



OFFLU AVIAN DATA PACKAGE FOR ZOONOTIC INFLUENZA TC

February 2024 to September 2024

SCOPE

In this document we present a summary of avian influenza A virus events reported from 1st February 2024 to 28th September 2024 and phylogenetic analysis of H5, H7, H9 and H10 viruses.

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Introduction, data sources, methods and acknowledgements

The epidemiological summary was generated using data from the Food and Agriculture Organization of the United Nations (FAO) EMPRES Global Animal Disease Information System (EMPRES-i+) and the World Animal Health Information System (WAHIS) portal provided by the World Organisation for Animal Health (WOAH). Only data for confirmed reports in environmental samples, wild birds, captive wild birds, domestic birds and mammals were used; suspect cases were excluded where results were based solely on serology.

We thank the OFFLU avian group for their expertise and for sharing data. We acknowledge the following national laboratories, research institutes and surveillance programs and are very grateful for their collaboration: *Agence National de Sécurité de Santé (France)*, *Animal and Plant Health Agency (UK)*, *Animal and Plant Quarantine Agency (Korea, Republic of)*, *Australian Centre for Disease Preparedness (Australia)*, *Bangladesh Livestock Research Institute (Bangladesh)*, *Canadian Food Inspection Agency (Canada)*, *Chittagong Veterinary and Animal Sciences University (Bangladesh)*, *Disease Investigation Centre Wates (Indonesia)*, *Friedrich-Loeffler-Institute (Germany)*, *Harbin Veterinary Research Institute (China)*, *Hokkaido University (Japan)*, *Institut Pasteur Cambodia (Cambodia)*, *Istituto Zooprofilattico Sperimentale delle Venezie (Italy)*, *Laboratório Federal de Defesa Agropecuária (Brazil)*, *National Centre for Foreign Animal Disease (Canada)*, *National Institute of High Security Animal Diseases (India)*, *Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (Mexico)*, *The National Institute of Agricultural Technology (Argentina)*, *University of Chile (Chile)*, *Wageningen Bioveterinary Research (Netherlands)* for sharing unpublished data and virus isolates via the OFFLU network. We would also like to thank research programs and contributors who upload their data in a timely fashion to GISAID and Genbank and acknowledge the authors, originating and submitting laboratories of the sequences. Report contributions were made by Amelia Coggon, Lorcan Carnegie and Francesco Bonfante.

We acknowledge and thank the FAO and WOAH reference laboratory and diagnostic laboratory teams, WHO CCs: St Jude Children's Research Hospital, the Centers for Disease Control and prevention, Worldwide Influenza Centre and Hong Kong University, as well as United States Department of Agriculture-Agricultural Research Service National Animal Disease Centre and Royal Veterinary College and for sharing their expertise and data. Phylogenetic and sequence analyses were performed using the RVC and CEIRR pipeline. Avian influenza A virus haemagglutination inhibition (HI) assay data in this reporting period was generated using harmonised protocols by IZSVe using ferret-origin reagents kindly provided by WHO CCs: CDC and WIC. We are also very grateful to a WHO-OFFLU initiative for the generation of additional ferret reagents at IZSVe which have been shared between WHO CCs and the OFFLU network.

Analyses were conducted by subtype. Sequences collected between 1st February 2024 and 16th September 2024 were downloaded from GISAID and Genbank. These were added to data kindly provided through the OFFLU network and reference datasets. Sequences were analysed preliminarily using the Augur pipeline by Nextstrain (Hadfield et al., 2018). Sequences were aligned using MAFFT v7.475 (Katoh and Standley, 2013) using default settings. Alignments were trimmed (HA1) from the start codon using H5 numbering to the start of the cleavage site. Incomplete, poor quality or duplicate sequences were removed from the dataset. Trees were run using IQ-TREE 2.1.3 (Nguyen et al., 2015) using an ALRT test (Guindon et al., 2010). Sequences were compared against the most similar CVV using the NADC IAV bioinformatic toolkit (<https://github.com/flu-crew>) for amino acid differences. Strains were selected for testing against reference ferret antisera in haemagglutination inhibition (HI) assays based on virus availability and *in-silico* analysis of changes in putative antigenic sites.

Summary

Avian influenzas have caused outbreaks in poultry and/or in wild birds in every continent within the last reporting period. Low pathogenic H5 viruses continue to circulate in poultry in Central America and multiple clade H5 Gs/Gd lineage viruses can be found causing disease in poultry throughout the world. Clade 2.3.4.4b activity has once again predominated with continued circulation in wild birds and spillover into poultry. Despite continued predominance of the H5N1 subtype, some other subtypes have been detected in poultry and/or wild birds, including H5N2, H5N6 and H5N8. In Mexico H5N2 was detected which arose from reassortment of the Mexican lineage low pathogenic H5N2 with clade 2.3.4.4b H5N1 viruses. H5N5 viruses are restricted to wild birds in the Northern Hemisphere and have been circulating in an under surveilled host since 2021. H5N6 have been detected in wild birds in China and Japan with NA genes similar to the H5N6 enzootic in poultry in China and which has caused infections in humans. H5N6 has also been detected in Poland but no genetic data was available. In Germany an H5N8 was detected which arose from an H5N1 clade 2.3.4.4b reassorting with low pathogenic viruses.

Reports of H5 in poultry are at their lowest since the 2018-2019 season. Genomic evidence indicates that the virus is continually circulating within poultry in some countries and that disease outbreak reporting may not accurately reflect this. In well surveilled areas, less outbreaks are attributed to secondary spread. Reports of H5 in wild birds are also at their lowest since the 2019-2020 season, although it is clear that clade 2.3.4.4b viruses continue to circulate in local wild bird populations year round and surveillance does not fully capture the extent of this. Decreasing detections are potentially affected by immunity acquired by some wild bird species. With the autumn migration outbreaks are expected to increase and there have been detections in wild birds and poultry along the Central Asian flyway (China, India) and East-Asia East-Africa flyway in an eastward direction since May.

Spillover into mammalian species continue to be reported with an ever increasing number of abhorrent hosts listed, although the frequency of these reports (with the exception of dairy cattle) is decreasing. This is probably associated with the decrease in infected wild birds and decreased viral infection pressure in the environment. The breeding season for sea mammals in South America is just starting and so it will soon become apparent as to whether there are continued cases in these species however populations have been severely depleted after mass-mortality events associated with H5N1 infections in the previous breeding season.

Clade 2.3.2.1a viruses are geographically restricted to South Asia where they can be routinely found in live poultry markets or causing outbreaks on farm and co-circulate with clade 2.3.4.4b.

Clade 2.3.2.1c viruses are geographically restricted to the Mekong delta where they co circulate with clade 2.3.4.4b viruses.

Clade 2.3.2.1e viruses are restricted to the Indonesian archipelago alongside clade 2.3.4.4b viruses although no sequence was gathered from these clades within this reporting period.

Many countries that have been vaccinating using G5 Gs/Gd lineage seed strains are considering updates to vaccines in response to clade 2.3.4.4b incursion and some countries which have relatively recently had incursions of clade 2.3.4.4b for the first time are considering vaccination.

Independent emergent events of high pathogenic H7 viruses in poultry have occurred in Germany (H7N5) and in 3 instances in Australia (H7N3, H7N8 and H7N9). Diverse lineage low pathogenicity H7 viruses continue to be detected in environmental samples, wild birds and occasionally poultry.

H9N2 viruses continue to be detected in healthy chickens and are often incidental findings when testing poultry for AIV.

Global avian influenza events in animals

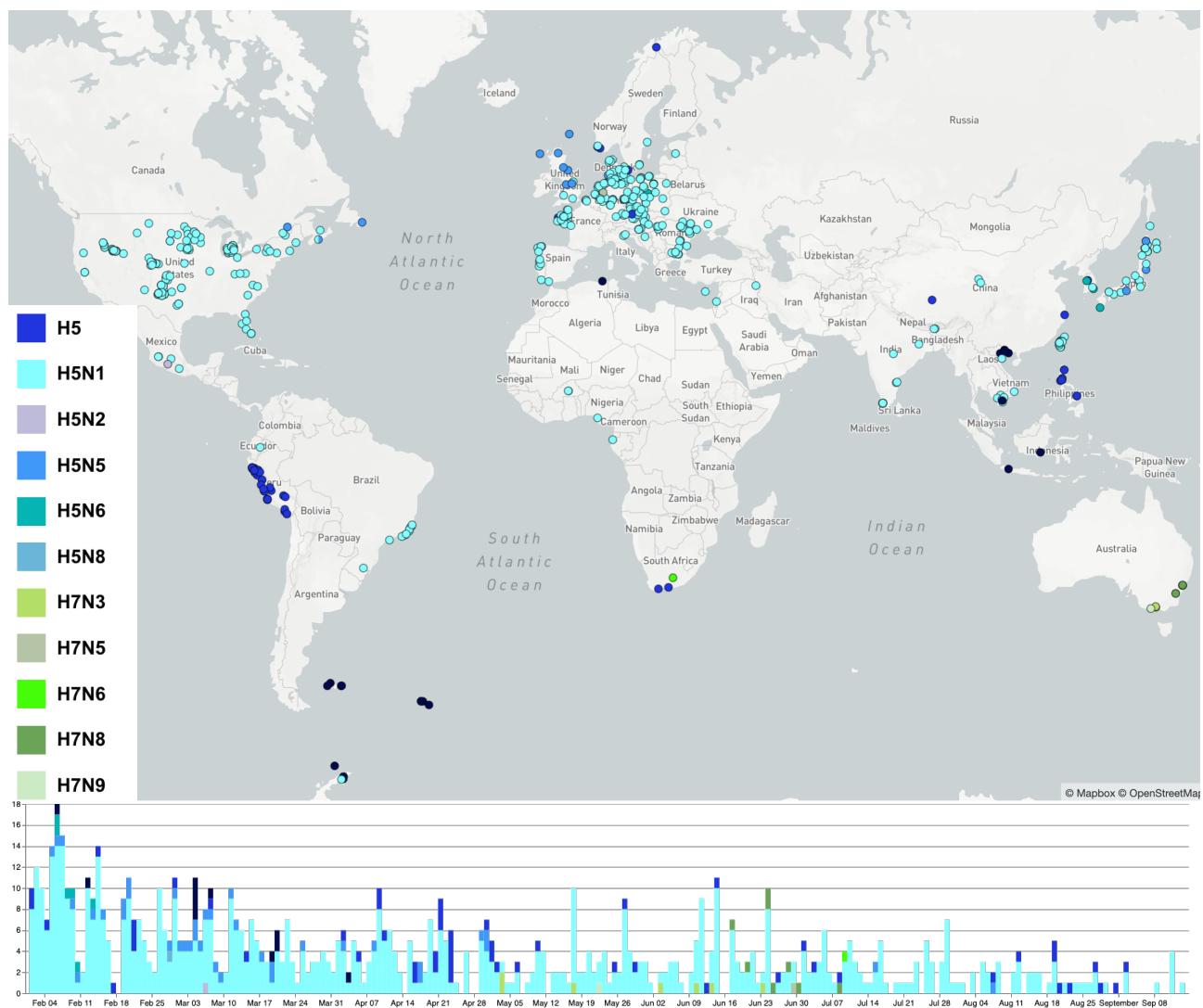


Figure 1: Geographical and temporal representation of outbreaks occurring between 1 February 2024 to 18th September 2024 reported to WOAH or national authorities downloaded from EMPRESi+.

