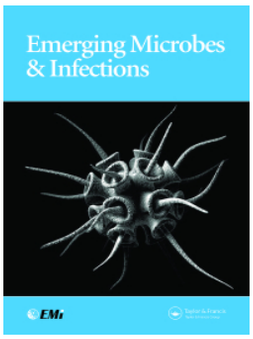

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Detection and phylogenetic analysis of contemporary H14N2 Avian influenza A virus in domestic ducks in Southeast Asia (Cambodia)

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ABSTRACT

Avian influenza virus (AIV) in Asia is a complex system with numerous subtypes and a highly porous wild birds-poultry interface. Certain AIV subtypes, such as H14, are underrepresented in current surveillance efforts, leaving gaps in our understanding of their ecology and evolution. The detection of rare subtype H14 in domestic ducks in Southeast Asia comprises a geographic region and domestic bird population previously unassociated with this subtype. These H14 viruses have a complex evolutionary history involving gene reassortment events. They share sequence similarity to AIVs endemic in Cambodian ducks, and Eurasian low pathogenicity and high pathogenicity H5Nx AIVs. The detection of these H14 viruses in Southeast Asian domestic poultry further advances our knowledge of the ecology and evolution of this subtype and reinforces the need for continued, longitudinal, active surveillance in domestic and wild birds. Additionally, *in vivo* and *in vitro* risk assessment should encompass rare AIV subtypes, as they have the potential to establish in poultry systems.

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KEYWORDS H14N2; surveillance; avian influenza; phylogenetics; Southeast Asia

Introduction

Wild aquatic birds in the order *Anseriformes* (ducks, geese, and swans) and *Charadriiformes* (primarily gulls and waders) are the natural reservoir of avian influenza viruses (AIV) [1]. These species play a major role in the epidemiology of low pathogenicity avian influenza (LPAI) viruses and, recently, high pathogenicity avian influenza (HPAI) H5N1 clade 2.3.4.4 viruses [2–4]. Critically, the wild birds-poultry interface is highly porous, facilitating the transmission of both LPAI and HPAI viruses between poultry (including *Anseriformes* and *Galliformes*) [5] and wild birds. This interplay results in complex patterns of circulation and evolution, often involving cross-species transmission [4]. Of particular concern are instances in which AIVs have undergone amplification in poultry systems, sporadically spilling over into humans and other mammals. Typically, such transmission occurs indirectly through contact with infected poultry [6].

Influenza A viruses are classified into subtypes based on their surface proteins, haemagglutinin (HA; H1-H18) and neuraminidase (NA; N1-N11), of which H1-16 and N1-9 are detected in wild birds [7,8]. While some subtypes are very common (e.g. H3, H9), some viral subtypes are notably underrepresented in surveillance systems [7], such as H14 and H15. To date, less than 70 unique H14 genomes are currently available in publicly accessible influenza sequence databases. The lack of sufficient data has created a substantial knowledge gap in our understanding of the ecology and evolution of H14 viruses, especially in regions beyond the Americas.

The first reported detections of H14 occurred in 1982 in three wild mallards (*Anas platyrhynchos*) and one herring gull (*Larus argentatus*) in Russia and Kazakhstan, respectively, although not specifically defined as H14 until 1990 [9]. Almost 30 years later, H14 was reported in sea ducks; two long-tailed ducks (*Clangula hyemalis*) and one white-winged

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scoter (*Melanitta deglandi*) in Wisconsin, USA in 2010 [10], in northern shovelers (*Spatula clypeata*) in California in 2011 [11], and blue-winged teals (*Spatula discors*) in Guatemala in 2011 and Texas in 2013 [12]. Following these detections, retrospective serology indicated that H14 was likely not circulating in North American wild bird populations prior to the first virus isolation in late 2010 [10,13], strongly suggesting an introduction from Eurasia. Unfortunately, beyond the initial description of H14 viruses collected in the 1980s, there have been only six additional viral sequences recovered from Eurasia and one viral sequence from northern Africa. Of these, six detections were in *Anseriformes* and a single detection in *Chadriformes*, including one garganey (*Spatula querquedula*) in Ukraine in 2006, one goose (*Anser spp.*) in Pakistan in 2014, two garganey, one sandpiper (*Scolopacidae spp.*) and one common teal (*Anas crecca*) in Russia in 2014 and 2019 and single garganey in Egypt in 2017 [14].

