraminidase and other internal viral protein exposures are also relevant agent-host interactions to consider.

B. Influenza A(H5Nx) context

The first HPAI outbreak involving domestic geese was reported in 1996 in Guangdong, China, due to an H5N1 subtype A/goose/Guangdong/1/1996 virus that was associated with unusually severe infection for aquatic poultry [2-4]. This was followed by 18 human cases and six deaths in Hong Kong in 1997 due to reassortment of the same Goose/Guangdong (Gs/GD) lineage with other local LPAI. Culling of the whole poultry population of Hong Kong essentially eradicated this clade 0 virus but domestic geese continued to be a reservoir for the Gs/GD lineage, becoming endemic in southern China by 1999 [3].

Early in 2003, two additional human cases of Gs/Gd lineage HPAI H5N1 were identified due to virus represented by A/Hong Kong/213/03 (clade 1) [3]. The patients, both of the same family, and another family member who died of pneumonia in Fujian province, may have been infected in Southern China where closely-related viruses were detected in poultry [3]. Thereafter, Gs/Gd descendant viruses expanded their geographic range to southeast Asia (clade 1, e.g. Vietnam/Thailand/Malaysia) and Indonesia (clade 2.1) with poultry HPAI H5N1 outbreaks occurring frequently during 2003-2005, including sporadic human infections [3].

Following large outbreaks affecting wild birds at Qinghai and Poyang Lakes in China in 2005, Gs/GD HPAI H5 lineage spread north and westward, intercontinentally beyond southeast Asia through the established flyways of migratory birds, diversifying through mutation, reassortment and/or natural selection [2-4]. Increasing types and numbers of avian host species (domestic and wild) and other animals were affected with spillover into mammals including humans [2-4]. Beyond birds, the Gs/GD lineage shows an exceptionally wide host range that is probably not yet fully known but with infection demonstrated in pigs, cats, dogs, donkeys, tigers, leopards, palm civets, foxes etc. [6]. In rodents, susceptibility may differ between species; experimentally infected rats sero-converted but did not demonstrate productive infection [6].

Prior to 2008, HPAI H5N1 virus reassortment involved genes encoding internal viral proteins but without switch in the surface neuraminidase. Beginning in 2008, the long stable Gs/GD H5N1 lineage began to reassort in other ways, leading to several new HPAI H5 non-N1 subtypes, called H5Nx [26]. H5Nx viruses have spread through the flyways of migratory birds, and, following reassortment with local LPAI, have triggered regional epizootics.

C. WHO classification of H5Nx diversified viruses

The unified classification for HPAI H5Nx viruses, each of which are predicated upon the same Gs/GD H5 backbone, is clade 2.3.4.4 [26,27]. Clade 2.3.4.4 viruses appear to have displaced many earlier clades [26]. The World Health Organization recognizes 8 further genetic subgroups of clade 2.3.4.4 labelled from a to h [28]. In general, but not exclusively, H5Nx viruses of clades 2.3.4.4a and d-h have been mainly confined to Asia, while clades 2.3.4.4b and c have spread globally through wild bird migrations during 2014-15 (2.3.4.4c) and from 2016 to the present (2.3.4.4b) [28].

D. H5N6 infections, including clade 2.3.4.4b, in China, 2014-2022

Among clade 2.3.4.4 H5Nx viruses, HPAI H5N6 comprises the majority of human infections to date (n=79) of which all but one (Laos, n=1 in 2021) have occurred within China (n=78, 2014-22) (**Supplementary Material 2**).

H5N6 has been mainly endemic among birds in China and southeast Asia since 2013 [29], diversifying through reassortment with local H9N2 and other avian influenza viruses [30]. As described in **Supplementary Material 3** and the accompanying phylogenetic analyses of available 2.3.4.4 viral sequences since 2014, an array of clades 2.3.4.4 a-h viruses contributed to human (**Supplementary Figure 1**) and non-human (**Supplementary Figure 2**) H5N6 infections between 2014 and 2020. Conversely, the majority of

H5N6 human cases in 2021 have been clade 2.3.4.4b. In our phylogenetic analysis, 8/10 human H5N6 sequences in GISAID/FluDB with collection dates in 2021 were clade 2.3.4.4b; two (collection dates January/February 2021) were clade 2.3.4.4h (more common then in birds). In separate publication by authors with access to additional H5N6 sequences from China [29], 18/20 H5N6 viruses in 2021 grouped with clade 2.3.4.4b. The WHO has made similar observations for 2021 [31].

From the epidemiological profile of H5N6 cases in **Supplementary Material 2**, noteworthy are: (a) the increase in cases in 2021; (b) unusual increase during the summer of 2021 whereas influenza viruses typically show a strong winter predilection (including human H5N1 cases historically) [32]; (c) ongoing elevated H5N6 cases in 2022; (d) relatively younger age (median 50 years) compared to H7N9 [17]; and (e) an overall high case fatality since 2014 (40%). Of the 79 human H5N6 cases reported in total since 2014, 49 (62%) accrued in 2021 (n=37) and 2022 (n=12) alone. All 49 human H5N6 cases with onset in 2021 and 2022 were hospitalized and at least 12 (24%) thus far have died. Given such severe profile, under-reporting of milder cases is likely a factor.

E. Inter-continental spread of clade 2.3.4.4 H5Nx viruses, 2014-2020

Clade 2.3.4.4 H5Nx viruses were first reported in migratory birds in eastern China in 2013 [28]. In late 2014, wild waterfowl spread 2.3.4.4 viruses from their breeding areas in Siberia to Europe and North America along migratory flyways [33]. In Europe and North America these initial HPAI H5Nx incursions through migratory birds in 2014 were foremost due to H5N8 subtype viruses [34,35]. Geographic spread of clade 2.3.4.4 viruses was accompanied by reassortment with local LPAI, resulting in multiple novel H5Nx subtypes and regional epizootics in domestic poultry.

The first HPAI H5 outbreak in North America was in a turkey farm in BC in December 2014 due to an H5N2 (clade 2.3.4.4c) reassortment virus derived from Eurasian HPAI H5N8 and LPAI of North American wild bird lineage [35]. Further reports of domestic and wild or captive bird infections with Eurasian HPAI H5N8, H5N2 and H5N1 reassortment viruses (clade 2.3.4.4c) followed in the Pacific Northwest of the United States (US) in December 2014 and January 2015 with additional detection of the same HPAI H5N1 reassortment virus in poultry in BC in February 2015 [35].