H5 Influenza A viruses

H5 Phylogenetic tree coloured by region

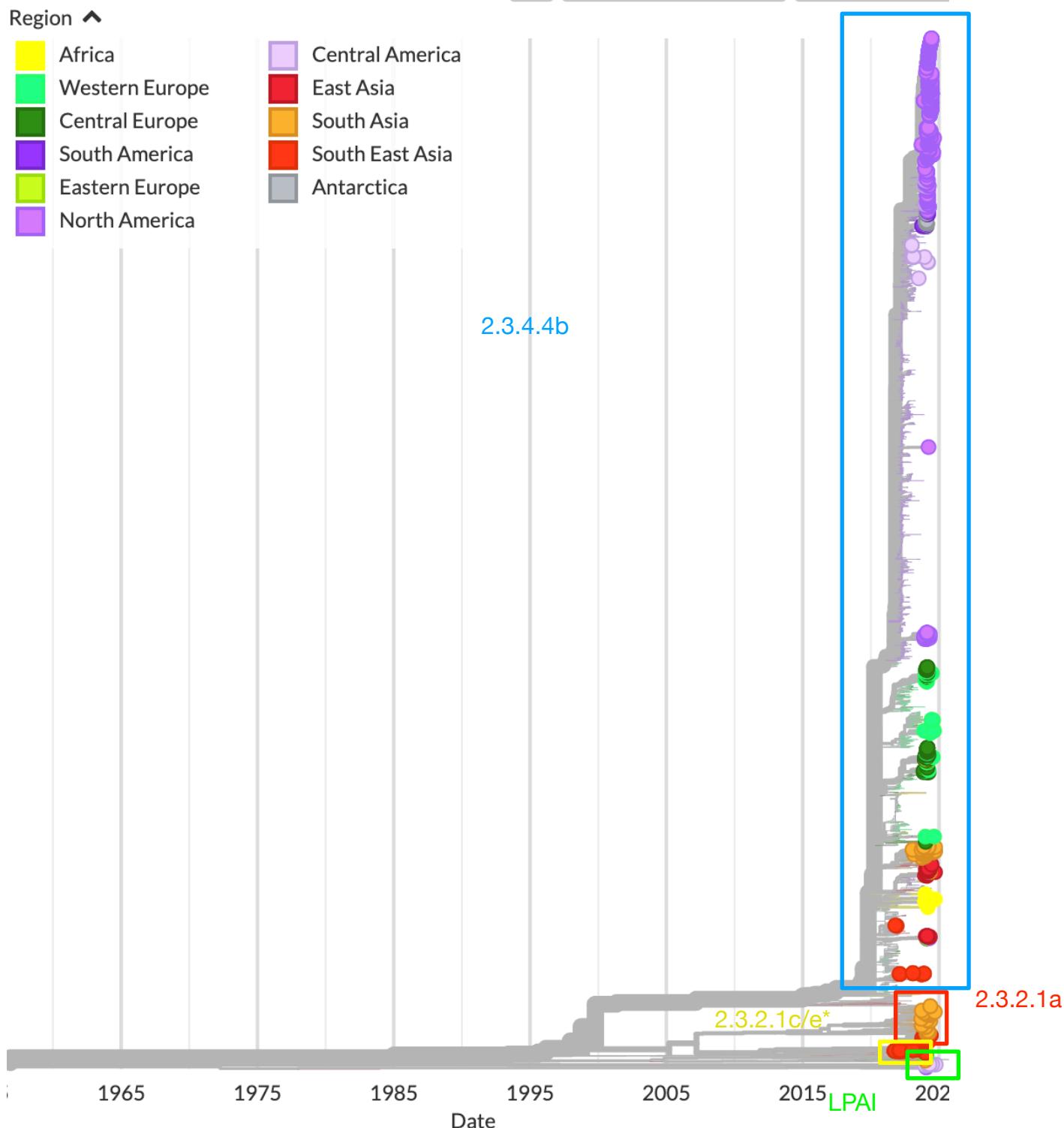


Figure 2: Avian H5 global summary time resolved phylogenetic tree. Analyses were conducted with reference sequences, sequences shared through the OFFLU network and data downloaded from GISAID (16th September 2024). Sequences from GISAID collected within the reporting period and **submitted** to OFFLU since February 2024 are coloured by region.

H5 Influenza A viruses- map

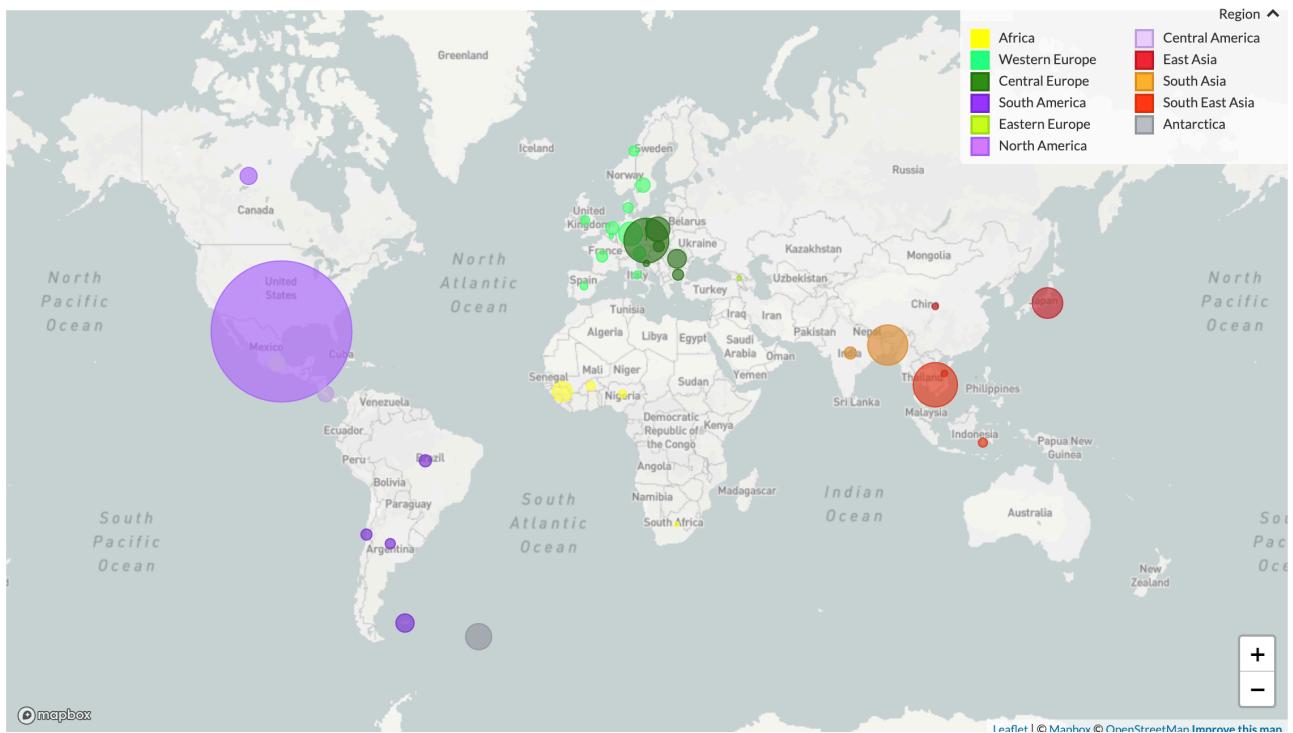


Figure 3: A map showing the geographic location of countries where the sequences from GISAID were collected within the reporting period and countries where sequences were **submitted** to OFFLU since February 2024. Points are coloured by region.