Cambodia is a developing country in Southeast Asia with a population of >16 million of which more than 70% are dependent on agricultural practices for their livelihood [15]. Estimates from 2015 indicate the presence of about ≈18 million chickens and ≈8 million ducks mostly raised in small backyard poultry flocks. Previous longitudinal surveillance at key live bird markets in Cambodia reveal year-round, high co-circulation of H5 and H9 and to a lesser extent H7 viruses [16–21]. Occasional detection of low pathogenicity H1, H2, H3, H4, H6, H10 and H11 have also been reported [22]. In 2022, H14N2 virus was detected in domestic ducks (*Anas platyrhynchos domesticus*) in Cambodia, a host species and location not previously known to harbour this subtype. To shed light into the geographic origin, temporality, segment diversity, and whether these H14N2 virus have undergone reassortment with endemic poultry AIV subtypes, the phylogenetic relationships of all genome segments of the Cambodian H14N2 viruses were compared with all H14Nx viruses and contemporarily AIVs.

Materials and methods

Ethics statement

Animal sampling was conducted by Institute Pasteur du Cambodge (IPC), Wildlife Conservation Society (WCS), and the National Animal Health and Production Research Institute (NAHPRI) under the direction of the General Directorate for Animal Health and Production, Cambodian Ministry of Agriculture, Forestry and Fisheries (MAFF) as part of disease surveillance activities focusing on the wild birds – poultry interface; thus, poultry sampling was not considered as experimental animal research. The analysis of poultry samples taken for surveillance for avian influenza testing was approved by the Cambodian National Ethics Committee for Health Research (approval #051NECHR). The Institut Pasteur du Cambodge (IPC) serves as a World Health Organization H5 Reference Laboratory and the Cambodian National Influenza Center, with approvals and infrastructure necessary to work on high pathogenicity avian influenza. No animal experimentation was performed at IPC.

Sample collection, detection, and isolation

As part of an active surveillance effort at the wild birds- -poultry interface, poultry farms, including free-ranging duck farms were generally identified as being in close proximity of known breeding sites of Asian openbill storks (*Anastomus oscitans*) and Sarus crane (*Antigone antigone*). Oropharyngeal and cloacal samples were collected from domestic birds (ducks and chickens) as described previously [19]. Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Maryland, USA) according to the manufacturer's protocol. All samples were screened by real-time reverse transcription PCR with M gene, as previously described [17,23]. Samples with a cycle threshold (Ct) of less than 40 were deemed positive. Positive samples with Ct < 30 were used for isolation using 10-day-old embryonated chicken eggs. Eggs were inoculated via the allantoic route and were harvested 3 days later. The presence of influenza virus in the allantoic fluid was tested using haemagglutination (HA) assay (REF). Both original samples and isolates were subtyped using influenza RT–PCR assays to test for H5, H7 and H9 HA genes and N1, N5, N6, N8, and N9 NA genes [16,17]. Finally, all positive original samples and isolates with Ct < 25 were selected for sequencing regardless of their subtype.

Sequencing

Whole genomes were amplified using custom Uni12/Inf-1 and Uni13/Inf-1 integrated barcoded primers [17,24,25] and SSIII One-step RT–PCR with Platinum Taq High Fidelity kit (Thermo Fisher, Massachusetts, USA). Sequencing libraries were prepared using ligation sequencing kit SQK-LSK109 (Oxford Nanopore Technologies, Oxford, UK) and sequenced on the GridION platform (Oxford Nanopore Technologies, Oxford, UK). Sequencing reads were de-multiplexed, quality trimmed, and filtered using Porechop software (https://github.com/rrwick/Porechop). Consensus sequences were generated using IRMA v1.02 [26] using default settings. Consensus sequences were manually inspected for errors such as INDELs and mixed bases, and corrected if required. A minimum depth coverage of 10 bp was set for all genes. A total

of 20 gene segment sequences obtained from three H14 influenza A viruses in this study were deposited to GISAID under accession numbers EPI-ISL: 18331156, 18331157, 18331158.

Phylogenetic analysis

All available non-human influenza A virus sequences from the last 5 years (2018-01-01 to 2023-01-01), regardless of subtype, and all known H14Nx sequences (regardless of sample date) were obtained from the GenBank (https://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html) and GISAID [27] databases (assessed on 2023-03-06). For each gene dataset, the top 50 BLAST matches from NCBI, closest to the Cambodia H14N2 virus gene sequences, were added. For the internal protein-coding genes, an additional set of contemporary Cambodian H7Nx ($n = 4$, accession numbers EPI-ISL: 18508229, 18508293, 18508230, 18508294) viruses were included to address questions of locally derived gene segments. Duplicates (based on strain name), laboratory-derived, mixed subtype (excluding H14Nx), and low coverage (<90% of full length) sequences were excluded from downstream analysis.

