

Surveillance of avian influenza through bird guano in remote regions of the global south to uncover transmission dynamics

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Avian influenza viruses (AIVs) pose a growing global health threat, particularly in low- and middle-income countries (LMICs), where limited surveillance capacity and under-resourced healthcare systems hinder timely detection and response. Migratory birds play a significant role in the transboundary spread of AIVs, yet data from key regions along migratory flyways remain sparse. To address these surveillance gaps, we conducted a study between December 2021 and February 2023 using fresh bird guano collected across 10 countries in the Global South. Here, we show that remote, uninhabited regions in previously unsampled areas harbor a high diversity of AIV strains, with H5N1 emerging as the most prevalent. Some of these H5N1 samples also carry mutations that may make them less responsive to the antiviral drug oseltamivir. Our findings documented the presence of AIVs in several under-represented regions and highlighted critical transmission hotspots where viral evolution may be accelerating. These results underscore the urgent need for geographically targeted surveillance to detect emerging variants, inform public health interventions, and reduce the risk of zoonotic spillover.

Recent global trends show a marked rise in highly pathogenic avian influenza (HPAI) outbreaks, especially among wild birds, with increasing crossover to mammals and humans^{1,2}. The continuous adaptation and mutation of avian influenza subtypes underscore the urgent need for improved monitoring and early detection systems. However, the threat is exacerbated in low- and middle-income countries (LMICs), where healthcare systems may be less equipped to handle the evolving threat² and surveillance programs are scarce.

To assess the potential of environmental surveillance in capturing AIV transmission dynamics, particularly within LMICs, we conducted a field-based surveillance study from December 2021 to February 2023. Fresh bird guano was collected from remote, uninhabited regions across 10 countries (Indonesia, Madagascar, the Maldives, Mozambique, Myanmar, Papua New Guinea, the Philippines, Somalia, Sri Lanka, and Yemen) in Global South, offering a unique lens into viral circulation beyond traditional monitoring systems. These locations were situated along major bird migration flyways: East-Asian Australasian Flyway (EAAF), Asian East-African Flyway (AEF), Central Asian Flyway

(CAF), Mediterranean & Black Sea Flyway (MBSF), and East Atlantic Americas Flyway (EAAF)³ (Fig. 1a).

Results

The results indicated that 1.33% (360/27036) of bird guano samples contained AIV RNA. The analysis of AIV positive sample percentages across sampling countries revealed notable geographic variations. In Myanmar, AIV positive sample percentages ranged from 0.90% to 1.76%, with the highest values recorded in Maungmagan and Taungywa. Papua New Guinea exhibited generally lower AIV positive sample percentages, mostly under 1%, with Bonvouloir Islands showing the highest at 1.04% and Watom Island the lowest at 0.38%. In Indonesia, values were moderate, ranging from about 0.40% in the Taka Bonerate Islands to 0.79% in sites such as Boo, Bulan, and Badas Islands. The Philippines displayed slightly higher AIV positive sample percentages, reaching up to 1.79% in Tumaguin, with several other locations like Manlot and Tanguingui Island also exceeding 1.4%. In stark contrast, the highest percentage of AIV positive samples were observed in

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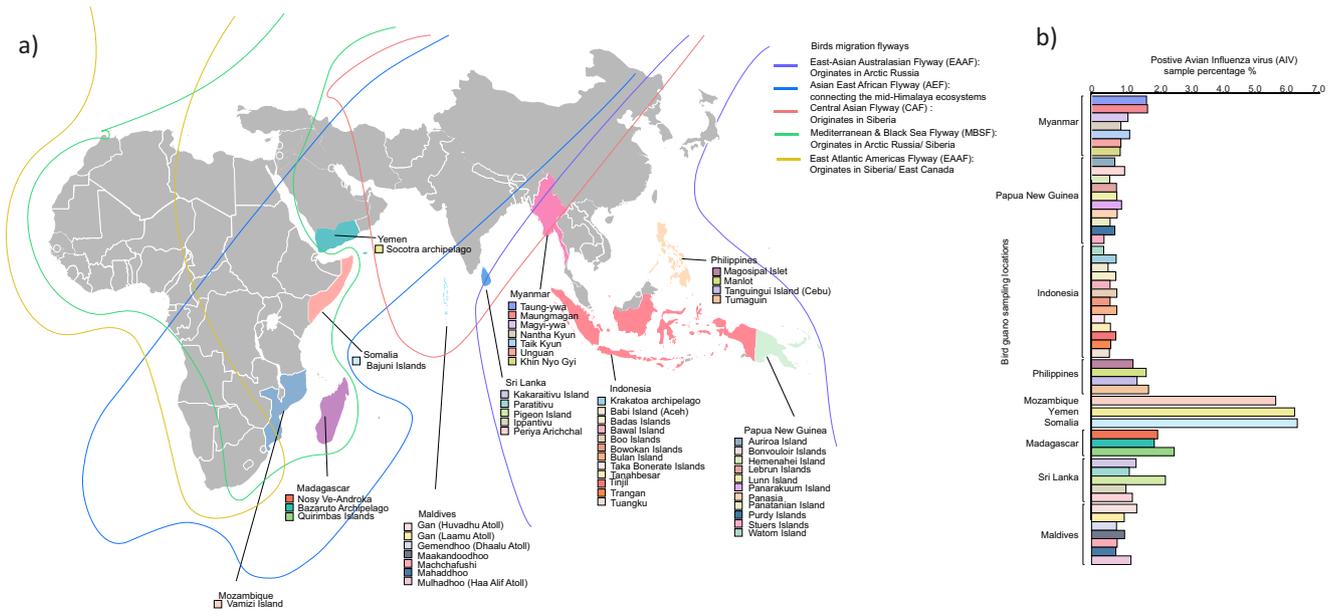


Fig. 1 | Avian influenza surveillance using bird guano in the Global South. **a** Map of sampling countries with bird migration flyways (Papua New Guinea ($n = 5792$), Sri Lanka ($n = 2657$), Indonesia ($n = 6205$), Yemen ($n = 503$), Myanmar ($n = 3709$), Philippines ($n = 2130$), Mozambique ($n = 502$), Madagascar ($n = 1540$), Somalia

($n = 512$), and Maldives ($n = 3486$)). **b** Percentage of avian influenza virus (AIV)-positive samples collected from different bird guano sampling locations. Source data are provided as a Source Data file.

