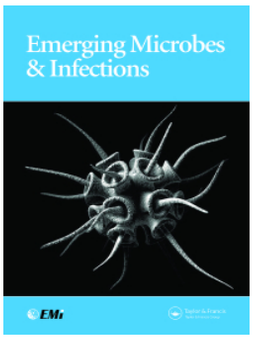

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Phylodynamics of high pathogenicity avian influenza virus in Bangladesh identifying domestic ducks as the amplifying host reservoir

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ABSTRACT

High pathogenicity avian influenza (HPAI) virus H5N1 first emerged in Bangladesh in 2007. Despite the use of vaccines in chickens since 2012 to control HPAI, HPAI H5Nx viruses have continued to infect poultry, and wild birds, resulting in notable mass mortalities in house crows (*Corvus splendens*). The first HPAI H5Nx viruses in Bangladesh belonged to clade 2.2.2, followed by clade 2.3.4.2 and 2.3.2.1 viruses in 2011. After the implementation of chicken vaccination in 2012, these viruses were mostly replaced by clade 2.3.2.1a viruses and more recently clade 2.3.4.4b and h viruses. In this study, we reconstruct the phylogenetic history of HPAI H5Nx viruses in Bangladesh to evaluate the role of major host species in the maintenance and evolution of HPAI H5Nx virus in Bangladesh and reveal the role of heavily impacted crows in virus epidemiology. Epizootic waves caused by HPAI H5N1 and H5N6 viruses amongst house crows occurred annually in winter. Bayesian phylodynamic analysis of clade 2.3.2.1a revealed frequent bidirectional viral transitions between domestic ducks, chickens, and house crows that was markedly skewed towards ducks; domestic ducks might be the source, or reservoir, of HPAI H5Nx in Bangladesh, as the number of viral transitions from ducks to chickens and house crows was by far more numerous than the other transitions. Our results suggest viral circulation in domestic birds despite vaccination, with crow epizootics acting as a sentinel. The vaccination strategy needs to be updated to use more effective vaccinations, assess vaccine efficacy, and extension of vaccination to domestic ducks, the key reservoir.

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KEYWORDS AIV; highly pathogenic avian influenza; surveillance; poultry; spill-over; wildlife

Introduction

High pathogenicity avian influenza (HPAI) H5N1 was first detected in 1996 in domestic geese (A/Goose/Guangdong/1/96 (gs/GD) lineage) in China [1,2]. Since then, the haemagglutinin (HA) gene of descendant lineages of HPAI H5Nx viruses have evolved into multiple HA clades and subclades [3]. Of these, clade 2 and, notably, clade 2.2 proved to be the most pervasive, with its recurrent spill-over from poultry into wild aquatic birds enabling episodic dispersal from China to other parts of Asia, Europe, and Africa, starting in 2005 [4]. Since 2014, with the emergence of clade 2.3.4.4, HPAI H5Nx, and more recently with another step change in October 2021 (clade 2.3.4.4b), the spread and impact on poultry and wild birds has been dramatic [5,6]. These HPAI H5N1 viruses, including the current panzootic clade 2.3.4.4b H5N1 viruses, have shown the capacity to jump the bird–mammal barrier and infect humans and other mammals [5], including carnivorous mammals (such as foxes, otters, skunks, coyotes, mink, and bobcats) [7,8], and a range of marine mammal species (such as South American sea lions *Otaria flavescens*, harbour seals *Phoca vitulina*, harbour porpoises *Phocoena phocoen* and dolphins, *Tursiops truncates* [9,10].

HPAI H5N1 emerged in poultry in Bangladesh in 2007, with the introduction of multiple clades, starting with 2.2.2 in 2007, followed by clades 2.3.4.2 and

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2.3.2.1 in 2011 [11]. Clade 2.2.2 established endemicity in poultry populations in Bangladesh and a range of other Asian and African countries, including China [12], Egypt [13], India [14], Indonesia [15], and Vietnam [16]. This clade disappeared from Bangladesh after the implementation of a vaccination programme against HPAI H5N1 in 2012 but was replaced by clade 2.3.2.1a, which is now endemic [17,18]. In Bangladesh, HPAI H5Nx has caused infections in a diverse range of domestic avian host species, including chickens, turkeys, ducks, geese, quail, and pigeons [19–21] as well as wild bird species, including migratory wild waterfowl and crows [22,23]. In addition to wildlife, outbreaks have had a profound impact on the poultry industry, posing challenges to the economy. For example, between 2007 and 2008, HPAI H5N1 impacted 547 commercial chicken farms in Bangladesh, which resulted in 1.7 million birds being culled with an estimated economic loss of US$746 million [20,24,25]. Since 2008, Bangladesh has documented eight human H5N1 cases, with one fatality reported [26]. Among these cases, three were individuals working at live bird markets (LBM), and it is presumed that they were exposed to infected poultry within that environment [19,26,27].

In order to develop effective mitigation strategies, it is crucial to comprehend the mechanisms by which HPAI H5Nx spreads within its host community and understand the role (i.e. amplifying, maintenance, dead-end) that the different host species play in these dynamics. While chickens and ducks have previously been identified as important reservoirs for HPAI H5Nx, major house crow (*Corvus splendens*) mortality events have been recorded in the Dhaka and Rajshahi districts of Bangladesh in 2011 and 2016, involving 44 and 38 casualties, respectively [22,28]. Crows are a common synanthropic species that share habitats with wildlife, poultry, and humans and could thus potentially act as bridge hosts to facilitate the spread of infectious diseases amongst wildlife, livestock, and humans. We here aimed to investigate and characterize the roles of both wild and domestic birds in the epidemiology and viral evolution of HPAI H5Nx virus in Bangladesh. As a first step, we constructed phylogenetic trees to assess the viral dynamics over time and the exchange of viruses between different host species as well as exchange between nations and regions across the globe. Next, we focused on three species that have been found affected by HPAI H5Nx most frequently in Bangladesh: chicken, domestic duck, and house crow. We investigated the contribution of these three important hosts to the evolutionary dynamics of HPAI H5Nx virus in Bangladesh using Bayesian modelling (discrete trait analysis) of the HA gene. To overcome a relative paucity of sequence data of HPAI H5Nx in crows, our study also investigated HPAI epidemiology of house crows over the years 2017–2023 in Dhaka City and generated 109 sequences related to these outbreaks. Herein we demonstrate the complex epidemiology of HPAI in Bangladesh and the role of crows as sentinels reflecting spread in ducks and silent spread in vaccinated chickens, pressing the need for more effective vaccination and post-vaccination monitoring in the region.