In Europe, further H5Nx incursions in 2016 and 2020 resulted in large epizootics foremost of H5N8 but also H5N1, with poultry outbreaks as well as mass mortality events in wild birds, including endangered species [36,37]. During the 2020-21 epidemic wave, clade 2.3.4.4b viruses diversified and the global spread and reassortment of this particular clade is reportedly greater and faster than observed with any prior H5 (or H7 or H9) clades [37]. Persistence of poultry outbreaks through the summer months in Europe and the United Kingdom (UK) was also unexpected and unprecedented [37]. Spillover H5N8 infection of other mammals was reported in 2020-21 such as in seals and foxes in the United Kingdom (UK), and seals in Denmark and Germany in September 2021. Moreover, in December 2020**, human HPAI H5N8 infections in seven poultry workers in Russia** due to clade 2.3.4.4b were reported with mild/asymptomatic infections.

F. Links to current avian influenza activity, globally and North America

The geographic distribution of affected domestic and wild birds is available globally from the United Nations Food and Agriculture Organization and in North America from the US National Wildlife Health Center. The latter includes geographic mapping of affected premises and wild birds in North America. Updates are provided at the following hyperlinks among Canadian domestic and Canadian wild bird populations. The list of affected premises in BC is regularly updated at the website of the BC Ministry of Agriculture and Food. In the United States (US), updates are provided at the following links for infected US domestic and US wild bird populations.

G. Clade 2.3.4.4b H5N1 epizootic, Europe and North America, 2021-22

Whereas clade 2.3.4.4b H5N8 viruses predominated in Europe from 2014 to 2020 [34,36], clade 2.3.4.4b H5N1 has taken over during the 2021-22 epizootic affecting both Europe [28] and North America. On both continents, this likely represents incursions during the fall migration of wild birds, followed by local reassortment events. Of note, HPAI H5N1 has also been the predominant subtype identified in domestic and wild birds in Asia between December 2021 and March 2022.

In Europe, the large and ongoing 2021-22 epizootic has been associated not only with outbreaks in domestic and wild birds but also confirmed clade 2.3.4.4b H5N1 infection among foxes and other mammals (e.g. otter, polecat, ferret and lynx), with neurological signs being a prominent feature. Moreover, the first **human HPAI H5N1 infection in the UK** (clade 2.3.4.4b) was reported in January 2022**,** an elderly man (>75 years) with asymptomatic infection in mid-December 2021 associated with very close contact with a large number of A(H5N1)-infected, domestically-kept birds. However, earlier in 2021, the first known human infections due to clade 2.3.4.4b H5Nx viruses pre-dated this UK report, with **at least three poultry workers in Nigeria** identified with mild or asymptomatic infection in March 2021 (7 reported, 3 confirmed). Poultry outbreaks due to both H5N8 and H5N1 were reported in Nigeria, and the Nx of human cases there has not been fully resolved; however, at least three are known to have been due to HPAI H5 clade 2.3.4.4b viruses.

In North America, 2021-22 HPAI H5N1 outbreaks began on the Atlantic Coast of Canada in December 2021 [38], with ongoing detections in Canadian domestic and wild bird populations. The first clade 2.3.4.4b H5N1 detection in a wild bird along the Pacific Coast was reported in February 2022 from British Columbia – a bald eagle. Carnivorous birds of prey, including raptors, have previously been identified as very susceptible to H5N1, with high lethality and intense CNS involvement [2]. However, the toll on bald eagles during the current outbreak in North America is exceptional. As of May 19, 2022, HPAI A(H5N1) has been detected in all 10 Canadian provinces including confirmed infection on 85 domestic bird premises and affecting >235 wild birds and waterfowl. As of May 19, 2022, 8 BC premises are listed by the BC Ministry of Agriculture and Food as having HPAI H5N1 infection among birds confirmed by the Canadian Food Inspection Agency.

In the US, from January and as of May 19, 2022 >335 commercial or backyard flocks have been confirmed with HPAI infections in 35 states with >1100 wild bird infections identified in 36 states as of May 16. As such, the number of HPAI-infected wild birds in North America associated with the current epizootic is unprecedented. The first locally-acquired **human HPAI H5N1 case in the US** (clade pending) was reported in April, 2022, a man <40 years of age with the only symptom being fatigue following poultry exposure. Prior report of HPAI H5N1 in a Canadian resident of Alberta (a fatal case in a woman <40 years with onset of respiratory and neurological manifestations) following brief visit to Beijing, China in December 2013 [16], was due to a different, earlier clade 2.3.2.1c virus.

Infections have also been detected in other mammals in North America during the current 2021-22 epizootic, including red foxes in Ontario and Minnesota and striped skunks in Alberta with presumptive H5 identification and strange neurological behaviours.

H. Genomic mutations associated with mammalian adaptation

Several published [39] and on-line resources [40] have provided an inventory of mutations among avian influenza viruses meaningful to risk assessment for humans.

As shown in **Supplementary Table 3**, among the 8 available H5N1 sequences from infected birds in BC, the clade 2.3.4.4b H5 genes are all 154N, 156A, and 223R which are markers associated with increased ability to bind to human (Sia-α2,6Gal) over avian receptors (Sia-α2,3Gal) [39-41]. They are also all 133A shown to increase pseudovirus binding to human receptors and 155D which may play a role in evading host immune responses [39,42]. Both the 189N and 218Q mutations found in all BC avian H5 sequences are believed to

alter receptor specificity and decrease HA binding ability but these changes are considered marginally effective [43]. Mutations in the PB2 gene associated with increased mammalian adaptation were not identified in any of the BC sequences from infected birds. Most sequences from elsewhere in Canada are still pending public posting and therefore unavailable for this sort of analysis.

Among viruses collected from human and non-human mammalian H5Nx cases, mutations associated with adaptation have been found, including in 2.3.4.4b viruses. However, the extent to which these mutations developed prior versus during mammalian infection is unclear and regardless have not yet conferred sustained transmissibility.

In the PB1 gene, the majority of available sequences from mammalian H5Nx cases are 3V and/or 622G, which have both been shown to increase polymerase activity and viral replication in mammalian cell lines [39]. All available BC sequences from birds are also 3V and 622G. The majority, including all BC bird sequences, also have one or more of 37A, 383D, and 409S within their PA gene, which have all been shown to increase polymerase activity in mammalian cells [39]. While there are some mutations present in the NP gene of a few of the reported HPAI A(H5Nx) human and non-human mammalian cases, they are not present in the majority. Those present (E434K and N319K) have been shown to increase polymerase activity and replication in mammalian cells. The majority of NS genes are 42S and/or 92E, which have been shown to increase virulence in various mammalian models [39]. An 80-84 deletion in the NS gene is found in most of the H5Nx cases analyzed, which has been shown to increase virulence in mice and swine [39]. Most are also 106M and/or 138F in the NS gene, both of which have been shown to increase replication in mammalian cell lines. All of the BC sequences from infected birds are also 42S, 106M, and 138F in their NS gene. Within the M gene, all available sequences, including those from the 2022 BC avian cases, are 30D, 43M, and 215A, shown to increase virulence in mice [39]. None of the ~10 neuraminidase mutations believed to potentially confer reduced oseltamivir susceptibility (not displayed) were found in any of the 8 H5N1 viruses from infected birds in BC. Additionally, none were prevalent among available human or non-human mammalian Nx sequences with the exception of the recently-defined and naturally-occurring I117T substitution [44], found in most (including 2.3.4.4b) sequences from human infections and half of those among other mammals. The significance of this finding requires further evaluation.