Eastern Africa and the Arabian Peninsula—particularly in Somalia’s Bajuni Islands (6.45%), Yemen’s Socotra Archipelago (6.36%), and Mozambique’s Vamizi Island (5.78%). Madagascar also had locations with elevated AIV positive sample percentages, notably in the Quirimbas Islands (2.59%). In South Asia, locations in Sri Lanka and the Maldives had moderate AIV positive sample percentages, ranging from just over 1% to 2.31% on Pigeon Island. The study areas are remote rural locations, often on uninhabited islands, small fishing communities, or indigenous tribal settlements that rely on hunting and gathering, with no history of poultry farming. Several of these regions are also affected by ongoing conflict and instability, which further limits access to health infrastructure and hinders routine disease surveillance.

AIV RNA concentrations in bird guano exhibited distinct spatio-temporal patterns across the studied countries, reflecting clear seasonal fluctuations (Fig. 2a). A Kruskal–Wallis test confirmed significant variation in viral concentrations across the four seasonal periods ($H = 56.05$, $p < 0.0001$), suggesting non-random temporal dynamics in AIV burden. Pairwise Mann–Whitney U tests revealed that December–February 2021–2022 had significantly lower concentrations compared to April–June 2022 ($p = 0.0170$), August–October 2022 ($p = 0.0485$), and December–February 2022–2023 ($p < 0.0001$). While no significant difference was found between April–June and August–October 2022 ($p = 0.5145$), viral levels rose sharply in December–February 2022–2023 compared to both previous periods ($p < 0.0001$ for both).

Country-level pairwise comparisons revealed substantial spatial heterogeneity in AIV RNA concentrations across all seasons. In December–February 2021–2022, Myanmar differed significantly from Yemen ($p = 0.0028$), Madagascar ($p = 0.0003$), and Maldives ($p = 0.0004$), while Papua New Guinea diverged from Indonesia ($p = 0.0312$), Yemen ($p = 0.0091$), Madagascar ($p = 0.0013$), Sri Lanka ($p = 0.0452$), and Maldives ($p = 0.0017$). Indonesia also showed marked differences from the Philippines ($p = 0.0072$), Mozambique ($p = 0.0071$), Yemen ($p = 0.0006$), Madagascar ($p = 0.0002$), and Maldives ($p = 0.0004$). During April–June 2022, significant differences persisted between Myanmar and Indonesia ($p = 0.0120$), Yemen ($p = 0.0055$), Sri Lanka ($p = 0.0008$), and Maldives ($p = 0.0001$). Papua

New Guinea also differed from Indonesia ($p = 0.0399$), Yemen ($p = 0.0085$), Sri Lanka ($p = 0.0045$), and Maldives ($p = 0.0023$). Other notable comparisons included Indonesia vs. Maldives ($p = 0.0176$) and Philippines vs. Maldives ($p = 0.0424$).

In August–October 2022, RNA concentrations in bird guano samples collected in Myanmar differed significantly from Indonesia ($p = 0.0046$), Yemen ($p = 0.0028$), and Somalia ($p = 0.0311$). Papua New Guinea also showed significant variation from Indonesia ($p = 0.0065$), Yemen ($p = 0.0039$), and Somalia ($p = 0.0448$). Indonesia diverged from Sri Lanka ($p = 0.0058$) and Maldives ($p = 0.0101$), while Yemen showed differences from Sri Lanka ($p = 0.0028$) and Maldives ($p = 0.0050$). Somalia also differed significantly from Sri Lanka ($p = 0.0072$) and Maldives ($p = 0.0255$). By December–February 2022–2023, fewer but still statistically significant differences remained, including Papua New Guinea vs. Indonesia ($p = 0.0061$) and vs. Mozambique ($p = 0.0011$). Mozambique differed significantly from Somalia ($p = 0.0437$) and Sri Lanka ($p = 0.0251$).

Temporal shifts in AIV RNA concentrations revealed considerable inter-seasonal variability. Between December–February 2021–2022 and April–June 2022, Madagascar (+274.09%), Yemen (+96.79%), and the Philippines (+79.32%) experienced the largest increases. Myanmar, Papua New Guinea, and the Maldives also showed moderate gains, while Indonesia (−25.85%), Sri Lanka (−46.75%), and Somalia (−13.83%) exhibited declines. The August–October 2022 period marked widespread decreases, especially in Yemen (−40.84%), Indonesia (−37.28%), and Madagascar (−28.92%). In contrast, Sri Lanka (+96.34%) and the Maldives (+180.90%) displayed strong rebounds. The final period, December–February 2022–2023, showed renewed increases in Indonesia (+134.07%), Yemen (+149.85%), Madagascar (+105.45%), and Somalia (+96.04%), while Maldives recorded a minor decline (−0.47%) following its earlier surge.

Eight of ten countries reached their highest mean AIV RNA concentrations during December–February 2022–2023, including Myanmar, Papua New Guinea, Indonesia, Philippines, Yemen, Somalia, Madagascar, and Sri Lanka. Mozambique peaked earlier in April–June 2022, while the Maldives recorded its highest concentrations in August–October 2022. The coefficient of variation (CV) across seasons