Materials and methods

Biological samples and data collection

House crow roosts in Dhaka were visited opportunistically throughout the year, with a frequency of at least twice a week. Upon finding the first casualties, that frequency was increased to daily visits throughout the winter season (November–March) to collect freshly dead and sick animals (Figure 1). We collected cloacal and oropharyngeal swabs from each dead or moribund individual found underneath the roost. The cloacal and oropharyngeal swabs from each individual were pooled and placed in viral transport medium (VTM) and stored in a cool box at approximately 4° C. Within four hours of collection, the samples were stored at −80°C until further testing. We performed necropsies on a random subset of 92 freshly dead carcases and collected tissue samples aseptically, including trachea, kidney, liver, lungs, and brains. For each carcase and tissue type, we stored one sample in VTM and another in Trizol and stored samples at −80°C until further analysis.

Laboratory testing and sequencing

We extracted viral RNA from VTM in which we stored swabs and tissues using the MagMAX 96 AI/ND Viral RNA isolation kit (Ambion, Inc. Austin, TX) using the KingFisher Flex 96-well robot (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. Viral RNA was screened for the presence of the avian influenza virus (AIV) matrix (M) gene using real-time reverse transcriptase PCR (rRT-PCR) with reference primers and probes, following procedures as reported by Spackman [29], CDC [30]. Positive samples were further tested for subtypes H5, H7, and H9 using subtype-specific primers using rRT-PCR as previously described [30,31]. M gene-positive samples that tested negative for H5, H9, and H7 were classified as AIV HA/untyped. H5-positive samples were further NA subtyped using N1, N2, and N6 specific primers and probes [32]. NA subtypes were confirmed using Sanger sequencing of full-length NA PCR products, as described by Hoffmann, Stech [33].

We used the Nanopore MinIon sequencing platform to sequence the H5 positive samples following

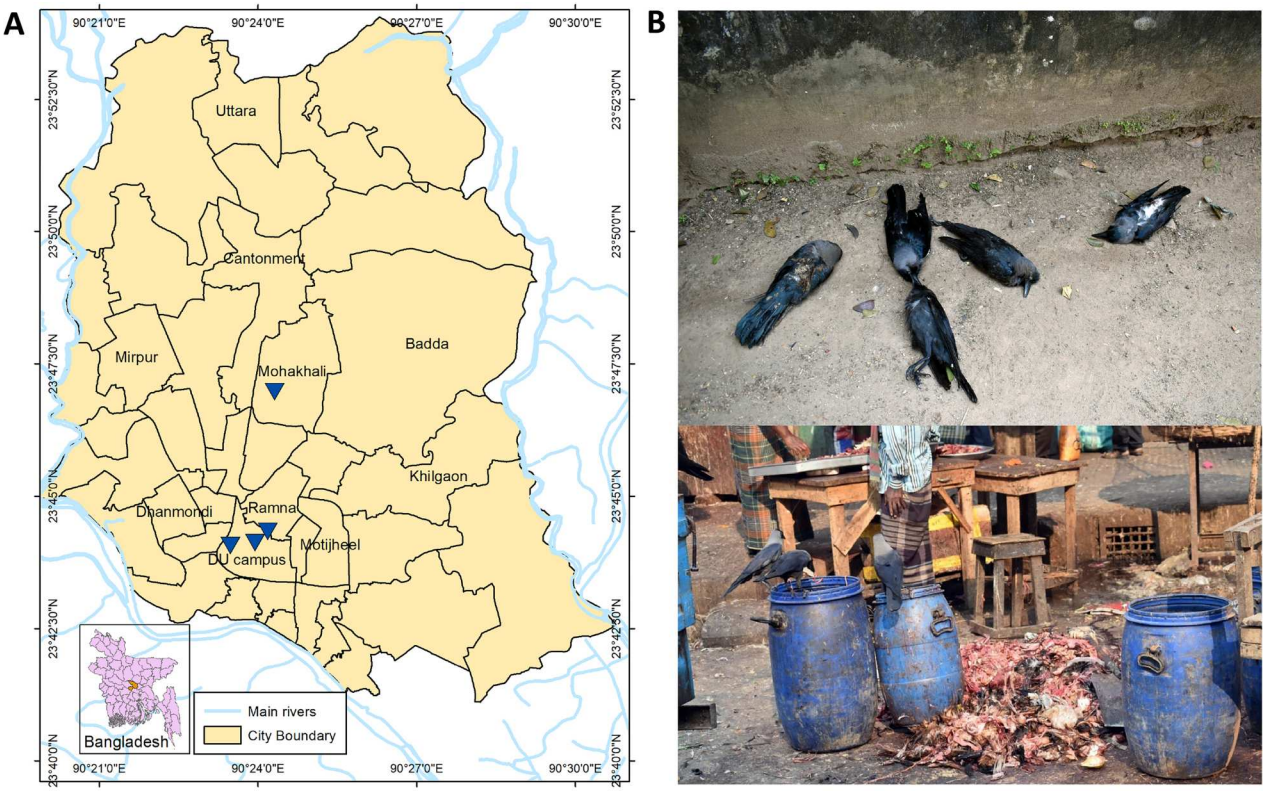

Figure 1. (A) Map showing four house crow roosts (navy blue triangles) in Dhaka city in Bangladesh that we monitored over the period 2017–2023 for house crow mortality. Lines delineate suburbs, with key suburbs named. Inset shows location of Dhaka city (orange) in Bangladesh. (B) Pictures showing typical causalities and moribund house crows and house crows feeding on the dead poultry carcases and offal around LBM.

our in-house sequencing methods and library preparations as previously described [34]. We created Nanopore sequencing libraries utilizing the ligation sequencing kit (SQK-LSK109) and barcoded them using the native barcode expansion packs (EXP-NBD104 and EXP-NBD114). To confirm HA subtypes and confirm nucleotide identity, we used basic local alignment (BLASTn) searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We used the H5 clade classification tool incorporated within the Influenza Research Database [35] to identify clade information. We further confirmed H5 clades through phylogenetic analysis, as described below.