I. Tally of human clade 2.3.4.4b H5Nx cases, 2020-22

Recognizing differences in internal gene segments, phylogenetic analysis of available 2.3.4.4 H5Nx viral sequences since 2014 shows H5N1 viruses from European and North American domestic and wild birds (including from BC) as well as human (**Supplementary Figure 1**) and non-human mammals (**Supplementary Figure 2**) in 2021-22 all cluster with the clade 2.3.4.4b H5 backbone.

Included within clade 2.3.4.4b are the human case from the UK (n=1) in December 2021 as well as the earlier H5Nx cases (n=3) from Nigeria in March 2021, and the H5N8 human cases (n=7) from Russia in December 2020. It is also highly likely the human H5N1 case in the US in April 2022, for which sequencing information is still pending (n=1), is clade 2.3.4.4b.

This means at least one dozen clade 2.3.4.4b H5Nx viruses have occurred in humans outside of China since December 2020 of which all to date have been reportedly mild. Also, per above, at least 18 of the H5N6 human infections in China for which sequencing was available in 2021 were clade 2.3.4.4b indicating at least 30 human clade 2.3.4.4b H5Nx infections including China since December 2020. Allowing 90% of the other 31 H5N6 cases in 2021-22 in China to most likely be clade 2.3.4.4b as per [29] would nearly double the tally of human cases due to clade 2.3.4.4b viruses (to 58) since December 2020. The accumulating infections due to clade 2.3.4.4b among other non-human mammals is further concerning.

J. Summary assessment and recommendations

In the 25 years since its first emergence in 1996, the Gs/GD ("Asian") lineage has become the ancestor of all HPAI H5 viruses circulating globally [3]. To date, the only continents spared its incursion are Australia, South America and Antarctica [33]. Since 1997, 885 human cases due to HPAI H5N1 have been reported globally of whom about half have died. Cases have been reported by 20 countries foremost in association with poultry outbreaks, including one from the UK in 2021 and one from the US in 2022. **Overall, this represents a very low number of human HPAI H5N1 cases worldwide, recognizing uncertainty in the number of people potentially exposed globally.**

However, the emergence of clade 2.3.4.4 H5Nx reassortment viruses since 2008, their inter-continental spread since 2014, notably including incursions of clade 2.3.4.4b viruses into Europe since 2016 and its unprecedented impact on birds and other animals, now widespread also in North America, signals a dynamic shift in the ecology of the Gs/GD lineage.

Notwithstanding a clinical spectrum that is mostly mild in the dozen or so humans infected outside of China since December 2020, there are reasons to be particularly concerned about clade 2.3.4.4b H5Nx viruses [37]. These include: widespread distribution with diversification and local reassortment over a broad geographic range, identification of mammalian adaptation mutations, transmission to humans, transmission to non-human mammals, detection in wild birds and backyard farms where there may be less awareness of the need or proper use of personal protective measures, and unusual detection in poultry elsewhere through the summer 2021 reinforcing the need for ongoing vigilance.

The risk to the general population from HPAI H5Nx viruses remains very low and ultimately, the risk that such viruses will trigger a pandemic constitutes what may be viewed as a "low probability, high impact" event. The US CDC has scored the potential pandemic threat associated with clade 2.3.4.4b H5N1 and H5N6 viruses as moderate. Prudence requires exposures to affected animals be minimized, the risk of acquiring infection be mitigated, and monitoring be undertaken to ensure timely identification and isolation of human cases and the gathering of critical information to inform real-time risk assessment.

Clinicians should have a low threshold for seasonal and avian influenza virus testing of individuals with clinically compatible symptoms[1] who report sick bird or other exposures of concern[2] within the ten days prior to onset. During patient assessment, testing and care, appropriate protective measures should be implemented which for novel influenza viruses include airborne, droplet and contact precautions unless otherwise advised. If there are questions or concerns, consult the appropriate infection control, clinical or public health authority. For testing of any suspect avian influenza in humans the BCCDC Public Health Laboratory (PHL) Medical Microbiologist should be consulted in advance (604-661-7033; 24/7). Separate nasopharyngeal and oropharyngeal specimens may improve detection and should be collected as close as possible to the onset of illness. Please complete the Virology Requisition and clearly flag "Suspect Human AI" on the form. For other testing details refer to the BCCDC PHL test menu on eLab and search "avian influenza". The local public health authority should be informed of human cases under investigation (24/7 contact information: Supplementary Material 4). Markers of virus susceptibility to the antiviral drugs oseltamivir or zanamivir are retained and these can reduce the duration and severity of illness if given within 48 hours of illness onset, preferably as soon as possible.

Visit the Public Health Agency of Canada website for general information on avian influenza and for periodic human emerging respiratory pathogen updates. Stay alert for updated Canadian guidelines for monitoring and management. The website of the US CDC is also a useful resource.

[1] **Clinical signs/symptoms:** conjunctivitis (red eye, discharge from eye) or acute respiratory or influenza-like illness with one or more of cough, sore throat, fever or feverishness, rhinorrhea, fatigue, myalgia, arthralgia, headache. May include mild, moderate (e.g. shortness of breath, difficulty breathing, altered mental status, seizures) or severe manifestations (e.g. pneumonia, respiratory failure, acute respiratory distress syndrome, multi-organ failure, meningo-encephalitis). Gastro-intestinal symptoms may also be present.

[2] **Exposures of concern:** Close exposure (within 2 meters) to a bird, animal or other human with confirmed avian influenza A virus infection. Exposures can include, but are not limited to: being in the same close airspace, touching or handling; OR consuming under- or uncooked poultry or egg products; OR direct contact with contaminated surfaces; OR being exposed to manure or litter containing high concentration of virus or being in a contaminated air space or environment; OR visiting a live poultry market with confirmed bird infections or associated with a case of human infection. Where avian influenza test results are not available but there is a high index of suspicion and other exposure criteria are met, also consider testing. Unprotected laboratory exposure also qualifies as testing indication.