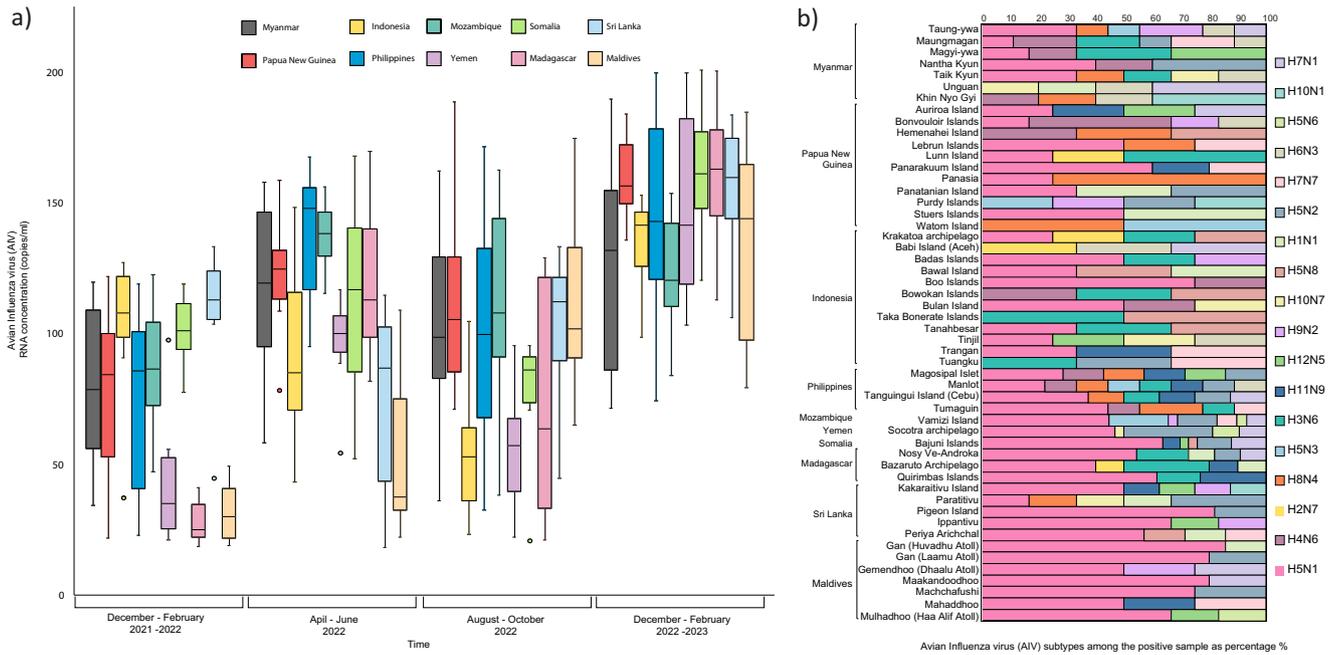


Fig. 2 | Avian influenza surveillance using bird guano in the Global South. **a** The boxplot of AIV RNA concentrations (copies/ml) in positive bird guano samples Papua New Guinea ($n = 41$), Sri Lanka ($n = 38$), Indonesia ($n = 40$), Yemen ($n = 32$), Myanmar ($n = 45$), Philippines ($n = 33$), Mozambique ($n = 29$), Madagascar ($n = 34$), Somalia ($n = 33$), and Maldives ($n = 35$). The lower and upper boundaries of the box (interquartile) represent the 25th and the 75th percentile, respectively. The line

within the box corresponds to the median, while the whiskers indicate the highest and the lowest AIV RNA copy values, except for the outliers that are represented by the rounds outside the whiskers. **b** Distribution of avian influenza virus subtypes among the positive samples Papua New Guinea ($n = 41$), Sri Lanka ($n = 38$), Indonesia ($n = 40$), Yemen ($n = 32$), Myanmar ($n = 45$), Philippines ($n = 33$), Mozambique ($n = 29$), Madagascar ($n = 34$), Somalia ($n = 33$), and Maldives ($n = 35$)).

highlighted substantial variability in viral dynamics. The Maldives (CV = 0.620), Madagascar (0.583), and Yemen (0.497) showed the highest seasonal fluctuations, suggesting episodic amplification or transient peaks. Moderate variability was observed in Indonesia (0.341), Sri Lanka (0.340), and Somalia (0.319). In contrast, Myanmar (0.194), Mozambique (0.199), and Papua New Guinea (0.266) demonstrated more stable, consistent viral levels across the study period.

H5N1 was the most frequently detected AIV subtype across surveyed sites (Fig. 2b), with the highest detection, with particularly elevated rates observed in Gan (Huvadhu Atoll) (Maldives, 85.7%), Pigeon Island (Sri Lanka, 81.8%), and Gan (Laamu Atoll) and Maakandoodhoo (Maldives, both 80.0%). Additional high-prevalence H5N1 detections were recorded in Boo Islands (Indonesia, 75.0%), Machchafushi (Maldives, 75.0%), and Ippantivu (Sri Lanka, 66.7%).

H1N1 reached its peak prevalence in Stuers Islands (Papua New Guinea, 50.0%), while H2N7 was most frequently detected in Babi Island (Aceh) (Indonesia, 33.3%). H3N6 and H4N6 both showed highest prevalence of 50.0% in Lunn Island (Papua New Guinea) and Bonvouloir Islands (Papua New Guinea), respectively. For H5N3, the highest prevalence was observed in Watom Island (Papua New Guinea, 50.0%), and for H5N6, it was Mulhadhoo (Haa Alif Atoll) (Maldives, 16.7%). H5N8 was most prevalent in Taka Bonerate Islands (Indonesia, 50.0%), while H6N3 reached its highest level in Babi Island (Aceh) (Indonesia, 33.3%). H7N1 was most prominent in Unguan (Myanmar, 40.0%), and H7N7 in Trangan (Indonesia, 33.3%). The top site for H8N4 was Panasia (Papua New Guinea, 75.0%), and for H9N2, it was Purdy Islands (Papua New Guinea, 25.0%). H10N1 was most frequently detected in Khin Nyo Gyi (Myanmar, 40.0%), and H10N7 in both Bulan Island and Tinjil (Indonesia, each 25.0%). H11N9 showed its highest detection in Trangan (Indonesia, 33.3%), while H12N5 was most prevalent in Magyi-ywa (Myanmar, 33.3%). Using a normalization approach based on subtype richness per positive sample, Papua New Guinea exhibited the highest subtype diversity, detecting approximately 0.39 subtypes per positive sample, followed by Indonesia (0.35) and the Philippines (0.33)

(Fig. 2b). In contrast, countries such as Yemen (0.16) and Somalia (0.18) displayed lower subtype-per-sample ratios, which may reflect more uniform subtype circulation or differences in sampling coverage and sensitivity.