Phylogenetic analyses

We constructed three phylogenetic trees of the HA gene. The first showcased the HA gene of all HPAI H5Nx clades found in Bangladesh since 2007, the second featured only clade 2.3.4.4 (H5N1, H5N6), while the third only featured clade 2.3.2.1a (H5N1) (for which we also inferred host migrations as explained below). For the first tree, we included 109 sequences acquired from house crows collected during our surveillance in Dhaka ($n = 66$), including sequences obtained from house crows in Rajshahi ($n = 26$), Bogura ($n = 11$), and Jashore ($n = 6$) (Supplementary Table 1) (Accession ID: OR777116-OR777135 & OR772954-OR773024). We downloaded (2 June 2023) all HA sequences of H5 viruses ($n = 564$) deposited in the Global Initiative for Sharing All Influenza Data (GISAID; https://gisaid.org/) [36] since 2007. We rejected sequences less than 1500 bp in length, as well as lab-derived sequences and sequences without or incomplete metadata. We only kept sequences from chicken, duck, and crow, resulting in a total of 529 sequences. Among them, there were 30 sequences from clade 2.2.2, 2 from clade 2.3.2.1c, 4 from clade 2.3.4.2, 33 from clade 2.3.4.4 h, and 15 from clade 2.3.4.4b and 445 from clade 2.3.2.1a. Sequences were aligned using the MAFFT [37], with a final alignment length of 1704 bp. The best fit substitution model was identified as the lowest BIC using model finder within IQ-TREE, and we inferred maximum likelihood phylogenetic trees, using IQ-TREE version 1.68, with 1000 bootstraps [38]. Focusing on only clade 2.3.4.4, we selected the 48 relevant sequences from the total number of sequences used in the HPAI H5Nx tree encompassing all Bangladeshi clades (see above). In addition to these sequences from Bangladesh, we used the BLASTn search tool to identify closely related sequences belonging to clade 2.3.4.4 in both GenBank [39] and the GISAID Epiflu database [36]. Using a final set of 138 HA sequences, we constructed a time-scaled

phylogenetic tree using BEAST v.1.10 [40] with an uncorrelated lognormal relaxed molecular clock, the HKY + G [41], and a Gaussian Markov random field (GMRF) Bayesian skyride coalescent tree prior [42]. Trees were run with 50 million steps sampled every 5000 steps.

Transition between hosts via discrete trait analysis

For the clade 2.3.2.1a tree, we only retained sequences from Bangladesh which originated from chickens, crows, and ducks, resulting in a final dataset of 445 sequences. Sequences were aligned and trimmed as described above. The degree of clock-like behaviour was confirmed through linear regressions of root-to-tip distances against year of sampling using TempEst [43]. Using BEAST v.1.10, data were analysed [40] with an uncorrelated lognormal relaxed molecular clock and the HKY + G, and a constant size tree prior [41]. To examine the ancestral host states and estimate the asymmetric viral exchange between host species, we used three host demes; crow, duck, and chicken. Discrete trait analysis was performed using the asymmetric substitution model, and social networks were inferred with Bayesian Stochastic Search Variable Selection (BSSVS) [44]. The migration events between hosts were inferred by logging the transitions between states along the phylogenetic branches (Markov Jumps) [45]. We ran three replicates of our analysis, and from each replicate, we isolated the maximum a posteriori (MAP) trajectory, which is the migration trajectory (along with associated parameters) that have the highest posterior support. There is a clearly defined mode in the posterior that overlaps with the MAP, for each replicate (Supp Fig 1). We plotted the MAP of each replicate in R studio version 2022.02.2 [46] to visualise the directionality of migrations, along with the temporal structure of migrations between hosts. We also calculated the time spent in the states between two transitions (Markov Rewards), which in addition to providing information pertaining to length of time in each host state, also provides insight into sampling bias. The parameters were analysed using TRACER v1.7.1(http://tree.bio.ed.ac.uk/software/tracer/). The maximum clade credibility (MCC) tree was generated using Tree Annotator within the BEAST software and visualized using FigTree 1.4.2. (http://tree.bio.ed.ac.uk/software/figtree/)

Ethics approval

The research was approved by the Chattogram Veterinary and Animal Sciences University Animal Experimentation Ethics Committee (Protocol:CVASU/Dir(R&E)AEEC/2015/751) and the Ethics Committee (Protocol:CVASU/Dir(R&E)EC/2019/126(1)).

Results

Epidemiology of HPAI epizootics in house crows in Dhaka

In response to an initial notification of unusual house crow mortality in a roost on 14 January 2017 [47,48], we commenced a crow mortality surveillance in four crow roosts in Dhaka city ending in May 2023 (Figure 1). Over the seven-year monitoring period, we observed a strong seasonal pattern in house crow epizootics, with numbers of dead and moribund birds peaking in December or January (Figure 2). Only a few dead crows were found during the spring season (March–May) and summer monsoon season (June–October).

HPAI was detected in 86% of dead and moribund house crows collected. In 2019/2020, 95.31% (61/64) of all collected birds were HPAI H5 positive, and 75.47% (40/53) in 2018/2019 (Table 1). HPAI H5-positive samples from house crows were further NA subtyped, and we found 97.24% were H5N1, 0.52% were H5N6, and 2.24% were H5Nx (where no NA result could be obtained). Phylogenetic analysis of the generated sequences demonstrated that the HPAI H5 lineage implicated in house crow epizootics was clade 2.3.2.1a in the G10 sub-group.