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Supplementary Material (with hyperlinks)

Supplementary Material 1. Global tally of influenza A(H5N1) cases by country and year, 1997-2022
Source: https://www.cdc.gov/flu/avianflu/chart-epi-curve-ah5n1.html

H5N1	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Total	Available clade information (extracted from various sources)
Azerbaijan	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	2.2
Bangladesh	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	3	1	0	1	0	0	0	0	0	0	0	8	2.2.2.1
Cambodia	0	0	0	0	0	0	0	0	4	2	1	1	1	1	8	3	26	9	0	0	0	0	0	0	0	0	56	1, 1.1, 1.1.1, 1.1.2; 2.3.2.1c
Canada	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	2.3.2.1c
China	18	0	0	0	0	0	3	0	8	13	4	4	8	2	1	2	2	2	6	0	0	0	0	0	0	0	73	2.2; 2.3.2.1, 2.3.2.1a, 2.3.2.b, 2.3.2.1c; 2.3.4; 2.3.4.1, 2.3.4.2, 2.3.4.3; Hong Kong SAR: 0, 1, 2.3.2.1c
Djibouti	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.2.1
Egypt	0	0	0	0	0	0	0	0	0	18	25	8	39	29	39	11	4	37	136	10	3	0	0	0	0	0	359	2.2, 2.2.1, 2.2.1.1, 2.2.1.2
India	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1
Indonesia	0	0	0	0	0	0	0	0	20	56	40	25	18	9	12	12	3	2	2	0	1	0	0	0	0	0	200	2.1.3, 2.1.3.2, 2.1.3.2a, 2.1.3.2b, 2.1.3.3, 2.3.2.1c; 2.1.2
Iraq	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2.2, 2.2.1
Laos	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	2.3.4, 2.3.2.1c
Myanmar	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Nepal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	2.3.2.1a
Nigeria*	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.2; three confirmed clade 2.3.4.4b H5N? in March 2021 not captured here
Pakistan	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	
Thailand	0	0	0	0	0	0	0	17	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	1, 2.3.4
Turkey	0	0	0	0	0	0	0	0	2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	2.2
United Kingdom	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2.3.4.4b
United States	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	
Vietnam	0	0	0	0	0	0	3	28	61	0	9	6	5	7	0	4	2	2	0	0	0	0	0	0	0	0	127	1, 1.1.1, 1.1.2; 2.3.2.1a, 2.3.2.1c; 2.3.4, 2.3.4.1, 2.3.4.2, 2.3.4.3, 2.3.4.4
Total	**18**	**0**	**0**	**0**	**0**	**0**	**6**	**45**	**100**	**114**	**86**	**45**	**71**	**48**	**62**	**35**	**39**	**52**	**145**	**10**	**4**	**0**	**1**	**1**	**1**	**2**	**885**	

Supplementary Material 2. Epidemiological profile of HPAI H5N6 human cases, 2014 - April 28, 2022 (n=79)

Since 2014, 79 laboratory-confirmed cases of human infection with influenza A (H5N6) have been reported by various sources with epidemiological profile as summarized below.

Person

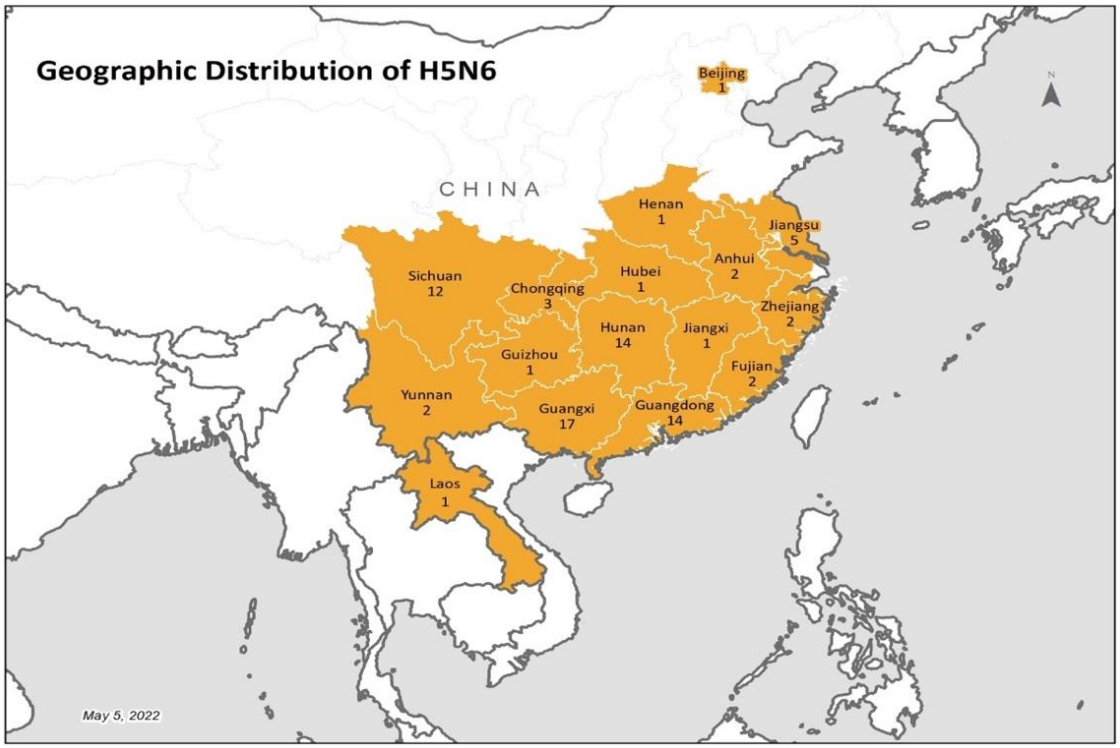
Of the 79 known human A (H5N6) infections, mean/median age was 44/50 years (range 1 – 81 years). Eleven were <20 years, 8 (73%) of whom were hospitalized. At least 32(40.5%) have died since 2014, involving all age groups, including four children. Exposure to avian species including poultry (live or meat), poultry market exposure, or exposure to dead wild birds was reported in 70 (88.6%) of cases, while the exposure of the remaining cases remained under investigation or was not yet clear at the time of reporting.