Sequences were aligned with MAFFT v.7.490 [28] and trimmed with TrimAL [29]. Large-scale maximum likelihood phylogenetic trees were inferred with Fasttree v2 [30] using the GTR nucleotide substitution model. Focused maximum likelihood phylogenetic trees were inferred in IQ-TREE v.2.2.0 [31] using the best-fit nucleotide substitution model (HKY + F + I chosen according to BIC). TempEst [32] was used to explore the temporal signal and clock-like behaviour of the HA gene, and a temporally structured tree was estimated using BEAST v1.10.4 [33] under an uncorrelated relaxed clock model [34], the HKY codon-structured nucleotide substitution model, and the Bayesian skyline coalescent tree prior GMRF [35]. Two MCMC chains were run for 10 million generations. Convergence was assessed using Tracer v1.6 [36], and maximum clade credibility trees were generated using TreeAnnotator v1.8. Trees were visualized and annotated in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and ggTree [37] in R (version 4.2.2).

Results

H14N2 viruses detected in Cambodian semi free-ranging duck farm

As part of an AIV surveillance study, which focused on the wild birds-poultry interface, three H14N2 viruses were identified from a single semi-free-ranging duck farm in February ($n = 1$) and March 2022 (n = 2), representing the only H14Nx viruses detected in domestic ducks to date. The farm is located in Prey Veng province, Cambodia (Figure 1(A)) and comprises stilted housing positioned above an artificial pond that houses approximately 4000 ducks (Figure 1(B, C)). Notably, the duck farm is located in close proximity to Boeung Sne, a 3557-hectare wild bird conservation area. During the sampling, there were no apparent clinical signs of influenza A virus disease observed in ducks. Environmental samples (faecal) samples collected from wild birds and other surrounding backyard poultry farms in this area did not reveal the presence of H14 viruses.

Detection of H14N2 viruses in Cambodian farmed ducks expands the known range of Eurasian lineage H14 viruses

Phylogenetic analysis of the HA gene of all H14 viruses available globally to date ($n = 71$) reveals two distinct contemporary lineages separated by geography: Eurasian and Americas (Figure 2). In Eurasia, five H14 viruses were sequenced from samples collected in 1982, and more contemporary strains include one collected in 2006, six between 2014 and 2019 from Eastern Europe/Western Asia, and a single sequence from Egypt from 2017. In contrast, all North American samples were collected between 2010 and 2019, and appear to originally derive from the Eurasian lineage [38], likely through a single introduction, sharing a common ancestor with A/garganey/Ukraine/2006 with a mean time of most recent common ancestor (tMRCA) of April 2004 (October 2002–August 2005, 95% highest posterior density (HPD)) and have been maintained in local bird populations.

The HA of the three Cambodian H14N2 viruses belonged to the contemporary Eurasian lineage, representing samples from a new geographic region, –Southeast Asia–. They were most closely related to each other (average 99.6% similarity) and shared a common ancestor November 2018 (May 2018–April 2019, 95% HPD) with an H14N3 virus identified in a common teal from Lake Chany, Russia, in 2019 (97.4–97.6% HA identity, Figure 2, Table 1).

The lack of sequence data makes it challenging to confirm the presence of H14 in Eurasia between 1982 and the emergence of the more contemporary Eurasian and North American clades. The long branch lengths are associated with under-sampling, which could potentially account for the limited detections. The sparsity of H14 detections can likely be attributed to under sampling of both known and unknown H14 host reservoir species within current surveillance schemes.

Most influenza A viruses depend on host trypsin-like proteases that facilitate cleavage of the immature HA into the mature HA subunits HA1 and HA2 to render the virus infectious and a key determinant