Activity table

Table 1: Gs/Gd lineage activity since 20th February 2024 as collected by OFFLU in this report until 6th September

Algeria	poultry	(HPAI)
Antarctica	mammal (Southern elephant seal)	(H5N1)
Austria	wild birds	2.3.4.4b(H5N1)
Bangladesh	poultry	2.3.2.1a, 2.3.4.4b(H5N1)
Belgium	wild birds	2.3.4.4b(H5N1)
Bhutan	poultry	(H5N1)
Brazil	wild birds	2.3.4.4b(H5N1)
Bulgaria	poultry	2.3.4.4b(H5N1)
Burkina Faso	poultry	2.3.4.4b(H5N1)
Cambodia	poultry	2.3.2.1c(H5N1)
	poultry	(H5N1)
Canada	mammal (Skunk, Raccoon)	2.3.4.4b(H5N5)
	wild birds	2.3.4.4b(H5N1)(H5N5)
China	wild birds	2.3.4.4b (H5N1/6)
Croatia	wild birds	2.3.4.4b (H5N1)
Czech Republic	poultry	2.3.4.4b (H5N1)
Denmark	wild birds	2.3.4.4b (H5N1)
Ecuador	poultry	(H5N1)
Falkland Islands (Malvinas)	wild birds	(HPAI)
France	poultry	(H5Nx)
	wild birds	(H5N1)
Gabon	poultry	(H5N1)
	poultry	(H5N1)
Germany	wild birds	2.3.4.4b(H5N1)(H5N8)
	mammal (Red Fox)	2.3.4.4b (H5N1)
Hungary	poultry	(H5N1)
	wild birds	(H5N1)
India	poultry	2.3.2.1a, 2.3.4.4b(H5N1)
Indonesia	poultry	(HPAI)
Iraq	wild birds	(H5N1)
Israel	poultry	(H5N1)
Italy	poultry	2.3.4.4b (H5N1)
	poultry	(H5N1)
Japan	wild birds	2.3.4.4b (H5N1/5)
Latvia	wild birds	(H5N1)
Mexico	poultry	2.3.4.4b(H5N1/N2)
Moldova, Republic of	wild birds	(H5N1)
Netherlands	wild birds	2.3.4.4b (H5N1)
Nigeria	poultry	2.3.4.4b (H5N1)
	wild birds	2.3.4.4b (H5N1)
Norway	mammal (Red Fox)	2.3.4.4b (H5N5)
Pakistan	poultry	(H5N1)
	poultry	(H5)
Peru	wild birds	(H5)
Philippines	poultry	(H5N1)
Poland	poultry	2.3.4.4b (H5N1)
Portugal	wild birds	2.3.4.4b (H5N1)
Republic of Korea	poultry	(H5N1)
	poultry	2.3.4.4b (H5N1)
Romania	wild birds	2.3.4.4b (H5N1)
Slovakia	poultry	2.3.4.4b (H5N1)
Slovenia	wild birds	(H5N1)
South Africa	wild birds	2.3.4.4b (H5N1)
South Georgia and the South Sandwich Islands	wild birds	(HPAI)
	mammal (South America fur seal, Southern elephant seal)	
Spain	wild birds	2.3.4.4b (H5N1)
Sweden	poultry	2.3.4.4b (H5N1)
Taiwan (Province of China)	wild birds	(H5N1)
U.K. of Great Britain and Northern Ireland	wild birds	2.3.4.4b (H5N1/N5)
Ukraine	wild birds	(H5N1)
	mammal (Alpaca, Bobcat, Cats, Cattle, Desert Cottontail, Goats, House mouse, Mink, Red Fox)	2.3.4.4b (H5N1)
United States of America	poultry	2.3.4.4b (H5N1)
	wild birds	2.3.4.4b (H5N1)
Viet Nam	poultry	(H5N1)

Gs/Gd H5 2.3.4.4b phylogenetic tree

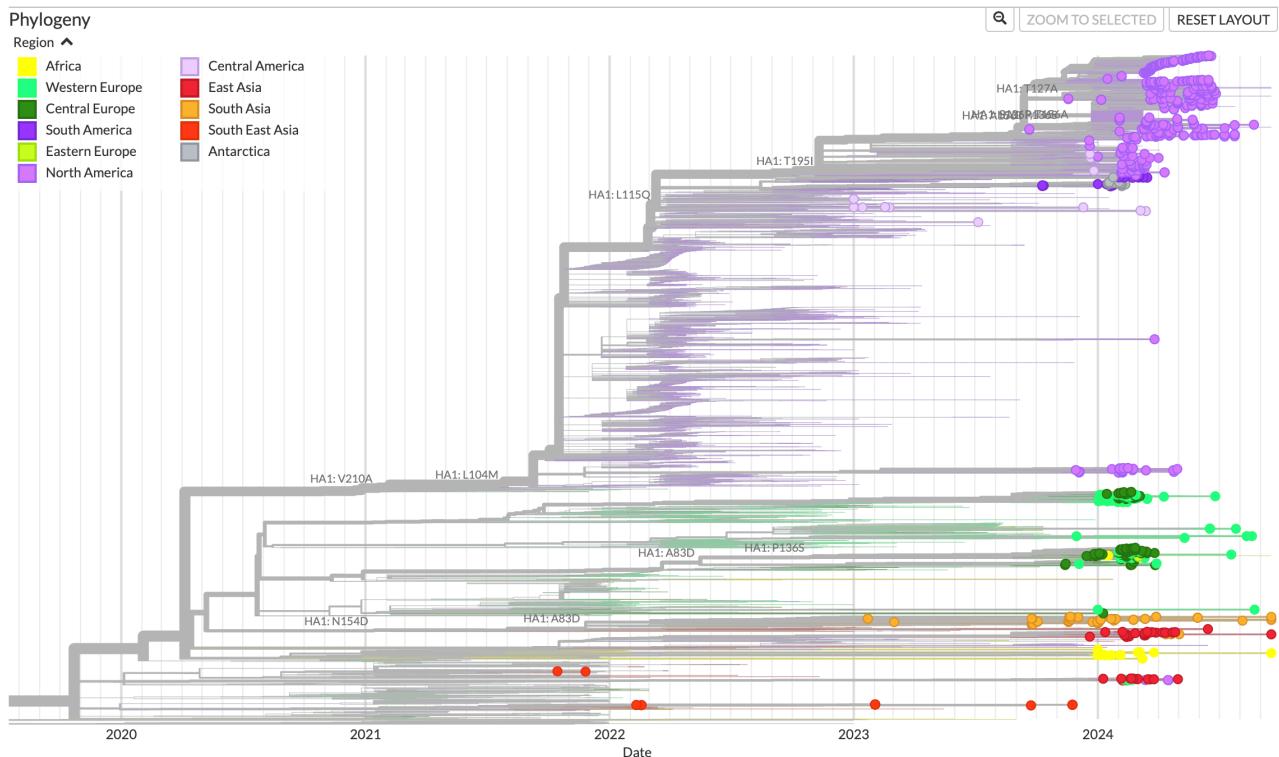


Figure 4: Gs/Gd lineage clade 2.3.4.4b time resolved phylogenetic tree. Sequences from GISAID collected within the reporting period and submitted to OFFLU since February 2024 are coloured by region. Amino acid annotations for major subclades are included along the branches.

Gs/Gd H5 2.3.4.4b subsampled phylogenetic tree

Human
CVV
OFFLU HI

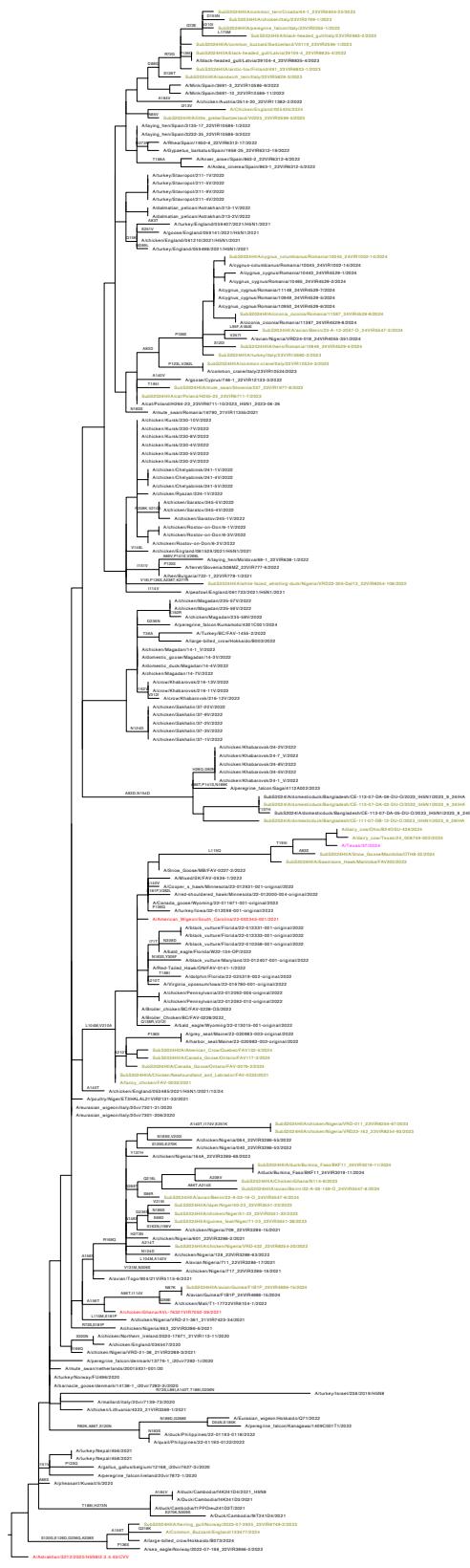


Figure 5: Gs/Gd lineage clade 2.3.4.4b phylogenetic tree, rooted by the astrakan CVV, with amino acid changes along the branches.

Gs/Gd H5 2.3.4.4b Amino acid difference tables

site	Antigenic														
83A	D	D	S			D	D								
86A															
87N															
94S															
95L															
104L	M		M	M	M	M	M			M					
114I															
115L		Q	Q							Q					
120S								G							
126E								D							
136P	S	S													
154N							D	D							
156A	T							T							
169R															
184A															
195T		I							I						
210V	A		A	A	T	T	T	A		A					
213I									V						
214A															
218Q								K							
236D									G						
238A									S						
288S															
Aadiffs from CVW															
Putative antigenic sites	1	2	2	2	2	2	3	5	2	2	2	2	6	1	4
	2	2	2	2	2	2	2	2	2	2	2	2	3	1	2

Gs/Gd H5 2.3.4.4b Antigenic data

Table 2: Avian H5 2.3.4.4b antigenic characterisation of viruses against CVV and reference post infection antisera. Fold changes are coloured.