	Sex		**Total**
Variables	**Males (n=42)**	**Female (n=37)**	
Age (years)			
Median age (range)	50.5 (3 – 79)	47 (1 – 81)	50 (1-81)
0-4	1 (2.4)	4 (10.8)	5 (6.3)
5-9	1 (2.4)	2 (5.4)	3 (3.8)
10-19	0 (0)	3 (8.1)	3 (3.8)
20-49	16 (38.1)	12 (32.4)	28 (35.4)
50-64	16 (38.1)	13 (35.1.)	29 (36.7)
65+	8 (19.0)	3 (8.1)	11 (13.9)
% Case fatality	ne	ne	32* (40.5)

* 2 deaths reported by WHO have not been linked to a specific case

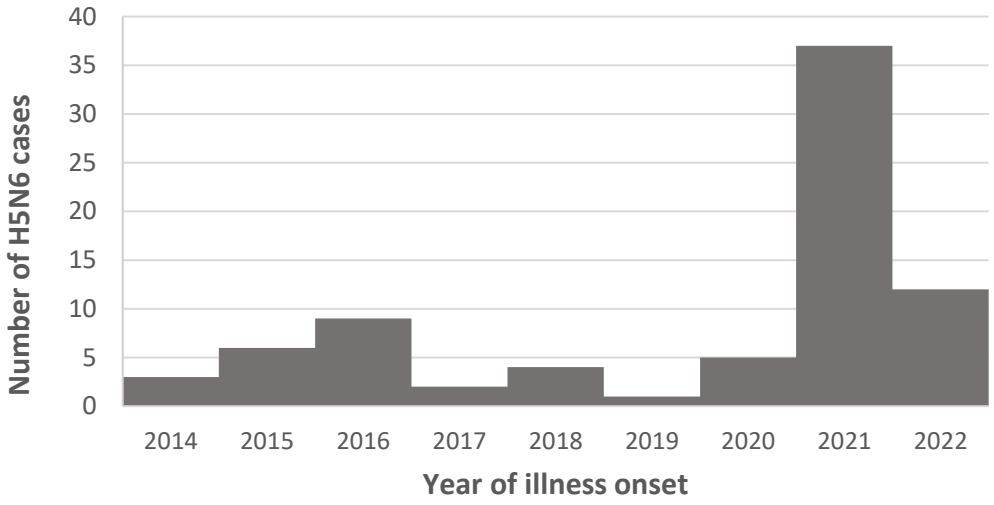
Place

Of 79 reported cases, one was identified in Laos in 2021 and the remaining in China. Cases reported from China (n= 78) mostly occurred in Southern China with one reported from Beijing in 2019, with geographic distribution as otherwise shown in the Figure below.

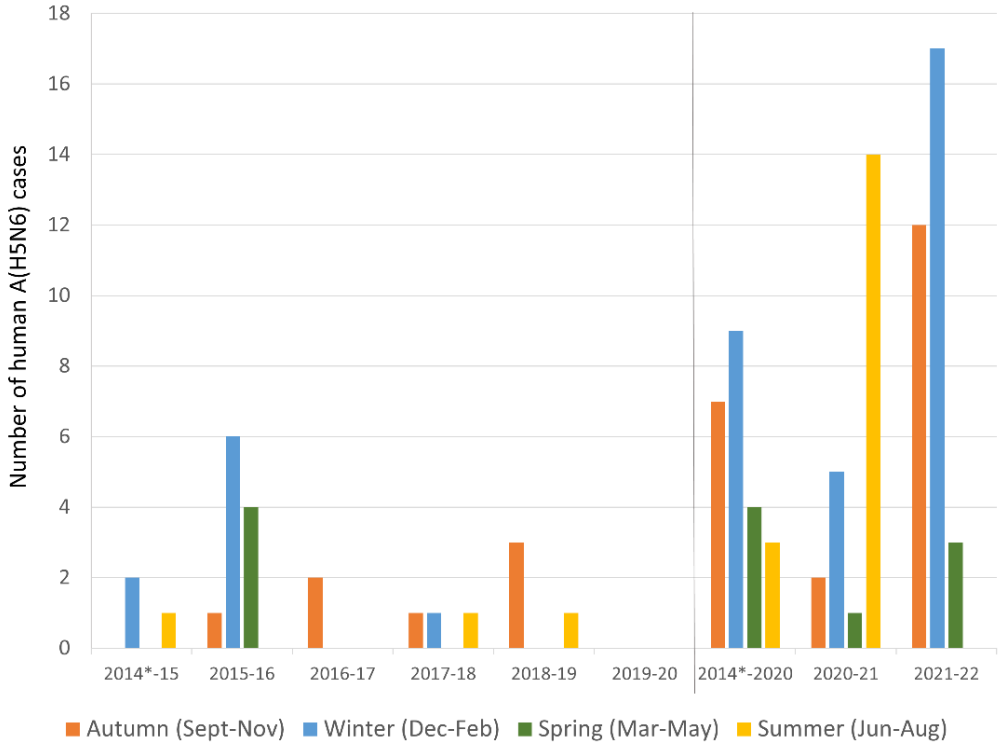


Time

While a majority of the 79 identified cases to date had onset in 2021 i.e., 37/79 (47%), there have already been 12 (15%) cases reported in the initial four months of 2022. The other 30 of 79 cases occurred as follows: 2014 (3 cases), 2015 (6 cases), 2016 (9 cases), 2017 (2 cases), 2018 (4 cases), 2019 (1 case), and 2020 (5 cases) and as displayed in the epidemic curve below.



Winter predominance is expected for human influenza infections and observed also in global analysis of H5N1 cases[1]. Although sample size is small for H5N6, similar seasonality is suggested for cases accruing between 2014 and 2020. However, an unexpected increase in cases occurred during the summer of 2021 with higher case counts continuing through autumn 2021 and winter and spring of 2022. Whether heightened detection continues in summer 2022 remains to be determined.



*Two cases in 2014 (February 2014 and April 2014), not included

[1] Mathur MB, Patel RB, Gould M, et al. Seasonal patterns in human A(H5N1) virus infection: analysis of global cases. PLOS ONE 2014;9:e106171. Available: https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0106171&type=printable

Supplementary Material 3. In-house BCCDC phylogenetic analysis of avian, human and non-human mammalian clade 2.3.4.4 A(H5Nx) viral sequences available in GISAID since 2014

All A(H5Nx) sequences and associated metadata from the Global Initiative on Sharing Avian Influenza Data (GISAID) and the Influenza Research Database (FluDB) submitted by April 28, 2022 were downloaded and duplicate strains were removed based on isolate names (n=17,800). Hemagglutinin phylogenetic clades were unassigned to more than half (n= 10,319) of the sequences, including all of the sequences from GISAID. These clades were therefore assigned based on an in-house curated blast library that includes 676 unique randomly selected sequences of at least 550 amino acids with known clades from FluDB meeting unique combinations of clade, HPAI/LPAI status, continent, avian or mammalian host, subtype and year to capture the diversity of all H5 clades worldwide.