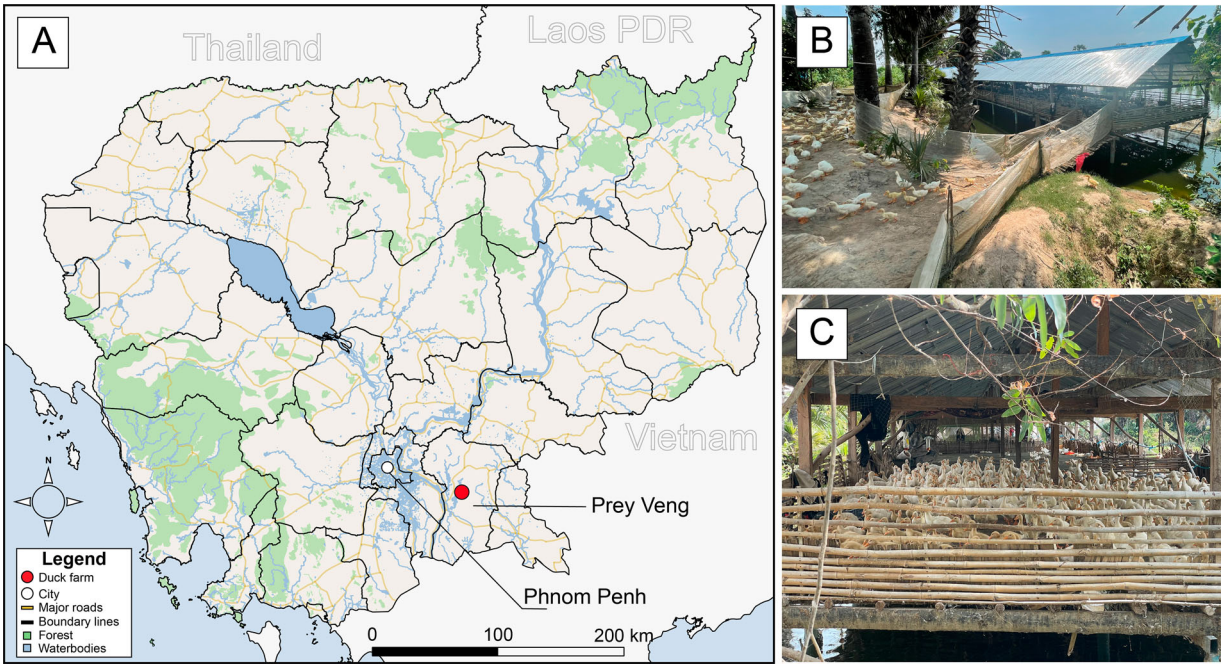

Figure 1. Location and characteristics of the duck farm in Cambodia. (A). Country map. Black lines denote provinces, and the capital Phnom Penh is indicated by a white circulate. Map was generated using QGIS (version 3.28.3). The duck farm from which H14 viruses were isolated is indicated in red. (B, C) Images of the semi free-ranging duck farm. Photographs were taken by Jurre Y. Siegers.

for pathogenicity and organ tropism [39]. The three Cambodian H14 sequences contain two different cleavage site motifs, specifically; PDKQT**K**↓GLF and PDKQT**R**↓GLF (Figure 2). Interestingly, the PDKQT**K**↓GLF motif has been observed in Eurasian H14 viruses before but the PDKQT**R**↓GLF motif has thus far only been observed in H14 viruses isolated from North American (Guatemalan) wild birds