Across the various tissue samples from house crows, we found similar H5 positivity in trachea and lung tissues as in the swab samples, with the positivity in the liver, kidney, and brain (tending) to be somewhat lower (Figure 3). As all tissue samples were from freshly collected birds, false negatives due to decomposition are an unlikely factor, rather, negative birds might reflect mortality due to other causes.

Epizootiology of HPAI in Bangladesh

For better appreciation of the diversity of HPAI viruses found in Bangladesh, we sequenced 109 viruses, in addition to downloading 564 sequences from GISAID. Since 2007, two HPAI H5Nx subtypes, H5N1 and H5N6, circulated in Bangladesh (Figure 4). H5N1 viruses comprised four clades: 2.2.2, 2.3.2.1c, 2.3.4.2, and 2.3.2.1a. Three of these clades were only present in 2011/12 (clades 2.2.2, 2.3.2.1c, and 2.3.4.2), such that all sequences since 2012 have fallen into clade 2.3.2.1a. Within clade 2.3.4.4, H5N6 fell into two distinct sub-clades, 2.3.4.4b and 2.3.4.4 h (Figure 5). Clade 2.3.4.4 h viruses were detected in waterfowl and were clustered with recent HPAI H5N6 viruses from whooper swans (*Cygnus cygnus*) in Xinjiang, western China, and Mongolia (MT872362, MT72354) (Figure 5). HPAI H5N6 clade 2.3.4.4b was detected in house crows, chickens, and ducks in Bangladesh and was related to the Middle Eastern/African cluster of clades 2.3.4.4 viruses. In addition to H5N6, seven H5N1 viruses were identified in the

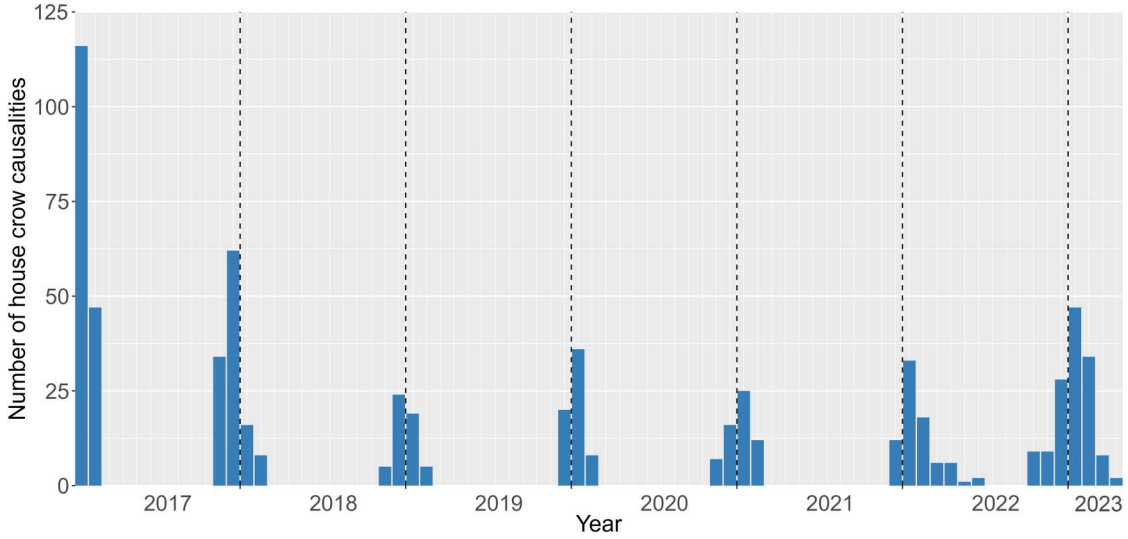

Figure 2. Number of causalities and moribund house crows collected across four crow roosts in Dhaka City from January 2017 to April 2023. Each column comprises a month. The dotted lines indicate the transition between years.

Table 1. Number and proportion of carcases positive for H5 in house crow swab samples (combined cloacal-oropharyngeal) over the years 2017–2023 in Dhaka, Bangladesh.

Host	Epizootic season	No of casualties	H5 prevalence (%)	95% CI
House crow	2016/2017	163	91.41	86.01–95.22
	2017/2018	120	85.83	78.29–91.53
	2018/2019	53	75.47	61.72–86.24
	2019/2020	64	95.31	86.91–99.02
	2020/2021	60	88.33	77.43–95.18
	2021/2022	78	84.62	74.67–91.79
	2022/2023	137	80.29	72.64–86.59
	Total	675	86.22	83.39–88.73

panzootic clade 2.3.4.4b in ducks. Sequences from this emerging clade clustered with white-tailed eagles (*Haliaeetus albicilla*) from Japan (Hokkaido) (LC730539), and chickens and domestic ducks from Africa (Nigeria and Benin) (MW961468, ON870420) (Figure 5). These six Bangladeshi ducks 2.3.4.4b H5N1 sequences share a similarity of between 98.65 and 98.97% with H5N1 viruses of clade from Japan, and a similarity of 99.30% with viruses from China. Time-structured phylogenetic analysis reveals that this clade was introduced to Bangladesh by the end of 2020 (2020.77–2020.93 95% highest posterior density [HPD]).