The phylogenetic trees reveal diverse clustering patterns among the sampled countries for both the HA and NA genes, showing close relationships with other geographical clusters from Asia, the Middle East, Europe, Africa, and North and South America (Figs. 3a-i and 4a-h). These clustering patterns also align with major bird migration pathways, reflecting spatial variations in AIV transmission dynamics. Sequences from the Maldives, Sri Lanka, and Myanmar exhibit clear clustering with those from Madagascar, Somalia, Yemen, and Mozambique, as these countries overlap with key bird migration routes (EAAF, AEF, CAF, MBSF, and EAAF) (Figs. 1, 3a-i, and 4a-h). Samples from Madagascar, Yemen, Mozambique, and Indonesia contained multiple subtypes. There is a notable diversity within the H3N6, H1N1, H5N1, and H7N9 subtypes in bird guano analyzed in this study.

H5N1 was dominant across multiple regions, including the Maldives, Sri Lanka, Madagascar, Somalia, Yemen, Mozambique, Philippines, Indonesia, and Papua New Guinea (Figs. 3a-i and 4a-h). Other subtypes, such as H5N6, H5N8, H5N2, and H5N3, were also detected alongside H5N1. The majority of H5N1 detections (2021–2023) were classified under Clade 2.3.4.4b which is similar to the strain currently circulating in dairy cattle in the United States and other wild bird or poultry samples from Asia, Middle East, Europe, Africa, North and South America (Figs. 3a-i and 4a-h). Interestingly, H5N1-positive samples from the Maldives, Sri Lanka, Somalia, Yemen, and Papua New Guinea in 2021, 2022, and 2023 showed a close relationship to sequences currently circulating in dairy cattle in the United States in 2024. Clade 2.3.4.4h, particularly associated with H5N2 and H5N3, was primarily found in Yemen, Myanmar, Papua New Guinea, and the Philippines. H3N6 was detected across several clades, with samples from Myanmar classified within Clades 3C.3a, 3C, and 3C.3a1. Samples from Lunn Island in Papua New Guinea, Clades 3C and 3C.3a1 were detected.

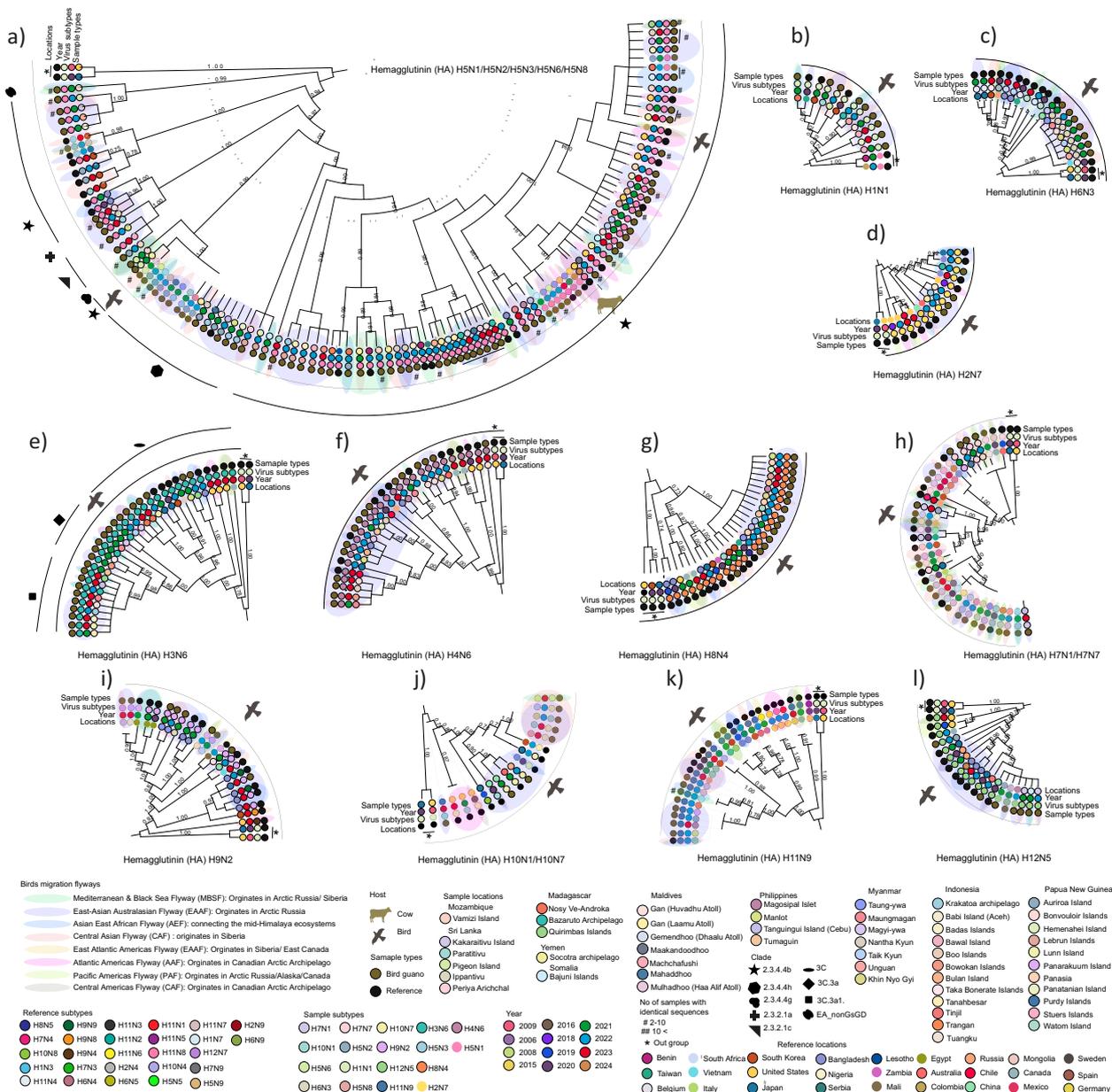


Fig. 3 | Phylogenetic analysis of avian influenza virus (AIV) hemagglutinin (HA) gene sequences obtained from bird guano samples, alongside reference sequences from GISAID. Panels show phylogenies for the following HA subtypes: a H5, b H1, c H6, d H2, e H3, f H4, g H8, h H7, i H9, j H10, k H11, and l H12. Evolutionary history was inferred using the Maximum Likelihood method and the

Hasegawa-Kishino-Yano substitution model for the hemagglutinin (HA) and neuraminidase (NA) genes. The trees with the highest log likelihood are shown. Next-clade (<https://clades.nextstrain.org>) were used for clade assignment, mutation calling and quality control for viral genomes.