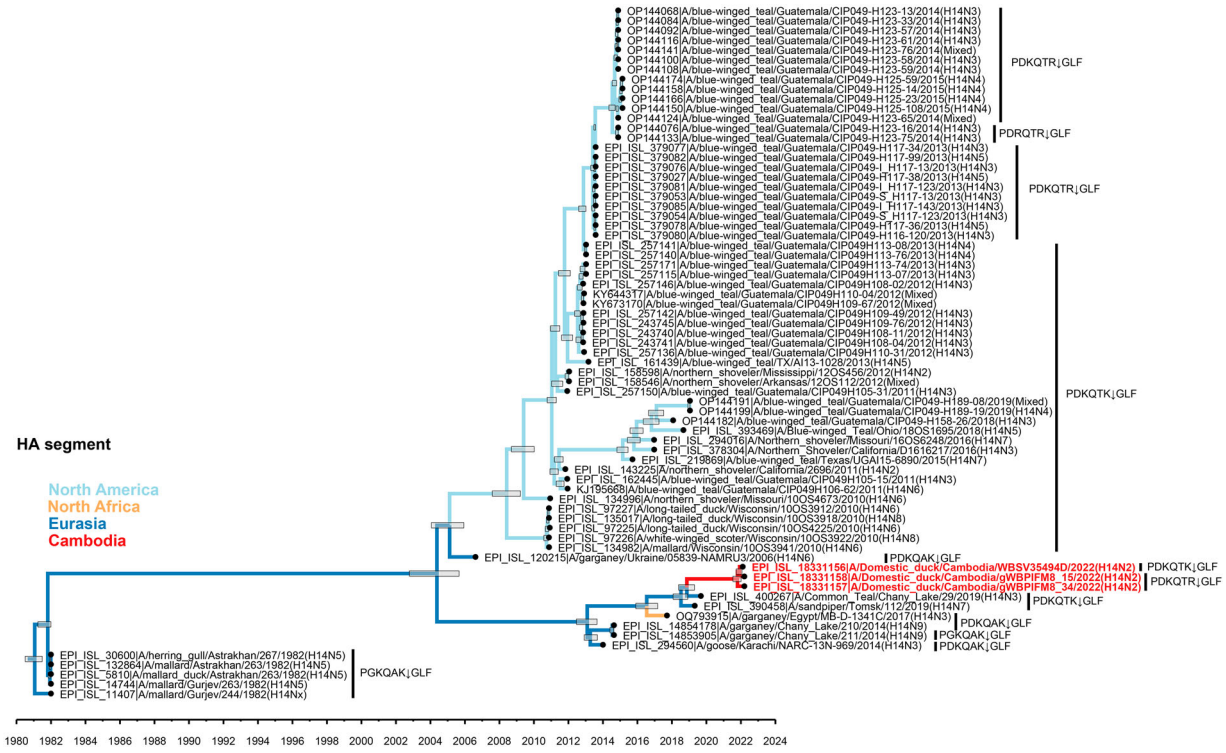

Figure 2. Evolutionary relationships and divergence of HA gene of H14Nx viruses. Phylogeny includes all H14 HA sequences available in public influenza databases (GISAID, NCBI, and BV-BRC) with sequences generated in this study shown in red. Branches are coloured by geographic location. Tree is scaled to time with node bars corresponding to the 95% HPD. HA cleavage site motif is shown for each isolate.

Table 1. Distance matrix of the haemagglutinin gene in percent identities of Cambodia H14N2 viruses versus the genetically closest ancestor.

	A/Common_Teal/Chany_Lake/29/2019	A/Duck/Cambodia/gWBPIFM8_15/2022(H14N2)	A/Duck/Cambodia/gWBPIFM8_34/2022(H14N2)	A/Duck/Cambodia/WBSV35494D_p1e1/2022(H14N2)
A/Common_Teal/Chany_Lake/29/2019		97.4%	97.5%	97.6%
A/Duck/Cambodia/gWBPIFM8_15/2022(H14N2)	97.4%		99.5%	99.6%
A/Duck/Cambodia/gWBPIFM8_34/2022(H14N2)	97.5%	99.5%		99.6%
A/Duck/Cambodia/WBSV35494D_p1e1/2022(H14N2)	97.6%	99.6%	99.6%	

GISAID accession numbers: EPI_ISL_400267, EPI_ISL_18331156, EPI_ISL_18331157. And EPI_ISL_18331158

GISAID accession numbers: EPI_ISL_400267, EPI_ISL_18331156, EPI_ISL_18331157. And EPI_ISL_18331158.

between 2013 and 2015. Other Eurasian cleavage site motifs include PD/GKQAK↓GLF where additional cleavage site motifs in North America include PDK/RQTR/K↓GLF. Subsequent analysis of all gene segments of the three Cambodian H14N2 viruses using FluSurver (https://flusurver.bii.a-star.edu.sg/) did not reveal any significant mutations classified as "warn level 3 (most significant)".

Cambodian H14N2 NA genes are locally derived but similar to other internal genes likely originate from Eurasian wild birds

Analysis of the NA and internal protein-coding genes of Cambodian H14 viruses is critical to clarify whether this detection represents a recent introduction into poultry, or if these rare viruses have been circulating and reassorting in Southeast Asian poultry systems. Specifically, it is important to ascertain if other H14 viral genes comprise those from circulating AIVs in Cambodian poultry, or the H14N2 viruses stem from direct spillover into domestic poultry from wild birds. To date, none of the other available H14 Eurasian lineage sequences is associated with the N2 subtype. The N2 NA sequences of the three Cambodian H14N2 viruses were similar to each other, and group with viruses detected in poultry and wild birds between 2019 and 2021 in Asia, including Cambodia, South Korea, Japan, Bangladesh, Mongolia, and Iran (Figure 3). Notably, they are most closely related to the NA of H7N2 viruses isolated from Cambodian-farmed ducks in 2020, suggesting localized NA circulation.

The internal protein coding gene segments of the Cambodian H14N2 viruses clustered together (Figure 4). The NA, PB1, and PA gene segments, were closely related to a H7N2 and H7N3 virus isolated in Cambodian poultry species in 2020 and 2022, other internal protein-coding genes were related to LPAI viruses predominantly detected in wild birds