All clade 2.3.4.4 viruses (n=7040; 40%) were further classified into sub-clades a to h using a secondary blast library of 27 reference sequences identified in published literature as belonging to 2.3.4.4 sub-clades a to h. All re-classifications were confirmed by phylogenetic analysis, and of these, only sequences from 2014 to *(28 April)* 2022 of avian or mammalian (human and non-human) origin were selected for further analysis (n=6531; 93%) (**Supplementary Table 1**).

Phylogenetic analyses of the hemagglutinin genes of all available clade 2.3.4.4 human (**Supplementary Figure 1**) and non-human mammalian (**Supplementary Figure 2**) sequences were conducted. This was done in the context of eight A(H5N1) British Columbia (BC) sequences from wild birds and poultry outbreaks in 2022 and a subset of global avian A(H5Nx) viruses. In order to create a common avian A(H5Nx) backbone for both phylogenies, 1% of all avian clade 2.3.4.4 viruses were randomly subsampled after removal of duplicate sequences. To emphasize the relatedness of BC avian A(H5N1), older and less geographically relevant subclades 2.3.4.4a, 2.3.4.4b prior to 2020 and 2.3.4.4 c to h were further randomly down-sampled and clade 2.3.4.4b viruses since 2020 were up-sampled while still retaining subtype and regional proportionality to the original data set (**Supplementary Table 2**).

Phylogenetic trees were created using the approximate likelihood method FastTree [1] based upon a generalized time-reversible (GTR) model and visualized in FigTree [2]. Sequences are labelled using a combination of GISAID/FluDB identification numbers and year of specimen collection. Unless sequence names are labelled as human or other mammalian, they are of avian origin; mammalian sequences include species and country. All BC sequences are from birds sampled in 2022 for which sequencing was performed by the BCCDC Public Health Laboratory on behalf of the Animal Health Centre and are distinctively labelled. Other sequences from Canadian clade 2.3.4.4 viruses were not available at the time of the current analysis.

[1] Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 2010; 5:e9490.

[2] Rambaut A. FigTree v1.4.0, a graphical viewer of phylogenetic trees. Edinburgh: University of Edinburgh; Available at: http://tree.bio.ed.ac.uk/software/figtree/

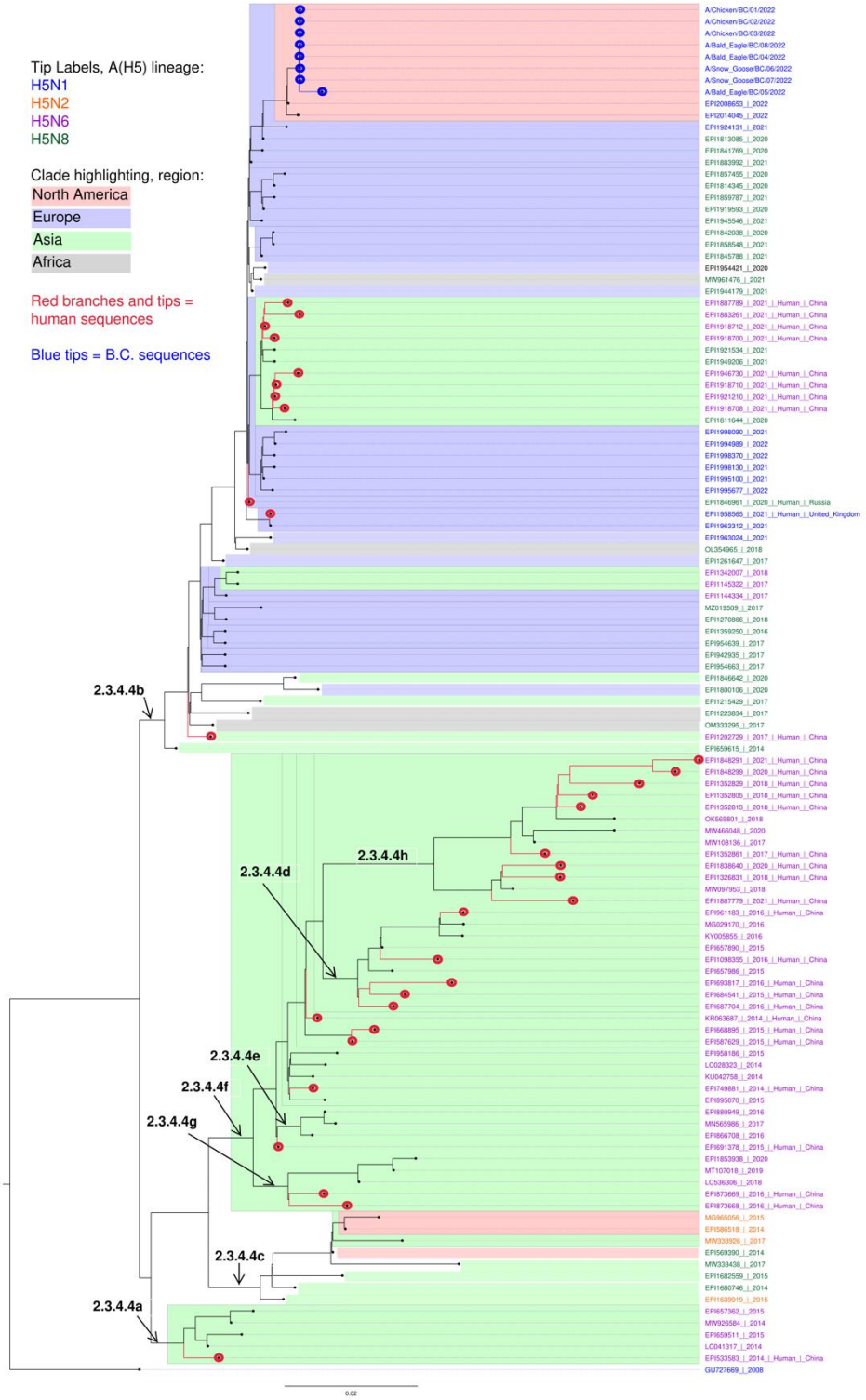
Supplementary Table 1. Subtype, species and global distribution of A(H5Nx) clade 2.3.4.4 sequences with collection dates from 2014 to 2022 and publicly available in GISAID and FluDB as of April 28, 2015*