Samples from Indonesia, exhibited clustering in Clade 3C.3a1, while samples from the Philippines and Madagascar were clustered in Clades 3C and 3C.3a1. We could not find any specific prior reports from the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) about AIV in Sri Lanka, Myanmar, Papua New Guinea, Mozambique, Madagascar, Yemen, Somalia, and the Maldives (www.offlu.org). However, the Philippines reported its first outbreak of highly pathogenic H5N2 bird flu among backyard ducks in January 2025⁵. Notably, an H5N2 subtype was detected in our dataset nearly two years earlier on Tanguingui Island (Cebu), Philippines (Fig. 3a). Additionally, H5N1-positive samples from the Bajuni Islands (Somalia), Socotra Archipelago (Yemen), and Maadhadhoo (Maldives) were found to carry the H275Y mutation in the neuraminidase (NA) segment, a known substitution associated with reduced sensitivity to neuraminidase inhibitors such as oseltamivir.

The analysis of hemagglutinin (HA) subtypes and their respective protease cleavage site consensus sequences revealed diverse patterns among different subtypes (Supplemental Table 1). For H1N1 and H2N7, 100% of sequences possessed the PSIQR/GLF and PQIESR/GLF cleavage sites, respectively. H3N6 showed two cleavage site variants: 52.17% of sequences had PEKQTR/GLF, while 47.83% carried PEKQTR/GLF, corresponding to clades 3C/3C.3a and 3C.3a1, respectively. H4N6 and H6N3 sequences were uniform, with 100% showing PEKASR/GLF and PQIKTR/GLF, respectively. H7N1 sequences showed variation, with 77.78% possessing PEPKGR/GLF, and 11.11% each with PEIPKGR/GLF and PEPKGRPRFR/GLF. H7N7 also exhibited heterogeneity, with 54.55% of sequences carrying PEIP-GKREK/GLF and 45.45% with PEIPKGR/GLF. All H8N4 sequences (100%) had the PSIEPK/GLF motif. Among H9N2 sequences, cleavage site diversity was evident: 55.56% carried PARSSR/GLF, 22.22% had PAKSKR/GLF, while PAASDR/GLF and

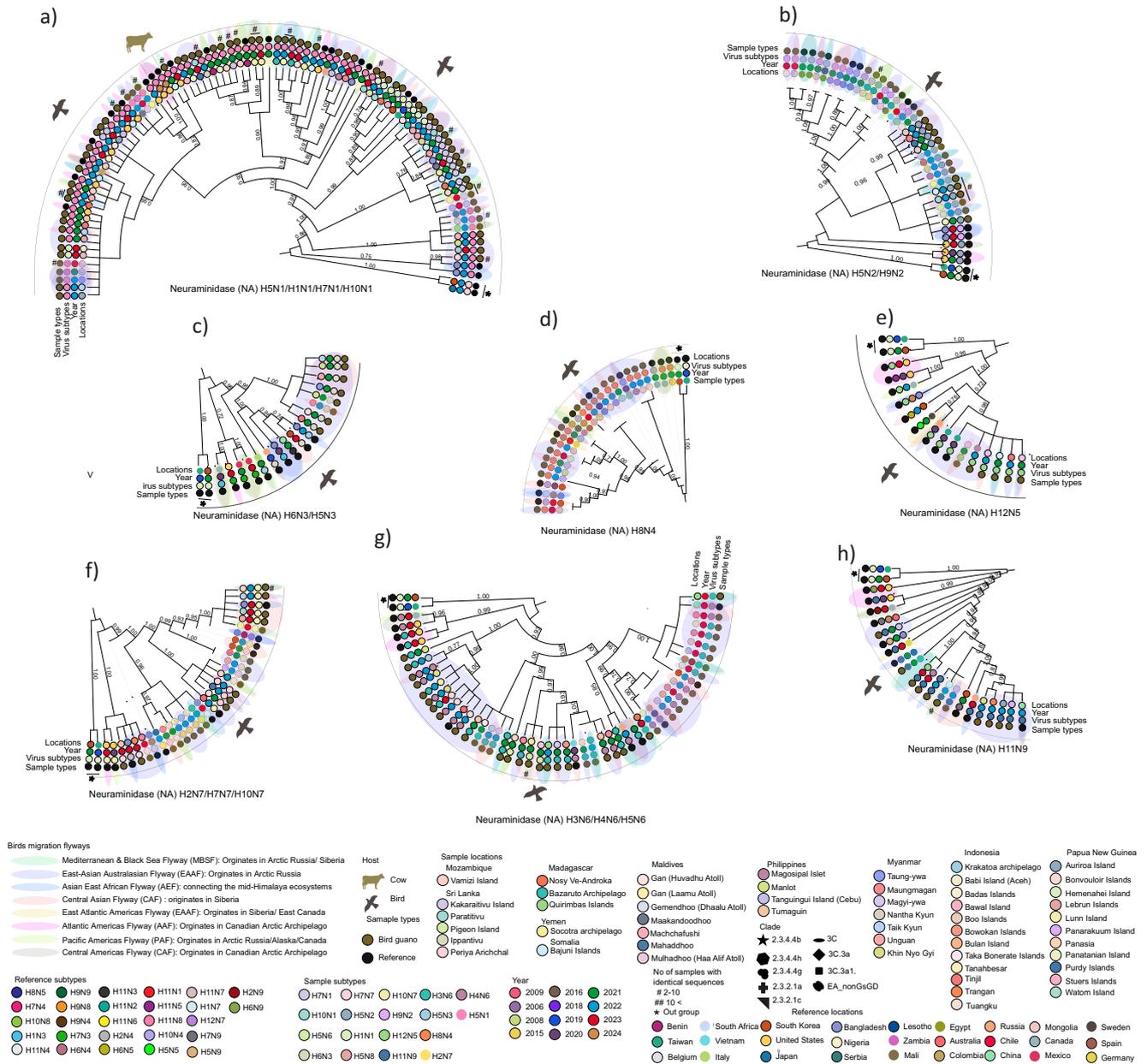


Fig. 4 | Phylogenetic analysis of avian influenza virus (AIV) neuraminidase (NA) gene sequences obtained from bird guano samples, alongside reference sequences from GISAID. Panels show phylogenies for the following NA subtypes: a N1, b N2, c N3, d N4, e N5, f N7, g N6, and h N9. Evolutionary history was inferred

using the Maximum Likelihood method and the Hasegawa-Kishino-Yano substitution model for the hemagglutinin (HA) and neuraminidase (NA) genes. The trees with the highest log likelihood are shown. Nextclade (<https://clades.nextstrain.org>) were used for clade assignment, mutation calling, and quality control for viral genomes.