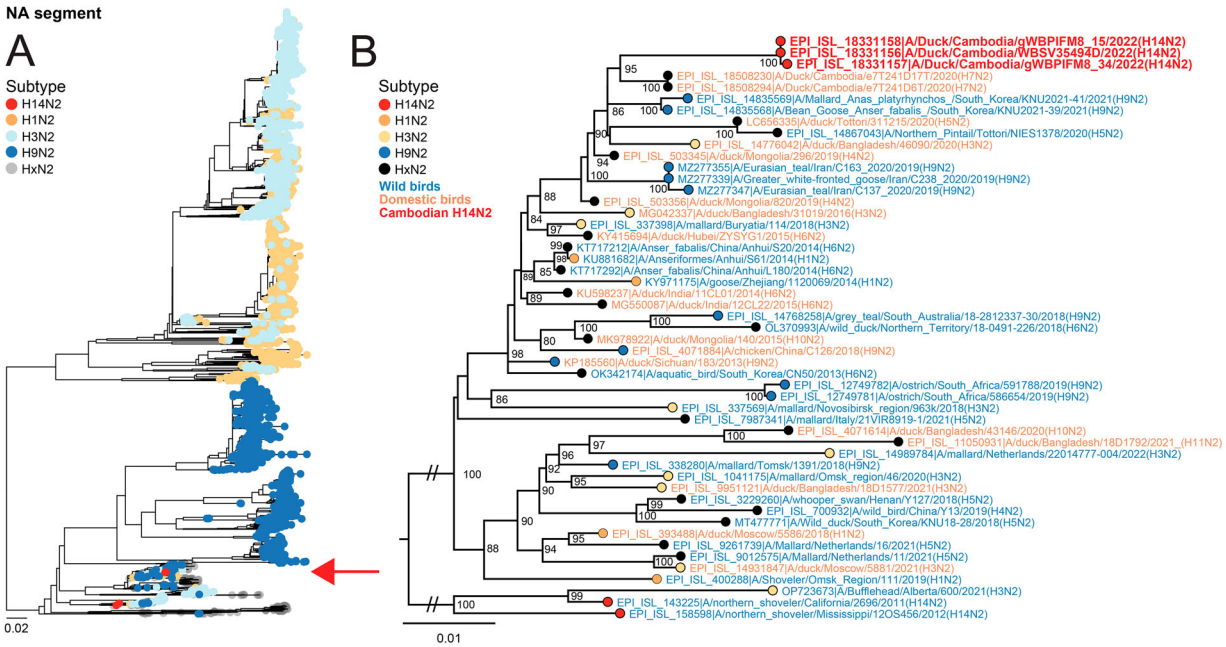

Figure 3. Phylogenetic relationship of the N2 gene sequences. Maximum Likelihood (ML) tree of Cambodian H14N2 viruses with HxN2 viruses. (A) Tree comprising all sequences in publicly available databases from 2018-2022. Red arrow indicates phylogenetic placement of Cambodian H14N2 in N2 tree for improved clarity. The tree was rooted to reflect geographic clades (B) comprising the >30 most closely related sequences. Sequences generated in this study are presented in red, with strain names emboldened. The tree was rooted against sequences from North America. Ultrafast bootstrap values (≥80) are presented. Scale bar indicates the number of nucleotide substitutions per site.

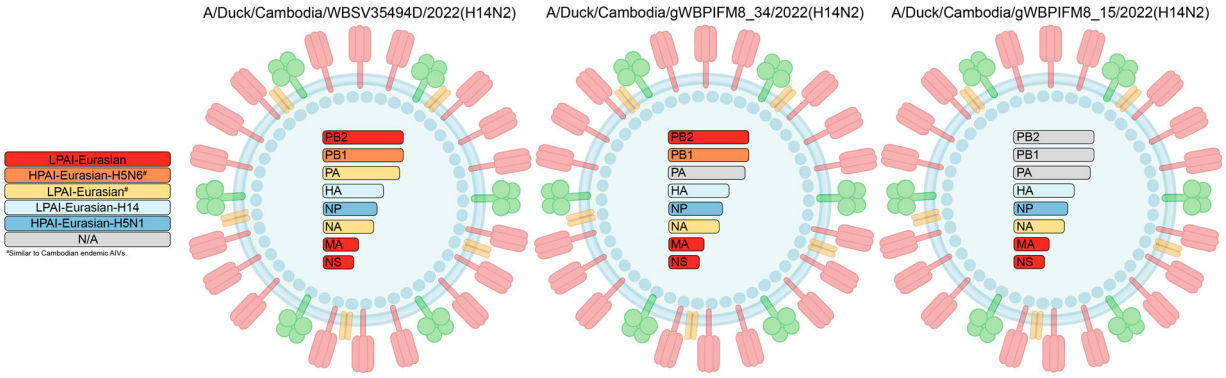

Figure 4. Genome constellation of contemporary Cambodian H14N2 viruses. Genome constellation of each of the Cambodian H14N2 viruses, when available, are colour coded according to their genetic origin.