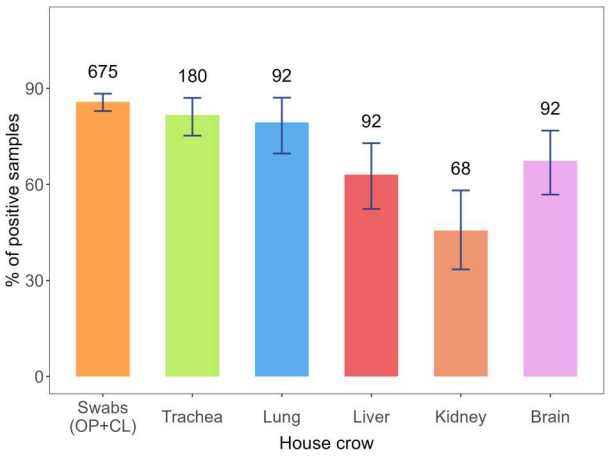

Figure 3. H5 positivity for swabs (oropharyngeal [OP] and cloacal [CL] combined) and in tissues collected from house crows, in Dhaka, Bangladesh from 2017 to 2023. The vertical error bar indicates the 95% confidence interval of the prevalence, non-overlapping confidence intervals identifying significant differences. Numbers above the error bar show the total number of samples tested.

Since 2012, clade 2.3.2.1a H5N1 viruses have become the most widespread among HPAI H5Nx clade group of viruses in Bangladesh (Figure 6). This endemic group of viruses consists of as many as 10 distinct genetic subgroups (G1-G10), as defined by a high posterior probability (>99%) in the Bayesian phylogenetic tree [49]. Most of these subgroups (G1-G9) have not been detected since 2016, indicating extinction or replacement of these genetic subgroups. However, the G10 subgroup has been consistently identified in poultry in Bangladesh since 2015, suggesting its continued circulation and persistence.

Viral transitions between host species in Bangladesh

To address the role of different hosts in the epidemiology of 2.3.2.1a, we used a discrete trait analysis to identify viral migrations (i.e. Markov jumps) between the key host groups, including chickens, ducks, and

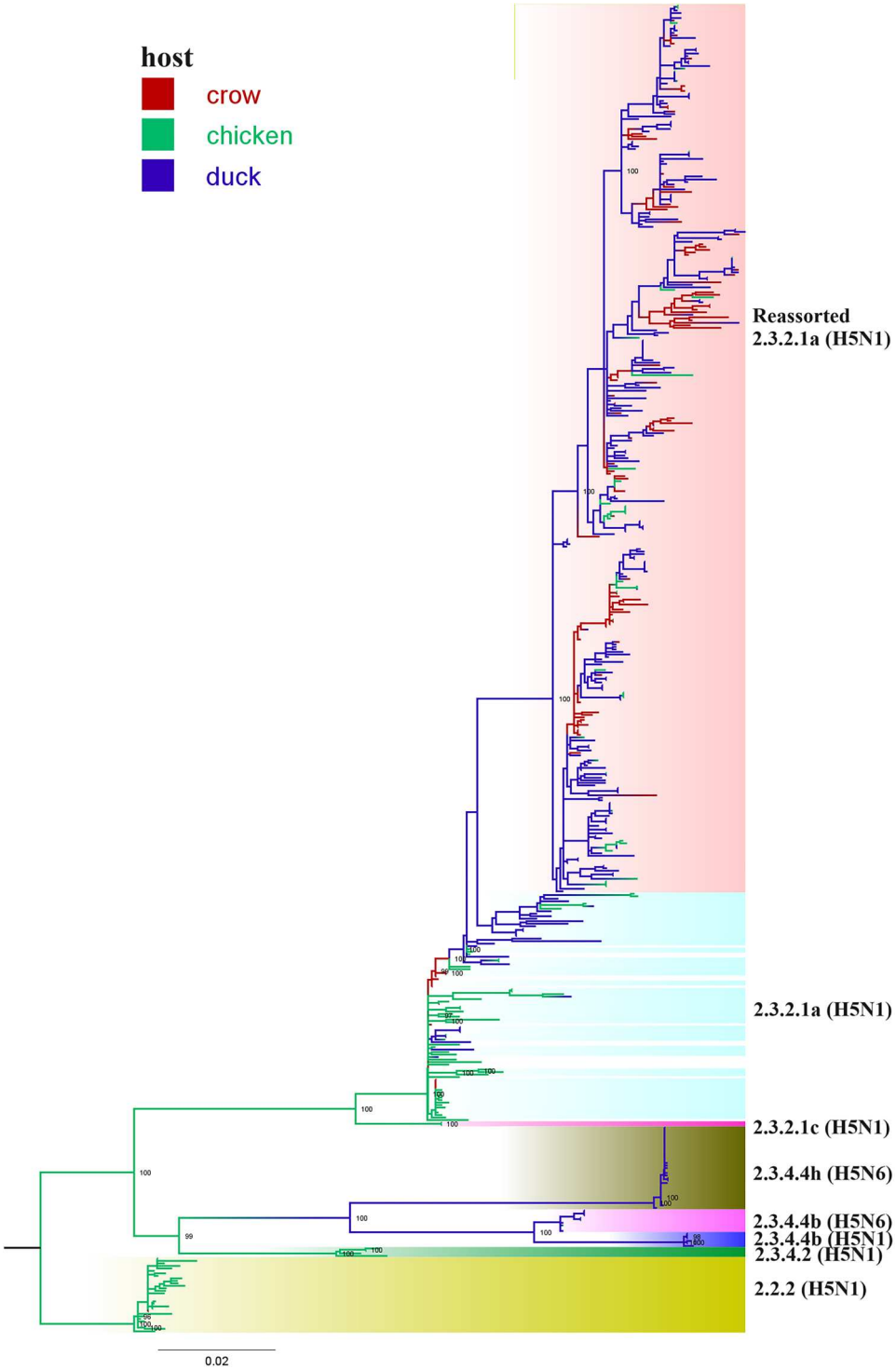

Figure 4. Maximum likelihood tree of the HA gene of HPAI H5Nx in Bangladesh from 2007-2023, depicting the various clades. While the large grouping at the top of the tree (in pink) technically still belongs to clade 2.3.2.1a, it appears quite distinct from the original 2.3.2.1.a clade, which may be due to reassortment. Scale bar indicates the number of substitutions per site. Tree was rooted against the most ancestral clade, 2.2.2. Coloured boxes indicate both HA clade and HA-NA subtype combination. Branches are coloured according to host type. Bootstrap values of the main clades are presented.