HARSSR/GLF were each found in 11.11% of sequences. H10N1 showed equal proportions of PELMQGR/GLF (50%) and PEVVQER/GLF (50%), while H10N7 sequences were composed of 66.67% with PEVVQER/GLF and 33.33% with PEVVQGR/GLF. All H11N9 sequences carried either PAIASR/GLF (100%). A H12N5 sequence (100%) displayed the PQVQNR/GLF cleavage motif. Among H5 subtypes, H5N1 exhibited high diversity in cleavage site motifs: 68.79% had PLREKRRKR/GLF (2.3.4.4b), 21.02% had PLRERRRKR/GLF (2.3.4.4b), 8.92% had PQRERRRKR/GLF (2.3.2.1c), 0.64% had PQRETR/GLF (EA_nonGsGD), and another 0.64% subset had PQKKGRRKR/GLF (2.3.4.4g). H5N2 sequences showed PLRERRRKR/GLF in 76.47%, while 23.53% had PLREKRRKR/GLI (2.3.4.4h). H5N3 sequences carried the PLRERRRKR/GLF motif (100%), with clades 2.3.4.4h, and the 100% H5N6 sequences also carried the PLRERRRKR/GLF motif, aligning with 2.3.4.4g/2.3.4.4b. Lastly, all H5N8 sequences (100%) contained the PLREKRRKR/GLF motif (clade 2.3.4.4b).

Discussion

This study identifies critical sites like Madagascar, Somalia, Yemen, Mozambique, Sri Lanka, Myanmar, and the Maldives along five major bird migration routes (EAAF, AEF, CAF, MBSF, and EAAF)³, which facilitate the spread of AIV across continents⁵. Moreover, the consistent presence of H5N1 in ecologically critical sites underscores its ongoing circulation and potential adaptation in wild bird populations across tropical and subtropical zones. This pattern reinforces global concerns about H5N1's expanding ecological footprint and the threats it poses to biodiversity and public health, especially along migratory bird pathways. The higher subtype richness per sample in Papua New Guinea, Indonesia, and the Philippines suggests a more heterogeneous viral population, potentially driven by diverse ecological niches, host species, or transmission interfaces. This diversity could reflect increased opportunities for viral reassortment or emergence of novel

strains. Conversely, lower subtype richness in Yemen and Somalia may indicate more stable or homogenous transmission patterns. These regions serve as convergence zones for migratory birds from different geographical origins, potentially increasing the probability of exchange and reassortment of viral strains³. This highlights the importance of considering both ecological dynamics and avian movement patterns when interpreting patterns of viral diversity across sites. Several high-risk wild bird species contribute to avian influenza transmission across Myanmar, Papua New Guinea, Indonesia, the Philippines, Mozambique, Yemen, Somalia, Madagascar, Sri Lanka, and the Maldives. Waterfowl, including the Lesser Whistling Duck (*Dendrocygna javanica*) (Myanmar, Indonesia, Philippines, Sri Lanka, Madagascar), Northern Pintail (*Anas acuta*) (found in all sampled countries), Garganey (*Spatula querquedula*) (migratory across all these regions), and Pacific Black Duck (*Anas superciliosa*) (Papua New Guinea, Indonesia, Philippines), are known carriers of H5N1 and H9N2 and frequently mix with domestic ducks in flooded rice fields and wetlands, especially in rural farming communities^{5,6}. Shorebirds and waders, such as the Common Sandpiper (*Actitis hypoleucos*) (found in all sampled countries), Great Knot (*Calidris tenuirostris*) (Indonesia, Philippines, Myanmar), and Whimbrel (*Numenius phaeopus*) (long-distance migrant across all these regions), follow major flyways like the East Asian-Australasian, Central Asian, and East African flyways, stopping at key wetlands, rice paddies, and coastal lagoons in these countries, where they may transmit AIV to local poultry and wild bird populations^{5,6}. Gulls and terns, including the Black-headed Gull (*Chroicocephalus ridibundus*) (Myanmar, Sri Lanka, Madagascar, Yemen) and Whiskered Tern (*Chlidonias hybrida*) (Indonesia, Philippines, Myanmar, Sri Lanka, Madagascar, Yemen), frequently scavenge at fish markets and coastal areas in low-income fishing communities, increasing disease spillover risks between wild birds, poultry, and humans⁶. Herons, egrets, and ibises, such as the Little Egret (*Egretta garzetta*) (all listed countries) and Glossy Ibis (*Plegadis falcinellus*) (Mozambique, Madagascar, Myanmar, Sri Lanka), inhabit rice paddies, irrigation canals, and marshlands, further intertwining wild and domestic bird populations⁵⁻⁷. Birds of prey, including the Brahminy Kite (*Haliastur indus*) (Indonesia, Philippines, Papua New Guinea, Myanmar, Sri Lanka, Madagascar) and Eastern Marsh Harrier (*Circus spilonotus*) (Myanmar, Indonesia, Philippines, Madagascar), scavenge infected bird carcasses, sustaining AIV in both natural and human-modified landscapes⁶. Meanwhile, urban-adapted species like the Common Myna (*Acridotheres tristis*) (Myanmar, Sri Lanka, Maldives, Madagascar, Mozambique), Eurasian Tree Sparrow (*Passer montanus*) (Myanmar, Indonesia, Philippines, Sri Lanka, Mozambique), and House Crow (*Corvus splendens*) (Sri Lanka, Maldives, Myanmar, Mozambique, Yemen, Madagascar) spread the virus in small village communities, informal markets, and backyard poultry farms, particularly where poultry waste, open food sources, and live bird trade create ideal conditions for viral persistence and mutation⁵⁻⁷.