Subtype	Asia	Europe	Africa	North America	Total
Avian	***3294***	***2267***	***494***	***410***	***6465***
H5N1	37	500	17	106	660
H5N2	235	13	4	282	534
H5N3	9	12	0	0	21
H5N4	0	5	0	0	5
H5N5	0	52	0	0	52
H5N6	1862	61	1	0	1924
H5N7	0	1	0	0	1
H5N8	1108	1622	461	22	3213
H5Nx	43	1	11	0	55
Human	***31***	***2***	***0***	***0***	***33***
H5N1	0	1	0	0	1
H5N6	31	0	0	0	31
H5N8	0	1[1]	0	0	1
Non-Human Mammalian	***21***	***12***	***0***	***0***	***33***
H5N1	3	4	0	0	7
H5N6	18	0	0	0	18
H5N8	0	8	0	0	8
Totals	**3785**	**2295**	**554**	**644**	**7278**

*Environmental samples excluded (n=379)

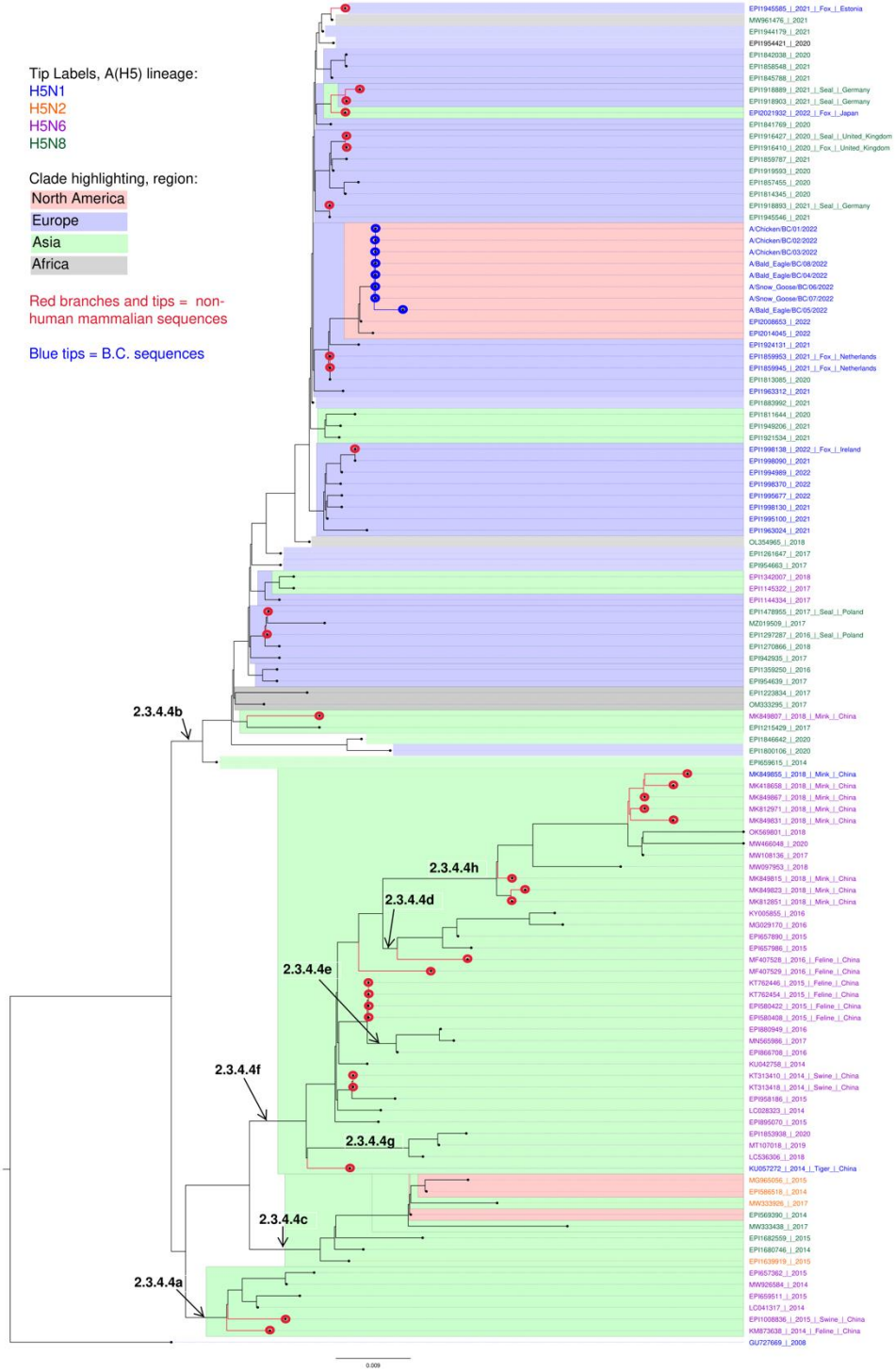
[1] Sequence is representative of seven human A(H5N8) viruses in the Astrakhan (Russian) outbreak, only one of which was made publicly available in GISAID.

Supplementary Figure 1. Phylogenetic tree of clade 2.3.4.4 H5 sequences for recent H5N1 avian detections in BC (n=8, blue dots) and all human A(H5Nx) clade 2.3.4.4 sequences since 2014 in GISAID and FluDB (n=33, red dots), against a backbone of avian A(H5Nx) sequences



BC avian and global human amino acid sequences are shown in a backbone of randomly sub-sampled avian A(H5Nx) clade 2.3.4.4 sequences from 2014 to 2022 and rooted on a 2008 clade 2.3.4.4 virus. Sequences are labelled using a combination of GISAID/FluDB identification numbers and year of specimen collection. Unless sequence names are labelled as "Human", they are of avian origin; human sequences include species and country. All BC sequences are from birds sampled in 2022 for which sequencing was performed by the BCCDC Public Health Laboratory on behalf of the Animal Health Centre and are distinctively labelled. Other sequences from Canadian clade 2.3.4.4 viruses were not available at the time of this analysis. The human case from Russia is representative of seven human A(H5N8) viruses in the Astrakhan (Russian) outbreak, only one of which was made available in GISAID. The two avian North American A(H5N1) sequences most closely clustering with the BC sequences are representative of 102 North American wild bird and poultry outbreak A(H5N1) viruses.

Supplementary Figure 2. Phylogenetic tree of clade 2.3.4.4 H5 sequences for recent H5N1 avian detections in BC (n=8, blue dots) and all non-human mammalian A(H5Nx) clade 2.3.4.4 sequences since 2014 in GISAID and FluDB (n=32, red dots), against a backbone of avian A(H5Nx) sequences



BC and non-human mammalian sequences are shown in a backbone of randomly sub-sampled avian A(H5Nx) clade 2.3.4.4 sequences from 2014 to 2022 and rooted on a 2008 clade 2.3.4.4 virus (the same backbone as **Supplementary Figure 1**). Sequences are labelled using a combination of GISAID/FluDB identification numbers and year of specimen collection. Unless sequence names are labelled with a mammalian host and country of origin, they are of avian origin. All BC sequences are from birds sampled in 2022 for which sequencing was performed by the BCCDC Public Health Laboratory on behalf of the Animal Health Centre and are distinctively labelled. Other sequences from Canadian clade 2.3.4.4 viruses were not available at the time of this analysis. Non-human mammalian sequences from infection studies were excluded from the phylogenetic tree. Identical amino acid sequences from multiple non-human mammalian hosts could not be confirmed as originating from the same animal and are presented as individual animals.