crows (Figure 7). Viral sequences were generated from both unvaccinated (sequences from 2011 to 2012) and vaccinated chickens (2012–2022), and both groups played a key role in the epidemiology of 2.3.21a. With the emergence of G9 and G10, which coincided with the start of vaccination of chickens, ducks became a primary reservoir species in Bangladesh, and we find a much larger number (0.34) of migrations from ducks, suggesting that they played a major role in the maintenance and transmission of HPAI H5N1 (Figure 7). Sequences from the house crow epizootics are interleaved with sequences from ducks in the G10 sub-group. Indeed, we found that most virus transition events into crows came from ducks (0.62) rather than chickens, indicating that the crow epizootics occur following spill-over events

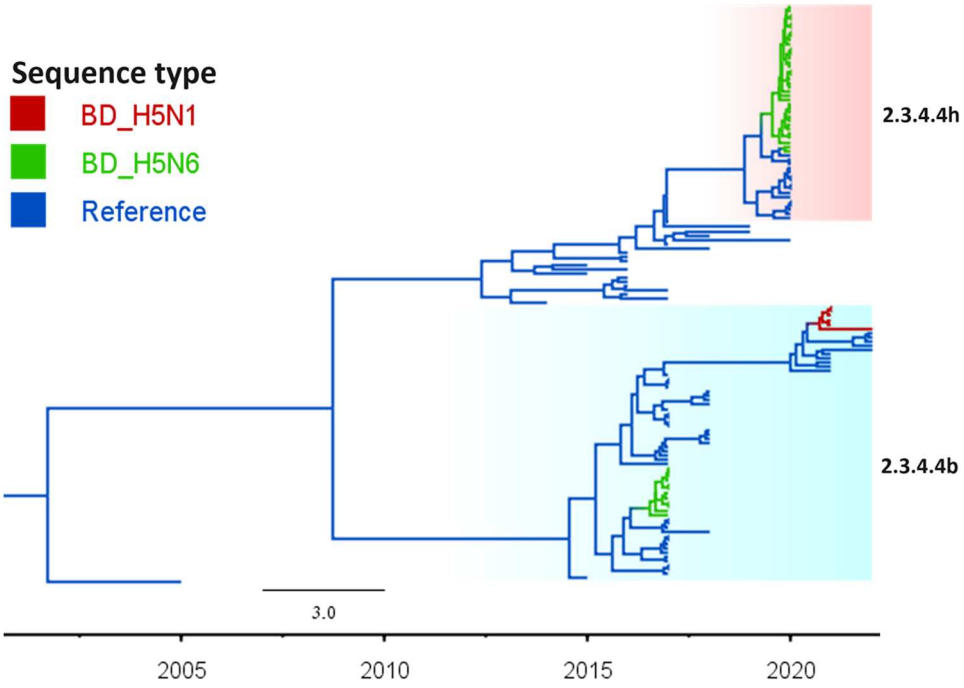

Figure 5. Bayesian phylogenetic MCC tree of HA gene of clade 2.3.4.4 H5Nx HPAIs in Bangladesh. Time scale in years. Coloured boxes indicate both HA clade and HA-NA subtype combination. Branches are coloured according to sequence clade 2.3.4.4 types. Here BD stands for Bangladesh.

from ducks (Figure 7). In addition to imports, we see several viral migrations from crows back to ducks (0.37) and chickens (0.42). Ducks had by far the longest Markov reward time, which is further evidence for long-term circulation in this host group (Figure 7, Supp Figure 1).

Discussion

Since 2011, house crow mortalities due to infection with HPAI H5N1 have been reported in Bangladesh [22]. However, the incidence of these has increased over time, and we recorded consecutive annual mortality events in winter over a period of seven years. The strong seasonality of these events is in accordance with previous reports of increased outbreak risk in poultry during winter months in Bangladesh [50]. That the crow mortalities were due to HPAI H5N1 infection was corroborated by evidence of systemic infection, with the vast majority of collected tissue samples of house crow casualties showing positive for HPAI H5N1. The infection rate amongst house crows might be higher than suggested by the number of dead and moribund birds found. In a previous study in Bangladesh, H5 antibodies were detected in apparently healthy crows, suggesting that at least some individuals recover from infection with the H5 virus [51].

Crows are scavengers and even engage in eating dead conspecifics, and have been found associated with HPAI outbreaks in Bangladesh, Germany, and Japan [22,52,53], wherein they are potentially facilitating viral transmission and aggravating enzootics through their scavenging behaviour. House crows are often found scavenging on offal in and around LBMs, which could be an important route for HPAI H5 infection, given the high prevalence of HPAI H5 found in LBMs in Bangladesh. Support of this hypothesis is the finding that there is a 99.9% nucleotide similarity between the HPAI H5Nx sequences from house crows, chickens, and ducks in Bangladesh. Additionally, the placement and the lack of clustering of host species, chickens, ducks, and crows, suggest a relatively good mixing of viruses within this host community. This is also generally supported by our Bayesian discrete trait analysis, although it did identify a vastly more important role in viral dynamics of domestic ducks compared to chickens and house crows. Indeed, viral transitions from ducks to crows and ducks to chickens were much more frequent than vice versa transitions. Our analysis also showed that the duration of time spent by the viruses in duck before transition to another host species are particularly long and suggests that the exchange rate from crows to ducks and chickens is limited.