REFERENCE FERRET ANTISERA			Clade	Subtype	Ferret ID	AA diffs compared to Astrakhan													
Semester	Reference Antigen	Clade	Subtype	ID	2.3.4.4b	H5N6	H5N8	H5N8	H5N8	H5N8	H5N1								
IZSVe Sep 23 - Feb 24	A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	2.3.4.4b	H5N6		80	1280	5120	640	1280	160	1280	2560	320	80					
	A/TURKEY/ITALY/17/VR576-11/2017	2.3.4.4b	H5N8		40	320	640	160	640	160	320	1280	160	160	320	1280	160	160	320
	A/Turkey/Israel/238/22/VR8411/2019	2.3.4.4b	H5N8		20	320	640	80	640	160	320	1280	160	160	320	1280	160	160	320
	A/ASTRAKHAN/3/12/2020	2.3.4.4b	H5N8		80	1280	2560	320	1280	320	1280	2560	320	80					
	A/duck/Cambodia/1/PPOrOeu241D3T/2021	2.3.4.4b	H5N8		40	640	2560	160	1280	160	640	1280	160	80					
	A/poultry/Niger/ET/HALAL/21/VR2131-33/2021	2.3.4.4b	H5N1		20	160	1280	40	640	40	320	640	80	40					
	A/avian/Togo/904/21/VR5115-6/2021	2.3.4.4b	H5N1		10	320	640	160	640	160	80	1280	160	40					
	A/Hen/Bulgaria/72-1/22/VR778-1/2022	2.3.4.4b	H5N1		20	80	2560	20	1280	80	1280	2560	80	160					
	A/chicken/Ghana/AVL-763/21/VR7050-39/2021	2.3.4.4b	H5N1		10	320	640	80	1280	320	160	2560	160	40					
	A/American wigeon/South Carolina/22-000345-001/2021	2.3.4.4b	H5N1		80	1280	5120	320	1280	640	1280	2560	320	160					
	A/American wigeon/South Carolina/22-000345-001/2021	2.3.4.4b	H5N1		80	1280	5120	320	1280	640	1280	2560	320	160					
APHA Sep 23 - Feb 24	Test antigen				20	80	1280	80	640	40	640	1280	80	80					
	1 A/cygnum-columbianus/Romania/10045_24/VR1002-14/2024	2.3.4.4b	H5N1		20	80	1280	80	640	40	640	1280	80	80					
	2 A/Ciconia_ciconia/Romania/11387_24/VR4529-8/2024	2.3.4.4b	H5N1		20	160	5120	40	1280	80	2560	5120	160	320					
	3 A/hens/Romania/10849_24/VR4529-4/2024	2.3.4.4b	H5N1		20	320	2560	80	640	40	320	1280	160	80					
	4 A/swainsons_Hawk/Manitoba/FAV200/2023	2.3.4.4b	H5N1		10	80	1280	40	320	20	320	640	40	40					
	5 A/Snow_Goose/Manitoba/OTH-32/2024	2.3.4.4b	H5N1		20	80	1280	40	640	20	640	640	40	80					
	6 A/American_Crow/Quebec/FAV132-5/2024	2.3.4.4b	H5N1		10	160	1280	40	640	20	320	1280	80	80					
	7 A/Canada_Goose/Ontario/FAV117-3/2024	2.3.4.4b	H5N1		20	160	2560	40	640	40	320	1280	80	80					
	8 A/Canada_Goose/Ontario/FAV-0076-2/2024	2.3.4.4b	H5N1		10	160	2560	20	640	20	320	2560	40	160					
	9 A/Chicken/Newfoundland_and_Labrador/FAV-0033/2021	2.3.4.4b	H5N1		80	2560	2560	640	5120	1280	2560	5120	640	320					
	Reference Antigen				80										10	80	640	1280	
	A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	2.3.4.4b	H5N6		80										10	80	640	1280	
	A/ASTRAKHAN/3/12/2020	2.3.4.4b	H5N8		20										160	320	320		
	A/duck/Cambodia/1/PPOrOeu241D3T/2021	2.3.4.4b	H5N8		160										160	640	2560		
	A/avian/Togo/904/21/VR5115-6/2021	2.3.4.4b	H5N1		20										40	160	1280		
	A/chicken/Ghana/AVL-763/21/VR7050-39/2021	2.3.4.4b	H5N1		20										40	320	2560		
	A/American wigeon/South Carolina/22-000345-001/2021	2.3.4.4b	H5N1		160										80	160	5120		
	Test antigen				20										160	320	1280		
	1 A/domestic_duck/Bangladesh/CE-111-07-DB-12-DU-O/2023	2.3.4.4b	H5N1		20										320	320			
	2 A/dominican_duck/Bangladesh/CE-113-07-DA-06-DU-O/2023	2.3.4.4b	H5N1		20										320	320			
	3 A/Common_Buzzard/England/133477/2024	2.3.4.4b	H5N5		10										80	320			
	4 A/Chicken/England/200543/2024	2.3.4.4b	H5N1		40										160	160			
	5 A/Bovine/Ohio/B/24/OSU-143/2024	2.3.4.4b	H5N1		10										40	80			
	6 A/dairy_cattle/Texas/24-008749-002/2024	2.3.4.4b	H5N1		40										20	40			
	7 A/chicken/Newfoundland_and_Labrador/FAV-0033/2021	2.3.4.4b	H5N1		20										40	80			
	8 A/Red_Tailed_Hawk/Ontario/FAV141/2022	2.3.4.4b	H5N1		20										20	40			
	9 A/Turkey/Ontario/FAV-0162/2022	2.3.4.4b	H5N1		40										80	80			
	10 A/Chicken/Alberta/FAV222-1/2022	2.3.4.4b	H5N1		80										80	40			
	11 A/Snow_Goose/Manitoba/FAV227-2/2022	2.3.4.4b	H5N1		10										20	20			
	12 A/Chicken/British_Columbia/FAV228/2022	2.3.4.4b	H5N1		10										20	10			
	13 A/Chicken/Alberta/FAV312-16/2022	2.3.4.4b	H5N1		20										40	80			
	14 A/Turkey/Saskatchewan/FAV936-1/2022	2.3.4.4b	H5N1		40										160	160			
	15 A/Turkey/British_Columbia/FAV1455-2/2022	2.3.4.4b	H5N1		80										160	160			

Gs/Gd H5 2.3.4.4b Antigenic data from previous report

Table 3: Avian H5 2.3.4.4b antigenic characterisation of viruses against CVV and reference post infection antisera from February 2023. Fold changes are coloured.



REFERENCE FERRET ANTISERA					A/FUJIAN-SANYUAN/21/09/2017 xA/PR/8/1934									
Semester	Reference Antigen	Clade	Subtype		A/FUJIAN-SANYUAN/21/09/2017 xA/PR/8/1934	A/ASTRAKHAN/3/21/2020	A/TURKEY/ITALY/17/IR576-11/2017	A/duck/Cambodia/f1PP0reu24103T/2021	A/turkey/Israel/238/2019	A/poultry/Niger/ET3/HALAL/21/IR2131-33/2021	A/chicken/Ghana/AVL-763/21/IR7050-39/2021	A/avian/Togo/904/21/IR5115-6/2021	A/american wigeon/South Carolina/22-000345-001/2021	
		Clade	Subtype	Ferret ID	2.3.4.4b	2.3.4.4b	2.3.4.4b	2.3.4.4b	2.3.4.4b	2.3.4.4b	2.3.4.4b	2.3.4.4b	2.3.4.4b	
APHA Sep23- Feb24	A/FUJIAN-SANYUAN/21/09/2017 xA/PR/8/1934	2.3.4.4b	HSN6		160	80	320	80	80	320	320	640		
	A/ASTRAKHAN/3/21/2020	2.3.4.4b	HSN8		10	160	320	160	320	160	320	160	320	
	A/TURKEY/ITALY/17/IR576-11/2017	2.3.4.4b	HSN8		20	40	640	320	320	160	640	320		
	A/duck/Cambodia/f1PP0reu24103T/2021	2.3.4.4b	HSN8		40	80	640	320	160	320	640	640		
	A/turkey/Israel/238/2019	2.3.4.4b	HSN8		20	40	160	80	320	160	640	160		
	A/poultry/Niger/ET3/HALAL/21/IR2131-33/2021	2.3.4.4b	HSN1		10	20	160	40	40	640	320	320		
	A/chicken/Ghana/AVL-763/21/IR7050-39/2021	2.3.4.4b	HSN1		0	20	320	320	160	320	640	640		
	A/hen/Bulgaria/722-1/22/IR778-1/2021	2.3.4.4b	HSN1		10	80	40	40	40	1280	640	640		
	A/avian/Togo/904/21/IR5115-6/2021	2.3.4.4b	HSN1		20	40	160	160	80	320	320	320	640	
	Test antigen													AA changes from 3212 putative antigenic sites
IZSVe Sep23- Feb24	A/Herring_gull/England/404234/2023	2.3.4.4b	HSN5		10	80	160	160	40	1280	320	640		S120G, E126D, A156T, D236N, A238S
	A/Duck/Bangladesh/CE-111-05-DA-05-DU-O/2022	2.3.4.4b	HSN1		20	80	160	80	80	640	640	1280		V210I, I299V
	A/Duck/Bangladesh/CE-111-05-DA-02-DU-J/2022	2.3.4.4b	HSN1		40	160	160	160	80	2560	1280	1280		V210I, I299V
	A/Kelp_Gull/Moltke_Harbour/133754/2023	2.3.4.4b	HSN1		0	0	40	10	10	320	320	1280		N84D, L104M, L115Q, Y137H, S163N, V210A
	A/Southern_fulmar/Falkland_Islands/133789/2023	2.3.4.4b	HSN1		10	20	40	10	20	640	320	640		L104M, L115Q, V137H, V210A, I294V
	A/Kelp_Gull/Harpon_Bay/133943/2023	2.3.4.4b	HSN1		0	20	20	40	40	1280	640	1280		
	A/Turkey/England/13330/2023	2.3.4.4b	HSN1		20	160	80	80	80	640	640	1280		
	A/Duck/Bangladesh/AV-23-071766/2023	2.3.4.4b	HSN1		10	80	320	160	80	640	640	1280		A83D, N154D
	Test antigen													
	A/chicken/Nigeria/VRD-011_23/IR8254-67/2023	2.3.4.4b	HSN1		<10	40	80	80	40	160	320	320	20	A146S, I115V, A156S, R169Q, D236N, A140T, A156S, R169Q, I174V, E251K
IZSVe Sep23- Feb24	A/chicken/Nigeria/VRD23-162_23/IR8254-93/2023	2.3.4.4b	HSN1		<10	80	160	80	80	160	320	640	40	A156S, R169Q, A214T
	A/chicken/Nigeria/VRD-432_23/IR8254-20/2022	2.3.4.4b	HSN1		10	80	640	640	320	640	1280	2560	R172G, D88G, P163Q	
	A/white-faced_whistling-duck/Nigeria/VRD22-305-Dai13_23/IR8254-10F	2.3.4.4b	HSN1		40	160	1280	640	320	5120	1280	5120	V16I, P136S, A238T, K277R	
	A/black-headed_gull/Latvia/29104-4_23/IR8835-4/2023	2.3.4.4b	HSN1		40	80	640	160	320	1280	1280	2560	R72G, D88G, P163Q	
	A/common-crane/Italy/23/IR10524-3/2023	2.3.4.4b	HSN1		40	160	640	320	160	2560	1280	2560	A83D, P123L, V282L	
	A/turkey/Italy/23/IR10680-2/2023	2.3.4.4b	HSN1		<10	80	80	40	40	1280	1280	1280	A83D, P123S	
	A/herring-gull/Norway/2023-07-2935_23/IR9749-2/2023	2.3.4.4b	HSN5		10	40	160	320	160	640	1280	1280	S120G, E126D, A156T, D236G, A238S	
	Test antigen													

Gs/Gd H5 2.3.2.1a phylogenetic tree

Feb2024-Sept2024

Sept2023-Feb2024

Human

CVV

HI



Figure 6: Gs/Gd lineage clade 2.3.2.1a phylogenetic tree, with amino acid changes along the branches.