Supplementary Table 2. Clade distribution of all 2.3.4.4 A(H5Nx) sequences collected from 2014-2022 from avian hosts and publicly available in GISAID and FluDB as of April 28, 2015*

Clade	Total Number of Sequences[1] (%)	Year Distribution (%)	Subtype Distribution (%)	Geographical Distribution (%)	Number in Subsampled Tree (% sampled)
2.3.4.4	0	2008-2013	-	-	1[2]
2.3.4.4a	151 (2)	2014-2015 (99)	H5N6 (96)	Asia (100)	4 (2.6%)
2.3.4.4b (<2020)[3]	1734 (27)	2016-2018 (94)	H5N8 (86) H5N6 (11)	Europe (54) Africa (25) Asia (21)	15 (0.9%)
2.3.4.4b (≥2020)	1729 (27)	2020-2022	H5N8 (60) H5N1 (36)	Europe (75) Asia (16) North America (6)	30 (1.7%)
2.3.4.4c	1192 (18)	2014-2015 (85)	H5N2 (42) H5N8 (57)	Asia (71) North America (26)	8 (0.7%)
2.3.4.4d	119 (2)	2014-2017 (99)	H5N6 (99)	Asia (100)	4 (3.4%)
2.3.4.4e	464 (7)	2016-2017 (97)	H5N6 (99)	Asia (99)	3 (0.6%)
2.3.4.4f	533 (8)	2014-2015 (89)	H5N6 (94)	Asia (100)	4 (0.8%)
2.3.4.4g	43 (1)	2019 (70)	H5N6 (98)	Asia (100)	3 (7%)
2.3.4.4h	500 (8)	2017-2020 (82)	H5N6 (92)	Asia (99)	4 (0.8%)
Total	**6465**	**2014-2020**	**H5N8 (50) H5N6 (30) H5N1 (10) H5N2 (8)**	**Asia (52) Europe (32) North America (9) Africa (8)**	**76**

*Clade distribution of sequences sub-sampled for phylogenetic analysis also shown in the last column.

[1] Error rate in absolute numbers for clades d to h is +/- 2.5%
[2] All 2.3.4.4 viruses were collected prior to 2014; this sequence was included to root the phylogenetic tree.
[3] Distinction made between clade 2.3.4.4b viruses prior to 2020 and after 2020 because of low prevalence of AIV clade 2.3.4.4b between the global epidemics in 2016-17 and 2020-21 which saw a shift from H5N8/H5N6 subtypes to H5N8/H5N1 subtypes.

Supplementary Table 3. Prevalence of single-point mutations that potentially increase adaptability to mammalian species, including humans, as observed in sequences of clade 2.3.4.4 HPAI A(H5Nx) viruses since 2014[1]

Gene	Mutation (H5 numbering)	Mutation (H3 numbering)	% in non-human mammals (N = 33[2])	% in human cases (N = 33[3])	% in human cases of 2.3.4.4b (N = 11[4])	% in BC sequences (infected birds) (N = 8)
PB2[5]	E627K	N/A	18.8%	28.1%	0%	0%
	D701N		12.5%	3.1%	0%	0%
	K526R		0%	3.1%	0%	0%
HA	D94N	D101N	59.4%	66.7%	0%	0%
	S133A	S137A	96.9%	100%	100%	100%
	S154N	S158N	100%	100%	100%	100%
	S155D	S159N	100%	97.0%	90.9%	100%
	T156A	T160A	96.9%	93.9%	100%	100%
	T188I	T192I	0%	24.2%	72.7%	0%
	K189N	K193N	93.8%	69.7%	100%	100%
	V210A	V214I	6.3%	3.1%	0%	100%
	K218Q	K222Q	100%	100%	100%	100%
	S223R	S227N	96.9%	66.7%	90.9%	100%
PB1	D3V	N/A	96.8%	96.9%	90%	100%
	N105S		0%	3.1%	10%	0%
	D622G		100%	100%	100%	100%
PA	S37A	N/A	93.5%	78.1%	100%	100%
	V63I		0%	21.9%	0%	0%
	K356R		9.7%	21.9%	0%	0%
	N383D		100%	100%	100%	100%[6]
	N409S		90.3%	78.1%	100%	100%[6]
NP	E434K	N/A	0%	3.6%	16.7%	0%
	N319K		6.3%	0%	0%	0%
NS[7]	P42S	N/A	100%	96.4%	100%	100%
	80-84DEL		43.8%	53.6%	16.7%	0%
	D92E		40.6%	53.6%	16.7%	0%
	I106M		93.8%	75%	100%	100%
	C138F		96.9%	82.1%	83.3%	100%
M	N30D	N/A	100%	100%	100%	100%
	I43M		100%	100%	100%	100%
	T215A		100%	100%	100%	100%

**Red indicates mutations that are present in contemporary B.C. avian sequences as well as the closely related 2.3.4.4b UK and Russian human AIV cases. For various reasons, analysis of neuraminidase (Nx) not included here.

[1] Only viruses made publicly available on GISAID are included in general analysis. All B.C. sequences are from birds sampled in 2022. Sequencing was performed by the BCCDC PHL on behalf of the Animal Health Centre.
[2]Some samples had no data for certain segments: n = 32 for PB2, HA, NP, NS, M; n = 31 for PB1, PA
[3] Some samples had no data for certain segments: n = 32 for PB2, PB1, PA; N = 28 FOR NS, M
[4] Some samples had no data for certain segments: n = 10 for PB2, PB1, PA; n = 6 for NS, M
[5] PB2 gene explored because of its importance in allowing AIVs to replicate after binding and entering mammalian/human cells
[6] Three sequences were ambiguous at this position
[7] Numbering is relative to A/Goose/Guangdong/1/1996

Supplementary Material 4. Regional public health authority, contact information (for health professionals only)

Fraser Health: 1-866-990-9941

Interior Health: 1-866-457-5648

Island Health:

South Island: 1-866-665-6626

Central Island: 1-866-770-7798

North Island: 1-877-887-8835

Northern Health:

Business hours: 250-645-3794

After business hours: 250-565-2000, press 7, ask for the MHO on call

Vancouver Coastal Health:

Business hours: 1-855-675-3900

After business hours: 604-527-4893