The overall implication of our analysis that domestic ducks play such a significant role in the persistence, dissemination, evolution, and replacement of genotypes of clade 2.3.2.1a HPAI in Bangladesh, is similar to findings for HPAI H5N8 in South Korea [54]. Previous research indicated that H5-infected domestic ducks might excrete large quantities of the virus through their respiratory and digestive systems [55]. Numerous prior investigations have implicated

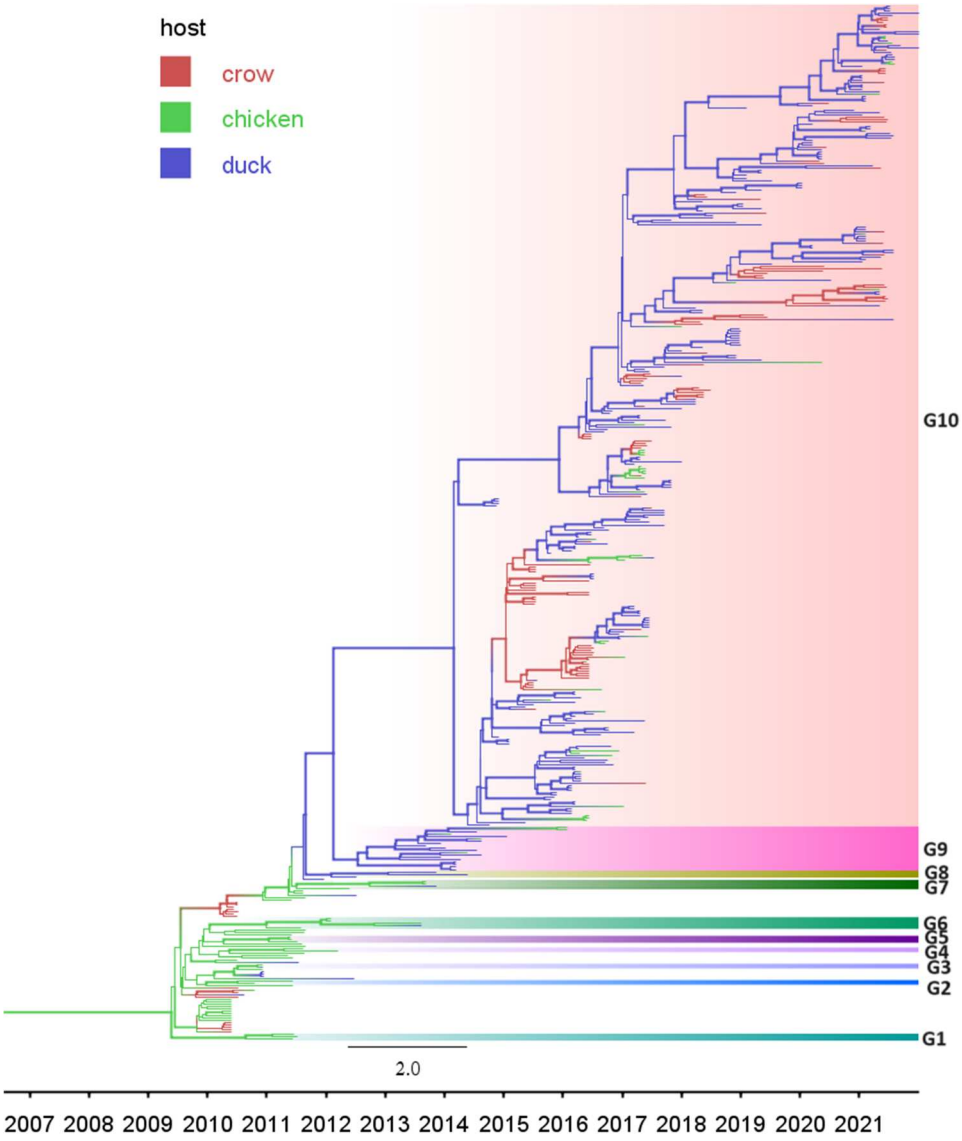

Figure 6. Time-scaled phylogenetic tree of HA sequences of clade 2.3.2.1a H5N1 HPAIs in Bangladesh. Branches are coloured according to host type and the thickness of branches indicates posterior probabilities of the ancestral host type. Genetic subgroups are identified by differently coloured boxes and marked G1-G10, as defined in Islam et al. 2023b.

domestic ducks as a significant host reservoir species for HPAI based on phylogenetic and epidemiological analysis [17,27,56]. In the Bangladesh setting, LBMs may play an important role in viral transmission between all three host species, but also poultry farms may play a significant role [57,58].

While our discrete trait analysis indicated that HPAI H5Nx is widely spread and endemic to Bangladesh, there are clear links with other countries in the region and further afield. The seven H5N1 2.3.4.4b sequences from Bangladeshi domestic ducks clustering with Japanese white-tailed eagles, Chinese domestic geese, African chickens and domestic ducks are an example in this case. Likewise, the HPAI H5N6 clade 2.3.4.4b detected in house crows, chickens, and domestic ducks in Bangladesh were related to the Middle Eastern/African cluster of clades 2.3.4.4 viruses. Finally, we also found that clade 2.3.4.4 h viruses detected in Bangladeshi domestic and wild waterfowl were clustered with viruses found in Chinese and Mongolian whooper swans. Thus AIV-wise, Bangladesh is well-connected to the rest of the world, conceivably both via wild bird movements and trade, which should be considered in its AIV mitigation strategies. For instance, the vaccination strategy in Bangladesh should induce broad-spectrum immunity to defend its avian population against both clade 2.3.2.1a and 2.3.4.4 viruses.

Although clades 2.2.2 and 2.3.4.2 HPAI viruses have disappeared in Bangladesh since the introduction of poultry vaccination in 2012, clades 2.3.2.1a viruses continue to be detected. Previous studies found that HPAI could replicate without inducing clinical signs in vaccinated chicken farms in Bangladesh, indicating that current vaccines do not provide complete protection against clade 2.3.2.1a A/H5N1 HPAIs in Bangladesh [59,60]. In addition, the H5 vaccination programme in Bangladesh has targeted commercial