across Eurasia but also in poultry in Asia. Their polymerase basic protein 2 (PB2) gene grouped alongside LPAI wild bird and poultry viruses from Eurasia isolated in 2019 to 2022 and genetically most closely related to a H5N3 virus from a Korean goose isolated in 2022 (Supplemental Figure 1B). Sequences of the polymerase basic protein 1 (PB1) gene and polymerase acid protein (PA) gene of the polymerase complex are closely related to H7N3 virus isolated from a Cambodian domestic duck in 2022 (Supplemental figure 2, supplemental figure 3). However, PB1 sequences were more distantly related to domestic poultry LPAI viruses (H7N7 and H6N6) and HPAI H5N6 viruses isolated from Vietnam in 2018 and 2021 (Supplemental Figure 2B). Unfortunately, full PA gene was only obtained from 1 virus, and was most closely related to H4N6 viruses isolated from wild birds in Eastern Russia and South Korea in 2020 and 2021, respectively, then the Cambodian H7N3 virus isolated from ducks in 2022 (Supplemental Figure 3B). The nucleoprotein (NP) gene sequences clustered with HPAI H5N1 and H5N8 viruses circulating in Asia in 2020–2022 (Supplemental Figure 4A), and most closely related to HPAI H5N1 viruses isolated from Chinese domestic poultry in 2021–2022 (Supplemental Figure 4B), whereas their matrix (M) gene segment sequences clustered within Eurasian LPAI viruses isolated between 2018 and 2022 (Supplemental Figure 5), and were most closely related to LPAI H5N3 and H3N8 viruses isolated from wild birds in Korea and Russia in 2022 and 2018, respectively (Supplemental Figure 5B). Non-structural (NS) segment sequences clustered with Asian LPAI viruses such as H7N3, H11N2, and H9N2 isolated from wild ducks in South Korea in 2020–2021 (Supplemental Figure 6B).

Discussion

H14 is an underrepresented and understudied AIV subtype. The ecology and evolution of this rare subtype is poorly understood. Indeed, following the first detection of H14 viruses in 1984 in Russia and Kazakhstan, 30 years passed before subsequent but infrequent detections in Eurasia, the Americas, and Africa. In 2022, H14 viruses were detected in three samples from domestic ducks in Southeast Asia, a subtype not previously reported or detected in this region or in domestic species. Overall, all viral segments, except the PB1, PA, and NA sequences of the three Cambodian H14N2 viruses were most closely related to viruses circulating in wild birds and domestic ducks in Eurasia and not associated with poultry associated LPAI and HPAI H5/H7/H9 viruses currently circulating in Cambodia. Together, this indicates that the Cambodian H14N2 viruses are likely wild bird viruses that have potentially reassorted with endemic poultry subtypes. The detection of these H14N2 viruses in Cambodian domestic ducks further advances our knowledge of the geographic distribution of this subtype, and its relatively rare global detection, suggests a possible ecological niche in an unknown reservoir species currently under sampled in Southeast Asia.

Several different LPAI and HPAI virus subtypes circulate concurrently in Cambodian poultry [16,22]. The peri-domestic nature of the Cambodian poultry system allows for frequent contact with wild birds leading to virus transfer from wild birds into the poultry system, and vice-versa. Phylogenetic analysis of the H14 viruses suggests a potential spillover event into domestic bird species from non-surveyed (wild) bird host reservoirs. This event resulted in a single introduction, amplification, and reassortment with locally, -Southeast Asian-, derived H7N2/N3 viruses, as evidenced by the PB1, PA, and NA sequences analysis throughout the poultry system. Future studies should address whether these H14 viruses are maintained in local (domestic) bird populations through longitudinal surveillance, acting as a potential reservoir. The limited availability of data on AIV circulating in wild birds in Cambodia and Southeast Asia makes it challenging to determine whether this recent detection of H14N2 in Cambodian domestic ducks originally resulted from a spillover event in Southeast Asia

from resident wild birds found year-round, or through migratory birds present only part of the year. The Greater Mekong Subregion is along the western edges of the East Asian Australasian Flyway, as well as in the Central Asian Flyway, such that numerous bird species may import novel viral subtypes and lineages from across the globe. For Southeast Asia, future studies on the prevalence of H14 viruses in migratory birds along these flyways and domestic birds are warranted [40].