Fluctuations in AIV RNA concentrations appear to be shaped by a complex interplay of seasonal migration patterns, environmental conditions, and avian community composition. The temporal dynamics revealed a biphasic pattern, with two distinct periods of heightened viral activity: one centered around April–June and the other during December–February. These peaks likely correspond to seasonal ecological triggers, such as rainfall, food availability, and the congregation of migratory birds which are especially relevant in tropical and subtropical ecosystems². This finding reinforces the importance of adopting regionally tailored seasonal frameworks in surveillance efforts, rather than applying temperate-biased assumptions.

Notably, the observed increases in AIV concentrations in countries like Madagascar, Yemen, and the Philippines between December–February 2021–2022 and April–June 2022, followed by widespread declines in August–October 2022, point toward episodic amplification events rather than continuous circulation. These brief

but intense surges in viral burden suggest that transmission is likely driven by ecological pulses such as migratory staging or breeding events rather than persistent endemicity. The December–February 2022–2023 period marked another significant resurgence, with eight of ten countries recording their highest concentrations during this time, further supporting the role of seasonal ecological dynamics in viral transmission.

Spatial heterogeneity was also pronounced, with countries showing divergent patterns of increase and decline across seasons. This reflects localized risk factors such as habitat type, species diversity, climate, and proximity to migratory flyways^{2,5}. For example, countries positioned along key migratory corridors or at ecological convergence zones such as Yemen, Madagascar, and Sri Lanka tended to show higher variability and sharper seasonal peaks. Interestingly, AIV concentrations were lowest during August–October 2022, despite its alignment with major migratory movements in the EAAF, AEF, CAF, and MBSF. This unexpected dip may reflect a temporal lag between bird arrival and viral shedding, low bird densities, or suboptimal environmental conditions⁵ (e.g., temperature, humidity, and UV exposure), for virus persistence and detection during this period. Moreover, many sampling sites were coastal or island habitats, where seawater exposure may inactivate AIV more rapidly than in freshwater environments⁵. Collectively, these findings underscore the dynamic, non-linear nature of AIV transmission in tropical and subtropical regions and highlight the need for flexible, frequent, and ecologically informed surveillance strategies.

Interestingly, we show that AIV RNA concentrations were highest during December–February 2022–2023, surpassing levels recorded in the same period of 2021–2022. This peak occurred before the widespread surge in avian influenza cases reported globally since 2023 until the present⁷⁻⁹. The subsequent global spread of the virus led to infections across multiple species, including wild and domestic birds, mammals, and even cases of spillover to humans⁷⁻⁹. Moreover, although the Philippines officially reported its first H5N2 outbreak in backyard ducks in January 2025⁴, our data indicate that the virus may have been present in the region much earlier. This highlights potential gaps in existing surveillance efforts, where early circulation of AIV can go undetected. The observed pattern highlights the importance of continuous environmental surveillance in underrepresented regions to improve the detection of AIV circulation and mitigate the impact of avian influenza on both wildlife and public health⁷. The observed diversity in cleavage site sequences across different HA subtypes reflects the evolutionary strategies of AIV in adapting to various hosts and environmental conditions¹⁰. The presence of polybasic motifs in several H5 and H7 sequences raises concerns regarding the potential emergence of highly pathogenic strains, particularly in regions where domestic poultry and migratory birds coexist^{7,10}. The detection of these subtypes along multiple migratory flyways highlights the need for enhanced surveillance and genetic monitoring in this region.

Additionally, the detection of the H275Y mutation in H5N1-positive samples from the Bajuni Islands (Somalia), Socotra Archipelago (Yemen), and Maakandoodhoo (Maldives) raises critical concerns about the potential emergence of antiviral resistance in avian influenza viruses circulating in the wild. This mutation in the neuraminidase (NA) gene is associated with reduced sensitivity to oseltamivir, one of the few antiviral treatments available for severe influenza cases¹¹⁻¹³. Notably, these locations lie along major migratory bird flyways (CAF, MBSF, AEF and EAAF) and serve as key stopover points where birds from different regions intermingle. The convergence of high H5N1 prevalence, seasonal viral amplification, and resistance-associated mutations in these ecologically strategic sites underscores the pressing need for sustained surveillance. Strengthening monitoring efforts in such settings is essential not only for local public and animal health but also for anticipating the transboundary spread of drug-resistant AIV strains.

Additionally, the expansion of mining, logging, and infrastructure projects in Indonesia, Papua New Guinea, Madagascar, and

Mozambique is pushing human settlements into remote forested regions and coastal islands, areas previously dominated by wild birds and migratory waterfowl. The increasing demand for nickel, cobalt, and rare earth metals to support the global electric vehicle (EV) revolution has led to rapid deforestation, wetland destruction, and the creation of artificial reservoirs and open-pit mines, which attract waterfowl and scavenging birds¹⁴. As workers, migrants, and displaced rural communities settle near these industrial sites, human-wildlife contact intensifies, increasing the risk of avian influenza spillover into domestic poultry and human populations^{7,14}.

At the same time, conflict zones in Myanmar, Somalia, and Yemen exacerbate the pandemic potential of HPAI. Ongoing civil unrest, displacement, and food insecurity have led to unregulated poultry trade, overcrowded refugee settlements, and weakened veterinary and public health infrastructure, all of which accelerate the risk of AIV transmission between humans, poultry, and wild birds¹⁵. In war-torn areas, military blockades, breakdowns in sanitation, and reliance on informal food sources force many communities to hunt wild birds or consume poorly regulated poultry, creating conditions for viral reassortment and the emergence of new AIV strains. Additionally, migratory birds that stop in these conflict zones, such as the Northern Pintail, Whimbrel, and Black-headed Gull, may act as silent carriers⁵, facilitating the long-distance spread of AI across unstable and poorly monitored regions.