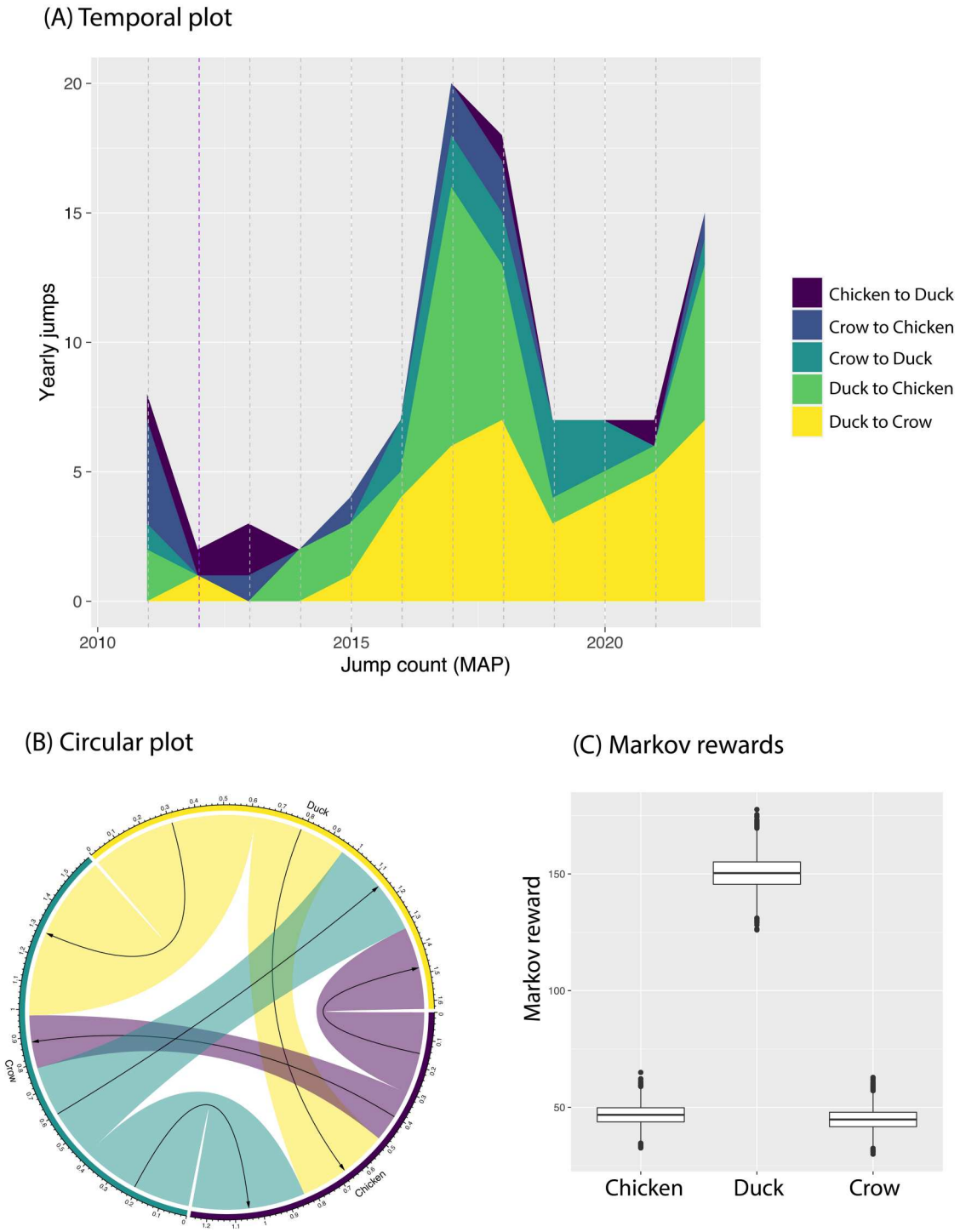

Figure 7. Visualization of the migration of Clade 2.3.2.1a H5N1 HPAI between hosts (vaccinated and unvaccinated chickens, domestic duck and free-living house crow) in Bangladesh. (A) The between-host number of migrations (based on the MAP) per year (indicated by dashed grey lines). The legend on the right describes the jump direction colours, including from chicken to duck (indigo), crow to chicken (blue), crow to duck (teal), duck to chicken (green), and duck to crow (yellow). Dashed purple line indicates the transition from unvaccinated chicken flocks (2011) to vaccinated chicken flock (2012–present). The yearly Markov jumps are plotted on the *y*-axis, and the *x*-axis represents time in years. (B) Circo splot representing the directionality of migrations between hosts, with the width of coloured bands reflecting the posterior median number of inferred migration events, and the arrows reflecting the migration direction. (C) Boxplots of the Markov rewards for chickens, domestic ducks, and free-living house crows for Clade 2.3.2.1a H5N1 HPAI, illustrating the total time spent in a particular host population.

chicken farms [60]. Backyard poultry and domestic ducks are thus not being vaccinated, while our results indicate that domestic ducks are instrumental to viral dynamics and likely also the epidemiology of HPAI viruses in Bangladesh.

In Bangladesh, vaccination has long been the principal method for controlling and preventing H5 subtype avian influenza. Although vaccines can largely prevent avian influenza outbreaks, they can also hasten the emergence of novel variants, leading to the

selection of some advantageous mutations. These positively selected mutations of viruses may be predominantly associated with so-called immune escape under vaccine selection, as well as the antigenic changes of viruses [61]. Vaccines are only available for a few clades of H5 and H7 subtype viruses, and they have been produced by only a few countries. The introduction of H5/H7 bivalent inactivated vaccine for chickens in 2017 resulted in a 93.3% decrease in the H7N9 detection rate in poultry. Additionally, only three human cases of H7N9 were reported in 2018, suggesting that the vaccination of poultry effectively eliminated human infection with the H7N9 virus [62,63]. The A/H5 vaccination was primarily targeted at commercial layer and breeder chickens, while domestic ducks were not a significant target for vaccination and biosecurity in the context of HPAI control. To mitigate the economic impact of future HPAI outbreaks, biosecurity must be reinforced [64,65], and the current vaccine strategy amended. This should include a review of the licenced vaccine's efficacy against current strains, a scheme to monitor their effectiveness against infection in vaccinated populations and an extension of vaccination to domestic ducks.

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

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Data availability

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

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