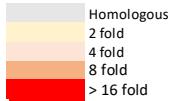
Gs/Gd H5 2.3.2.1a amino acid difference table

Table 4: Amino acid comparison of the HA1 compared to within-clade CVVs. Putative antigenic sites are highlighted in grey.

H5 numbering site	Putative antigenic site													
14 K	E	E												
20 M			V											
21 E			K											
22 K			R											
23 N			R											
24 V			I											
31 D				N										
36 T										A				
51 I	V	V			V									
115 R				Q		Q		Q						Putative antigenic site
124 D			N	N	N		N	N	N					Putative antigenic site
126 E	D	D			D									Putative antigenic site
129 L		R								M				Putative antigenic site
137 Y									H					
141 S	P	P			P									Putative antigenic site
154 N	D		D	X	D		D	D	D					Putative antigenic site
156 A	T	S	S			S								Putative antigenic site
163 S			N					N	N					Putative antigenic site
175 L								M						Putative antigenic site
185 A	T							T						Putative antigenic site
189 R	N	N				N			Q					Putative antigenic site
195 T	I	I			I				S					Putative antigenic site
210 V	I	I			I					R				Putative antigenic site
212 K	R	R			R									
273 N	H							D						Putative antigenic site
276 T		A								A				
285 I					V									
312 V					I									
AA diff	28	2	11	10	2	7	3	3	9	4	2	5	7	5
Putative antigenic site	13	2	8	7	1	2	2	3	6	3	2	4	4	2

Gs/Gd H5 2.3.2.1a Antigenic data

Table 5: Avian Gs/Gd-lineage 2.3.2.1a antigenic characterisation of viruses against CVV and reference post infection antisera. Fold changes are coloured. Amino acid changes compared to the within-clade CVV are annotated.



Semester		REFERENCE FERRET ANTISERA			AA changes comapred to 2018 CVV
		Clade	Subtype	Clade	
		Subtype	H5N1	2.3.2.1a	
		Ferret ID		A/HUBEI/1/2010	
APHA Feb 24 - <u>Sep 24</u>				A/HUBEI/1/2010	
		Reference Antigen		2.3.2.1a	
		A/HUBEI/1/2010	2.3.2.1a	H5N1	2560 640 160
		A/DUCK/BANGLADESH/19097/2013	2.3.2.1a	H5N1	2560 320 160
		A/DUCK/BANGLADESH/17D1012/2018	2.3.2.1a	H5N1	640 160 640
					N154D, A156T
		Test antigen			
		A/domesticduck/Bangladesh/CE-116-07-DA-03-DU-O/2023	2.3.2.1a	H5N1	320 80 320
		A/domesticduck/Bangladesh/CE-115-07-DB-10-DU-O/2023	2.3.2.1a	H5N1	1280 160 80
		A/domesticduck/Bangladesh/CE-113-04-DA-05-DU-O/2022	2.3.2.1a	H5N1	320 160 80
		A/domesticduck/Bangladesh/CE-111-04-DA-04-DU-O/2022	2.3.2.1a	H5N1	640 80 80
		A/domesticduck/Bangladesh/CE-115-04-DA-02-DU-O/2022	2.3.2.1a	H5N1	160 10 320
					K40M, D124N, I200V

LPAI H5 phylogenetic tree

Feb2024-Sept2024

Sept2023-Feb2024

Human



Figure 7: Low pathogenicity H5 phylogenetic tree. Sequences were downloaded from GISAID and collected through OFFLU. Sequences are coloured according to collection date. Sequences from humans are pink.

H7 Influenza A viruses

Feb2024-Sept2024
Sept2023-Feb2024
CWV

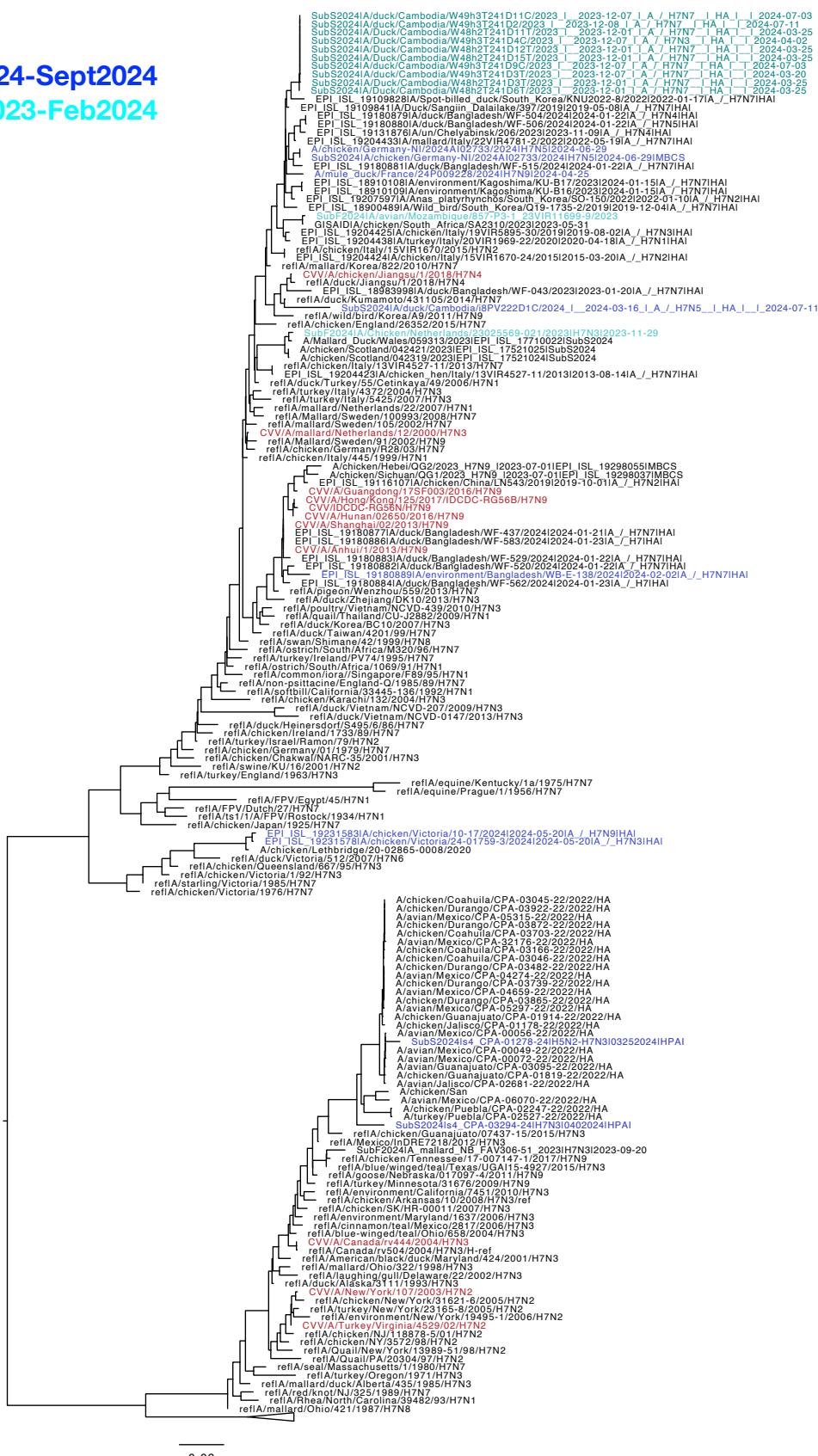


Figure 8: H7 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data downloaded from GISAID, Genbank and kindly shared through the OFFLU network. Sequences are coloured according to collection date. CVVs are red.

H7 Eurasian lineage and american lineage amino acid difference tables

Table 7: Amino acid comparison of the HA1 compared to within-clade CVV. High path strains are highlighted in red.