This study provides some of the earliest available AIV surveillance data from countries like Sri Lanka, Myanmar, Papua New Guinea, Mozambique, Madagascar, Yemen, Somalia, and the Maldives. The presence of HPAI subtypes^{1,9,16,17} like H5N1 in these regions highlights the need for targeted surveillance in migratory zones to mitigate spillover risks. A study conducted between 2006 and 2019, involving serological surveys of unvaccinated domestic ducks in Myanmar, revealed persistent yet intermittent circulation of H5 avian influenza viruses, even in years and regions without reported outbreaks¹⁸. This suggests silent or subclinical virus circulation in Myanmar and aligns with our findings. The diversity of detected clades, especially for H3N6 and H5N1, underscores the importance of global surveillance in tracking the spread and evolution of dominant and emerging subtypes². One of the key aims of this study was to highlight the glaring inequities in global infectious disease surveillance. Current systems are disproportionately concentrated in developed regions or limited to select locations or large-scale poultry farms, leaving vast areas of high zoonotic potential under-monitored. Our study spans a large geographical area with civil conflicts, ethnic strife, and political instability, providing a comprehensive overview of influenza virus diversity and spread in regions historically excluded from global surveillance networks. This study emphasizes the importance of these regions and demonstrates their role in shaping global AIV epidemiological patterns. While we acknowledge the limitations of only obtaining HA and NA gene sequences, the logistical and technical challenges of fieldwork in these remote settings often necessitate prioritization of the most epidemiologically informative genes. The HA and NA segments remain critical for monitoring vaccine strain efficacy and antigenic drift, which ensures the data retains its practical value despite the absence of complete genomes.

While this study highlights the potential role of tropical and subtropical regions in AIV detection, we caution against interpreting these areas as singular sources of viral emergence. AIV circulation is shaped by complex, multidirectional migratory and ecological dynamics, and no region should be framed as solely responsible for downstream outbreaks. Our goal is not to attribute origin or blame but rather to emphasize the value of equitable and regionally tailored surveillance systems, particularly in regions that have been historically under-monitored despite lying along key avian flyways. Strengthening global surveillance capacity is a shared priority that benefits all regions.

In conclusion, by incorporating targeted environmental surveillance in these high-risk locations, as demonstrated in this study, the

detection of novel AIV subtypes can be achieved, ensuring timely global awareness and preparedness for emerging threats. The high inter-seasonal variability in several countries suggests that AIV risk is neither static nor uniform and underscores the value of integrating ecological, climatological, and behavioral data into viral surveillance frameworks. By aligning molecular findings with migratory ecology, we can move toward more predictive and targeted surveillance strategies that are better suited to dynamic, tropical systems.

Methods

A total of 27,036 samples were collected between December 2021 and February 2023 from 52 islands in 10 countries (Sri Lanka, Indonesia, Myanmar, the Philippines, Papua New Guinea, Mozambique, Madagascar, Yemen, Somalia, and the Maldives) with the help of citizen scientists; local fishermen, sailors, and volunteers (Supplementary file). AIV RNA concentrations were measured using real-time RT-PCR (M gene), and the hemagglutinin (HA) and neuraminidase (NA) genes were sequenced to validate the results^{19–24} (Supplementary Methods and Supplementary Table 2). Data were analyzed and plotted using the ggplot 2 3.3.5, packages of R program version 4.1.0²⁵. Avian influenza virus (AIV) RNA concentrations from bird guano samples were analyzed across ten countries and four seasonal periods. Kruskal–Wallis tests assessed overall seasonal and country-level differences, with Mann–Whitney U tests for pairwise seasonal comparisons and Dunn's test (Bonferroni-adjusted) for country comparisons within seasons. Percent changes in mean concentrations were calculated between seasons. Seasonal peaks were defined by the highest mean per country, and coefficient of variation (CV) was used to assess seasonal variability. Spearman's rank correlation tested for temporal trends in viral concentration. A normalization approach was applied by calculating the number of virus subtypes detected per positive sample to account for differences in sampling effort across countries. (Supplementary Methods).

Phylogenetic analysis was conducted using avian influenza virus (AIV) hemagglutinin (HA) gene sequences derived from bird guano samples, alongside reference sequences obtained from GISAID (Supplementary Methods)²⁴. The evolutionary history was inferred using the Maximum Likelihood method, applying the Hasegawa-Kishino-Yano (HKY) substitution model for both HA and neuraminidase (NA) genes²⁴. Phylogenetic trees with the highest log-likelihood scores were selected for interpretation. Clade assignments and mutation profiling were performed using the Nextclade web tool (<https://clades.nextstrain.org>), enabling comparative placement of field isolates within globally recognized AIV lineages.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The authors declare that all data supporting the findings of this study are available in the article, its Supplementary Information, and its Source Data. The genome sequences and associated metadata generated in this study have been deposited in the Global Initiative on Sharing All Influenza Data (GISAID) EpiFLU database under the flu category under accession's numbers from EPI_ISL_19613337 to EPI_ISL_19613573/EPI_ISL_19812857-EPI_ISL_19812874) [<https://gisaid.org>] and National Institutes of Health (NIH) GenBank database under accession's numbers PV422873-PV423054, PV422241-PV422430, and PV440337-PV440372 [<https://www.ncbi.nlm.nih.gov/genbank/>]. Source data are provided with this paper.

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Author contributions

D.L.W., M.A., C.H., P.H., and S.A. conceived the idea, acquired funding, and supervised the investigation. D.L.W., M.A., C.H., P.P., and P.H. contributed to data curation, formal analysis, project management, and writing the original draft. C.M., J.J.V.B., K.M., L.C., S.F., A.T.H., P.O., W.W., A.H., P.V., D.J., T.S., S.L., P.K., W.T., R.T., B.C., S.V., N.N., H.I., T.F., and Y.W. were responsible for data collection, technical support, supervision, and manuscript review. N.K.D.R., A.C.S., K.S., A.N., A.L., T.K., H.H., and A.K. provided technical support, resources, and critical review of the manuscript. K.S., A.N., A.K.2, S.T., J.O.V., J.B., and D.S. supervised technical work for viral detection and environmental surveillance, contributing to critical review and editing. J.O.V. and J.B. supervised technical work for bird migration route identification, guano analysis for environmental surveillance, and bird species identification. P.G.H., A.C.S., T.C., J.O.V., J.B., and K.S. contributed to the supervision, conception, critical review, and manuscript editing. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Ethics

The institutional review board at Yamagata Prefectural Central Hospital, Yamagata, Japan, waived ethical approval for this work as it determined that our project is exempt under the type of environmental surveillance study.

Additional information

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