While there are several extensive surveillance systems for AIV in Asia [41], there are several possible factors associated with limited H14 and other rare subtype detections in commonly sampled birds and poultry. First, AIV surveillance systems have mainly focused on subtypes of agricultural concern, such as subtypes H5, H7, and H9 with limited interest and/or funding for other LPAI subtypes. Second, previous studies reveal that the divergence of H14 viruses is consistent with continuous circulation in a host species despite a large "genetic sequence gap" and provides evidence for an ecological niche that is under surveyed in current surveillance efforts [42]. Despite AIV being detected in >100 avian species globally, surveillance efforts are often unable to comprehensively represent the true diversity of wild birds and their abundance, –both on a species and AIV level–, at specific times and dates [43]. Current AIV surveillance systems in Eurasia are often biased towards domestic poultry and wild bird species that can be readily caught and accessed. As a result, if an AIV subtype is uncommon in the populations of domestic ducks and Mallards that are routinely monitored, it is highly improbable that existing surveillance efforts will detect these rare AIV strains, since they primarily circulate in other wild duck species or avian populations that are not commonly surveyed [43]. H14 viruses are rarely but predominantly detected in difficult to reach species (such as sea ducks), thus the low prevalence of AIV in some species of waterbirds compared to the high prevalence of other AIV subtypes leaves gaps in our understanding of rare AIV subtypes [44,45].

Finally, since domestic ducks and wild birds can harbour many different AIV subtypes, it is possible that transfer and circulation of AIV subtypes between wild birds and domestic birds is occurring [16,22], resulting in some immunological cross-protection. Furthermore, there could be genetic differences in duck species in the region affecting susceptibility and/or cross-reactive antibodies from infection with genetically related and more prevalent "sister subtype" H4 LPAIV [42]. One study found that cross protective antibodies against commonly circulating LPAI H4N6 in wild waterfowl provided heterosubtypic immunity against HPAI H5N1 infection [46]. Another study found that the rare subtype H15 and the more commonly detected sister subtype H7 appears to follow a similar evolutionary relationship as the rare H14 and sister subtype H4 [42].

While the exact introductory pathway is unknown, the detection and potential future establishment of H14Nx viruses within the poultry value chain in Southeast Asia is concerning for several reasons: (i) the apparent subclinical course of infection in both wild birds and poultry could result in cryptic spread across the region [9,42]; (ii) the impact and extent of reassortment of undetected H14 viruses with endemic H5, H7, or H9 viruses is currently unknown, but does occur as shown by the PB1 and NA sequences of the H14 viruses in this study; (iii) H14 viruses are able to attach abundantly to duck epithelial cells [47] and replicate *in vivo* in ducks, chickens, and ferrets without prior adaptation to mammals, indicating a relatively broad host range [9,48]; and (iv); similar to H5 and H7 subtypes, H14 viruses are compatible with a multi-basic cleavage site, resulting in a HPAI phenotype; however, this phenotype has never been observed in nature [49]. Similar to the first H14 sequences from Guatemala, the first Cambodian H14 contained the PDKQTK↓GLF cleavage site motif, which is quite unusual amongst IAVs. Cambodian H14 viruses sequenced one month later, at the same duck farm, however contained the PDKQTR↓GLF cleavage site motif, a more "classic" motif [38]. Currently, it is unknown how these differences in cleavage site motifs of H14 viruses affect HA functioning in terms of replication, virulence, and transmission in wild birds and their spillover hosts such as poultry and potentially mammalian species [49]. Taken together, continued active, longitudinal surveillance for all AIV subtypes in addition to H5, H7, and H9 viruses is critical to fully understand the epidemiology of AIV in the Greater Mekong subregion, Southeast Asia, and the globe. In addition, further serological studies in wild and domestic bird populations in Southeast Asia could shed light on the prevalence of H14Nx and other understudied but potentially important LPAI viruses [13]. Finally, and crucially, *in vivo* and *in vitro* risk assessments of H14 viruses are warranted, both in regards to zoonotic potential as well as economic burden.

Conclusion

The identification and phylogenetic analysis of contemporary H14 viruses from domestic ducks in Cambodia reinforces the need for vigilance in longitudinal active surveillance in domestic poultry and wild birds to include subtypes beyond those which cause high mortality and/or morbidity in poultry systems and occasional mammalian spillovers. In addition, detection in domestic species warrants further exploration into the role these birds play in the ecology and epidemiology of rarer influenza A virus subtypes.

Continued spillover and circulation of H14 viruses in domestic species could result in endemic adaptation, and, therefore poses a potential zoonotic and economic risk. Careful assessment of the pathobiology of H14 viruses in wild birds, poultry, and mammalian species is there for warranted. Early warning systems utilizing novel strategies such as multiplex testing and rapid genomic sequencing and epidemiology for monitoring and emergence of all potential AIVs are critical for One Health.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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