H9 Influenza A viruses

H9 Y280 (B4.7) phylogenetic tree

Feb2024-Sept2024

Sept2023-Feb2024

CV

Human

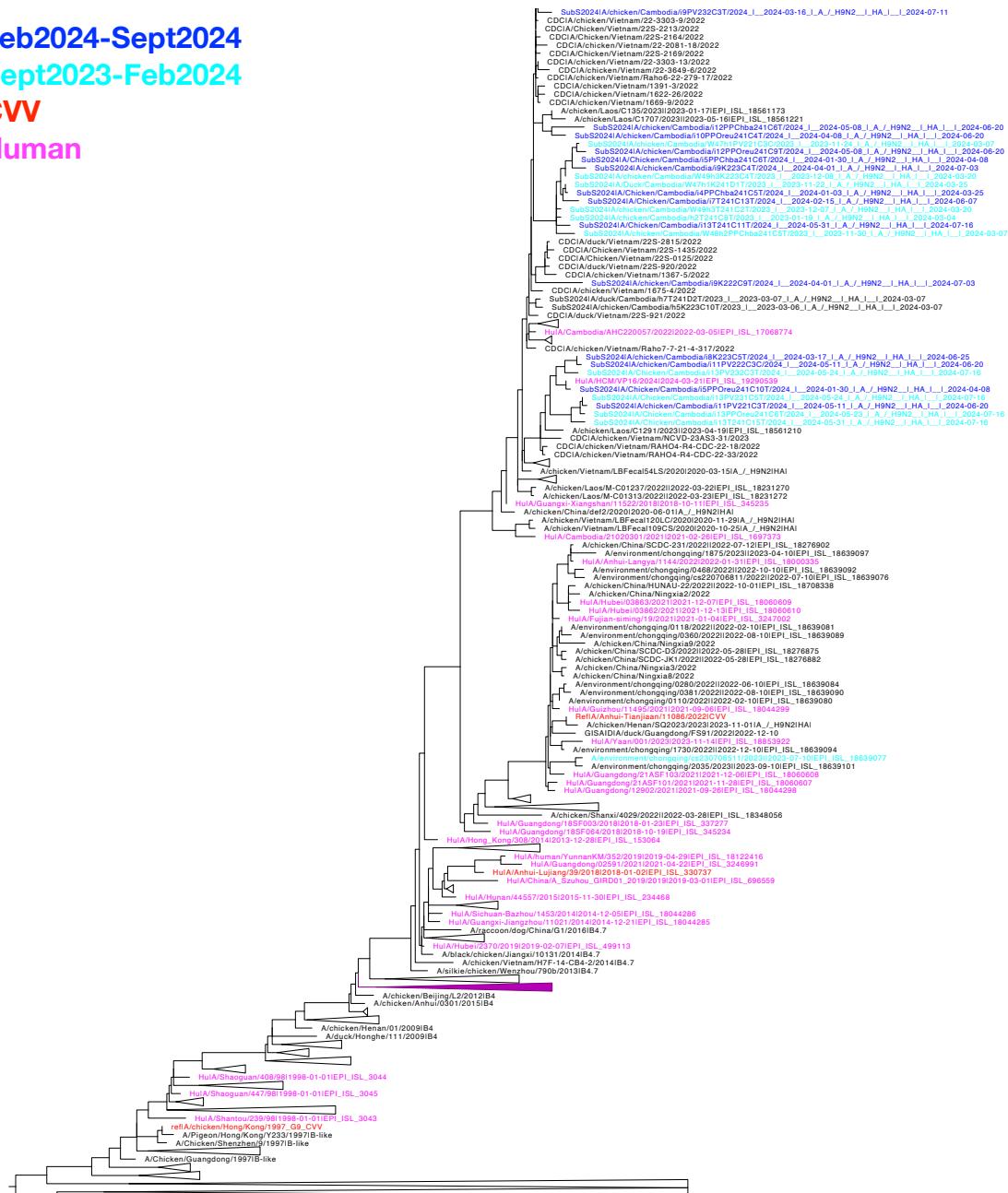


Figure 9: H9 phylogenetic tree. Analyses were conducted with reference sequences, data downloaded from GISAID, Genbank and shared from HKU, CDC and the OFFLU network. Sequences are coloured according to collection date. Sequences from humans are pink and CVs are red. Branches of the B4.7 clade according to the Proposal for a Global Classification and Nomenclature System for A/H9 Influenza Viruses, are coloured purple.

H9 Y280 (B4.7) amino acid difference table

Table 8: Amino acid comparison of the HA1 compared to within-clade CVVs. Putative antigenic sites are highlighted in grey.

site	Antigenic																									
22 N																										
23 N																										
36 E																										
45 S																										
51 V	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
53 E	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
57 I	V																									
60 L																										
68 E	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
69 P	L																									
70 L	S																									
71 P	L																									
72 E	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
73 E	G																									
74 K	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
79 V	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	
84 S																										
86 V	I																									
87 N	D																									
109 R																										
112 R	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	
115 Q	L	L	L																							
120 T																										
131 N	T																									
132 T	A																									
135 G	D																									
139 R																										
140 N	S																									
145 N	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	
146 R																										
148 N	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
149 N	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	
153 I	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	
158 Y	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	
162 Q																										
163 G	E	E																								
165 S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
174 H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
178 E	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
179 D																										
180 V	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	
183 T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
188 T	I	I	I																							
191 T																										
199 I	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
202 I																										
207 I																										
226 V																										
228 K	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
242 I																										
246 Y																										
248 Y	H																									
251 S	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
260 R	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	
264 K																										
265 R																										
288 V																										
302 I																										
315 P																										
Aaddfs from CVV	25	20	20	25	25	24	24	22	24	24	25	28	23	21	21	21	23	23	21	25	26	20	20	21	21	19
Putative antigenic sites	8	6	9	10	10	9	9	9	9	9	9	11	9	9	9	9	9	8	8	8	8	8	7	7	6	6

H9 Y280 (B4.5) phylogenetic tree

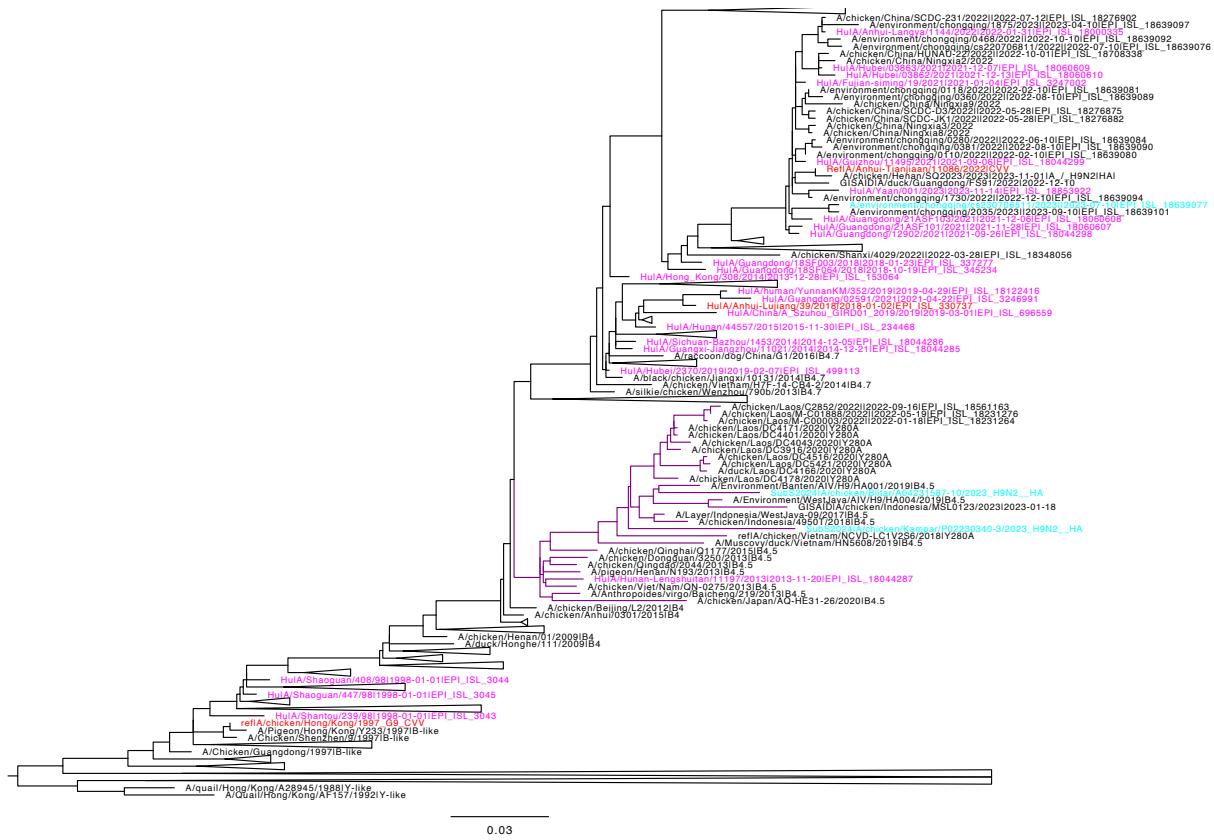


Figure 10: H9 phylogenetic tree. Analyses were conducted with reference sequences, data downloaded from GISAID, Genbank and shared from HKU, CDC and the OFFLU network. Sequences are coloured according to collection date. Sequences from humans are pink and CVVs are red. Branches of the B4.5 clade according to the Proposal for a Global Classification and Nomenclature System for A/H9 Influenza Viruses, are coloured purple.

H9 Y280 (B4.5) amino acid differences

Table 9: Amino acid comparison of the HA1 of B4.5 lineage viruses compared to CVV.

site	A/Anhui-Lujiang/39/2018 CVW	Ref A/Anhui-Tianjianan/11086/2022 CVW	SubS2024 A/chicken/Kampar/P02230340-3/2023_H9N2_H	SubS2024 A/chicken/Solok/P02230452-1/2023(H9N2)_HA	SubS2024 A/chicken/Blitar/A04231587-10/2023_H9N2_H
32 L	M	M			
34 H			Q		
37 H		X			
45 S	G	G			
48 Q	H	H	H		
51 I	V				
53 D	E				
57 V	I			I	
68 D	E				
69 L	P	P	P	Q	
70 S	L	L	L	L	
71 L	P				
73 G	E				
74 R	K				
79 I	V				
80 V		I	I		
87 N	D	D			
112 Q	R				
114 I		T	T		
115 Q		L	L	L	
125 S	T		T		
127 D	S	S	S		
131 T	N	K	K	K	
132 A	T				
135 D	G				
140 S	N				
145 T	N				
146 R		Q	Q	Q	
148 D	N	N	N	N	
149 G	N	N	N	N	
150 N	A	A	A	A	
153 T	I				
163 G				E	
165 N	S				
178 D	E				
179 D		T	T	T	
180 T	V	A	A	A	
182 R		T	T	T	
183 N	T				
202 I		T	T	T	
238 D	N	N	N		
248 H	Y				
260 K	R				
279 L	F	F			
298 K	R	R			
302 I	T	T			
309 V	I	I			
320 X		R			
	48	25	25	27	18

H9 G1 (G5.7) phylogenetic tree

**Feb2024-
Sept2024
Sept2023-
Feb2024
Human
HI strain
CVV**

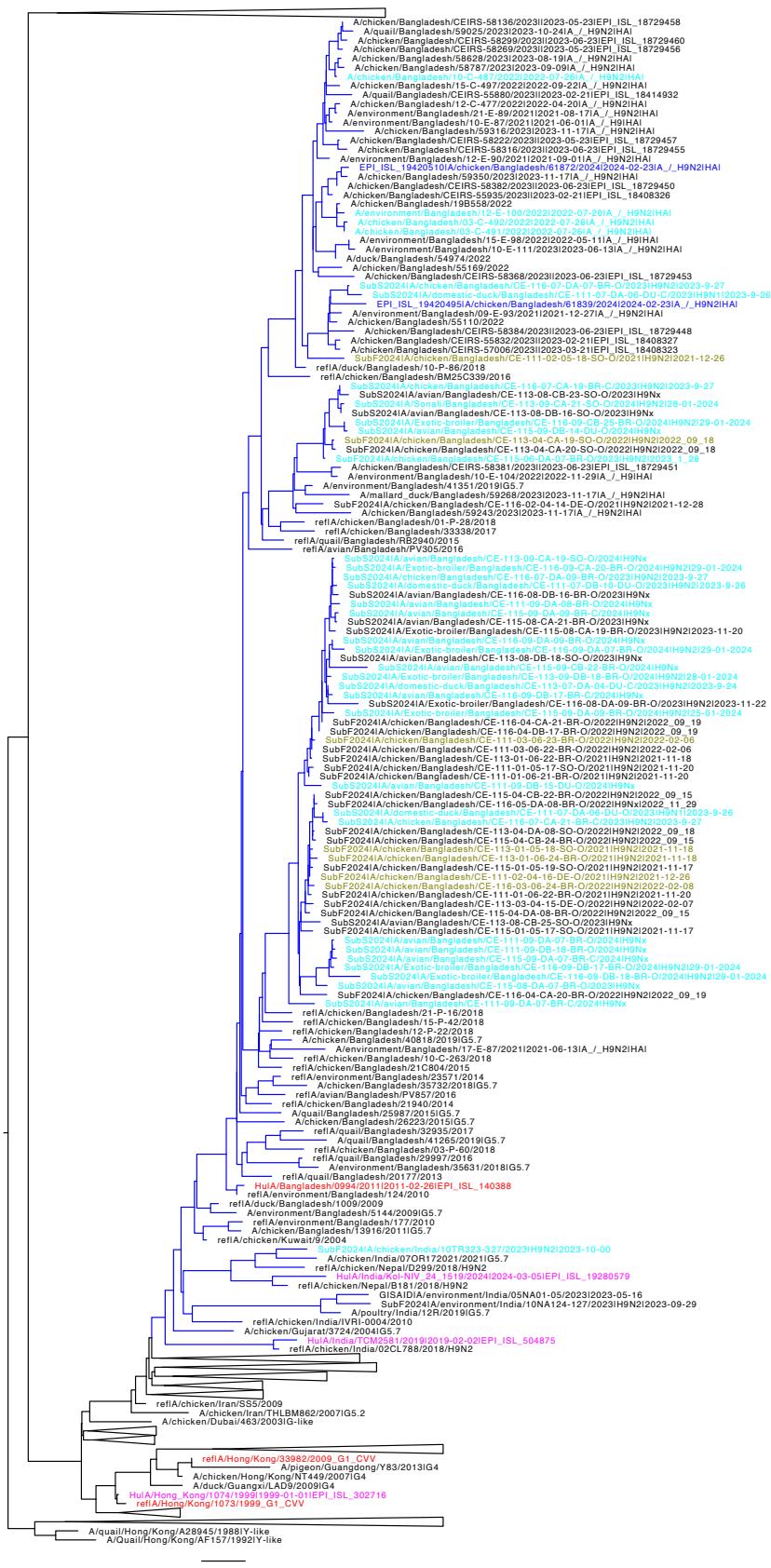


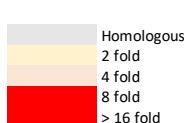
Figure 11: H9 phylogenetic tree. Analyses were conducted with reference sequences, data downloaded from GISAID, Genbank and shared from HKU, CDC and the OFFLU network. Sequences are coloured according to collection date. Sequences from humans are pink and CVVs are red. Branches of the G4.7 clade according to the Proposal for a Global Classification and Nomenclature System for A/H9 Influenza Viruses, are coloured blue.

H9 G1 (G5.7) amino acid difference table

Table 8: Amino acid comparison of the HA1 compared to within-clade CVVs. Putative antigenic sites are highlighted in grey.

H9 G1 (G5.7) antigenic data

Table 10: Avian H9 G5.7 antigenic characterisation of viruses against CVV and reference post infection antisera from February 2023. Fold changes are coloured.



Semester	Reference Antigen	REFERENCE FERRET ANTISERA				A/Oman/2747/2019	A/Bangladesh/0994/2011
		Clade	Subtype	Ferret ID	Subtype		
		G1	H9N2	G1	H9N2		
	A/Oman/2747/2019	G1	H9N2	640	40		
	A/Bangladesh/0994/2011	G1	H9N2	320	1280		
<u>APHA Sep23-</u>	<u>Test antigen</u>						
<u>Feb24</u>	<u>1</u> A/chicken/Bangladesh/CE-111-02-04-16-DE-O/2021	G1	H9N2	320	40		
	<u>2</u> A/chicken/Bangladesh/CE-113-04-CA-19-SO-O/2022	G1	H9N2	160	20		
	<u>3</u> A/chicken/Bangladesh/CE-111-06-DB-16-BR-O/2023	G1	H9N2	160	80		
	<u>4</u> A/chicken/Bangladesh/CE-116-03-06-24-BR-O/2022	G1	H9N2	320	40		

H9 G1 (G5.5) phylogenetic tree

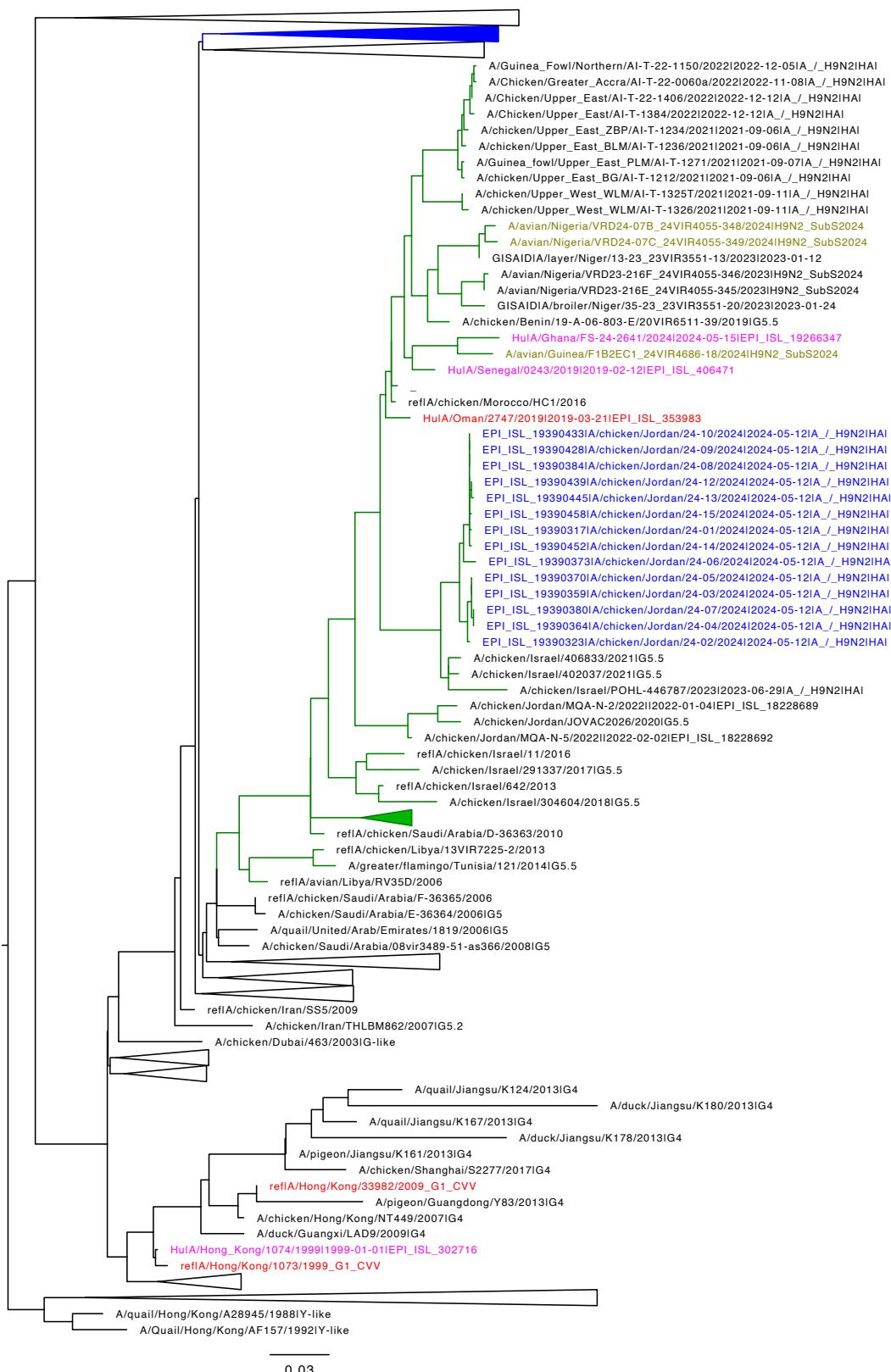


Figure 12: H9 phylogenetic tree. Analyses were conducted with reference sequences, data downloaded from GISAID, Genbank and shared from HKU, CDC and the OFFLU network. Sequences are coloured according to collection date. Sequences from humans are pink and CVVs are red. Branches of the G4.5 clade according to the Proposal for a Global Classification and Nomenclature System for A/H9 Influenza Viruses, are coloured green.

H9 G1 (G5.5) amino acid difference table

Table 11: Amino acid comparison of the HA1 compared to within-clade CVVs. Putative antigenic sites are highlighted in grey.

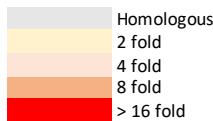
	A/Oman/274/7/2019 CVV			
site				
5	I	V V	H	H
28	Q			
36	E		N	D
40	M	K K	K K	K
45	N	K K K K		K K K
48	R	H	H	
54	T		K	
56	T		N	
57	I			V
69	M		I S	I S
72	G		E	I
77	S	A A A A A A		
86	V		A E	
95	V	I I		
103	T	A		
104	L	F F F F		
112	Q		K	
114	V	I I	I	I
116	L		I	I
120	S		T	T
121	I	X		
127	T	S S		
135	D		G	
148	N	S		
149	G		S	S
150	G	N N N N N N	L	L
153	I		V	V
158	Y		F	F
161	N	K		
180	A	T T		
188	T	I		V
198	T		A D	E
214	N	K K		
219	R	X		
226	V		I	I
238	N		T	T
246	F		Y	Y
249	V	I I		
254	S	X		
260	R		K	K
262	D	N N N N		
264	S	K K	N	N
265	S	N I I X		
266	G		S S S S S	S S S S S
283	M		L	L
288	I			V
295	T	I	N N N N N N	N N N N N N
298	K	X		
305	L	X	M M M M	M M M M
311	L		P	P
315	H		M M M M	M M M M
316	A	T		
317	R	K K	K	K
319	S		K	K

Aadiffs from CVV
Putative antigenic sites

8 11 9 8 8 14 10 4 25 4 4 4 5 3 24 4 4 4 4 5 5

H9 G1 (G5.5) Antigenic data

Table 11: H9 G5.5 antigenic characterisation of viruses against CVV and reference post infection antisera. Fold changes are coloured.



Semester <u>IZSVe Sep 23 - Feb 24</u>	REFERENCE FERRET ANTISERA			Ferret ID <i>A/BANGLADESH/0994/2011</i> <i>A/Oman/2747/2019</i>
	Clade	G1	G1	
	Subtype	H9N2	H9N2	
	Reference Antigen			
	A/BANGLADESH/0994/2011	G1	H9N2	320
	A/Oman/2747/2019	G1	H9N2	320
	Test antigen			
1	A/avian/Nigeria/VRD23-216B_24VIR4055-342/2023	G1	H9N2	80
2	A/avian/Nigeria/VRD23-216D_24VIR4055-344/2023	G1	H9N2	80
3	A/avian/Guinea/F1B2P_24VIR4686-16/2024	G1	H9N2	20
				640
				640
				160

H10

Feb2024-Sept2024
Sept2023-Feb2024
Human
CVV

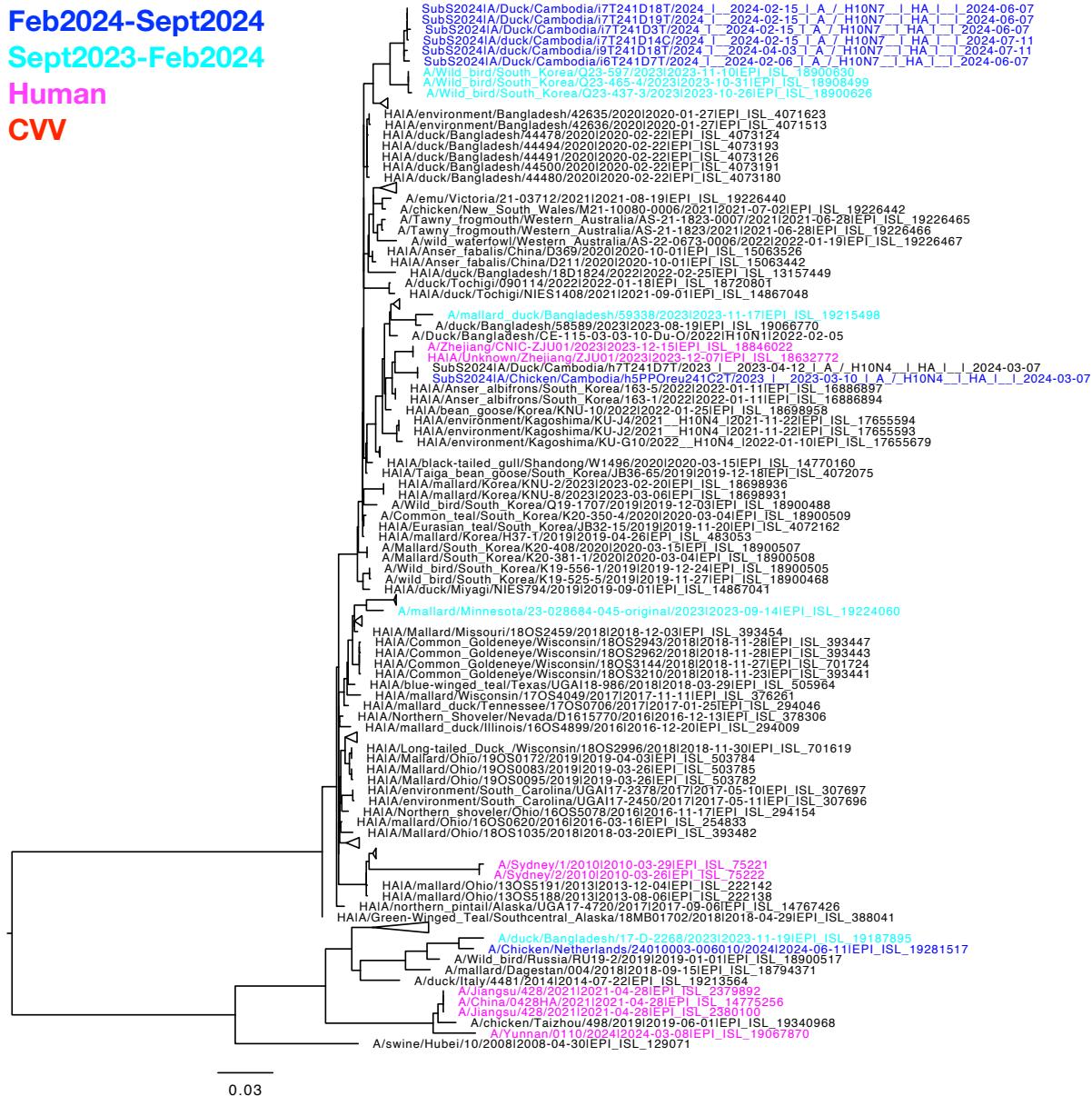
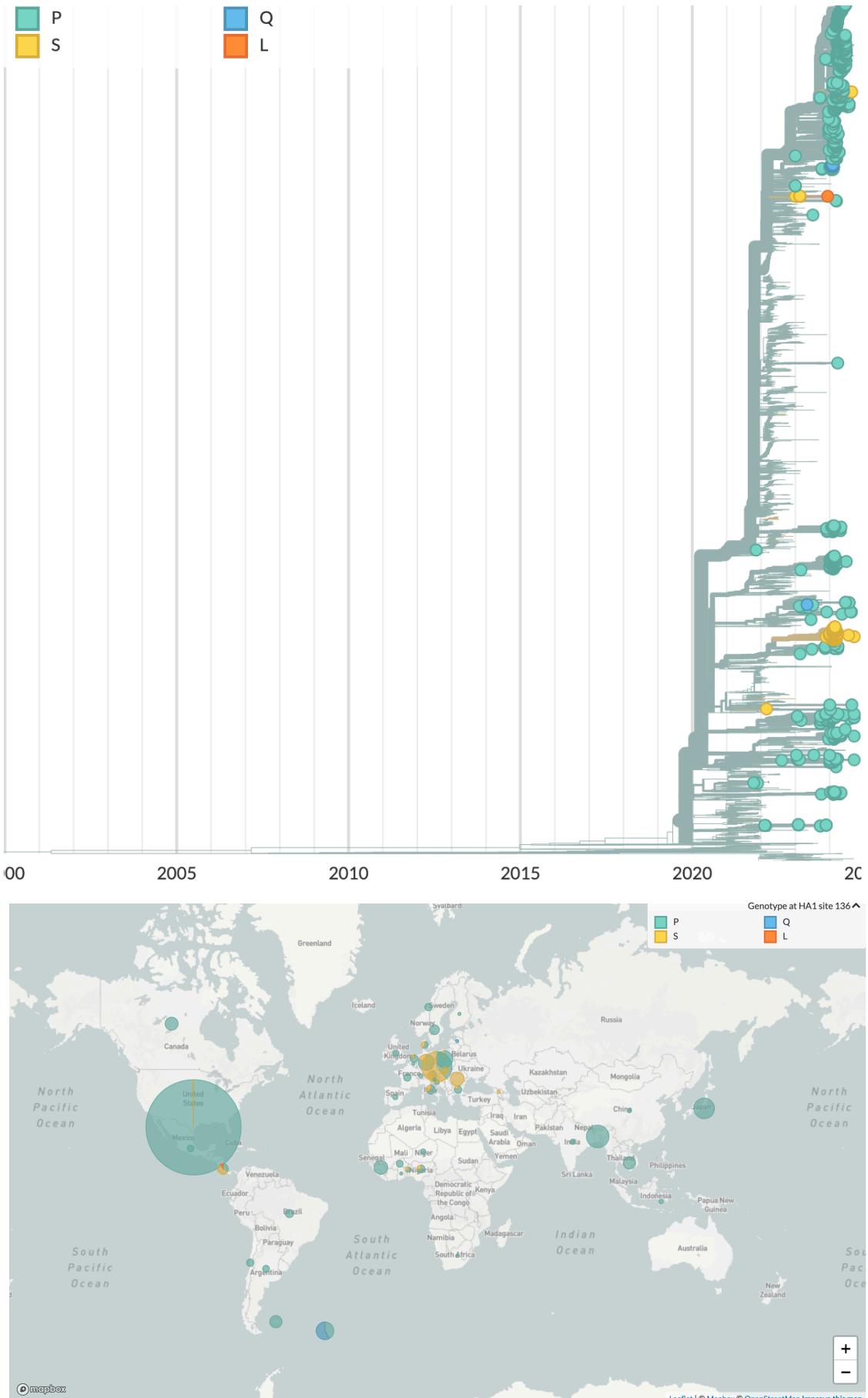


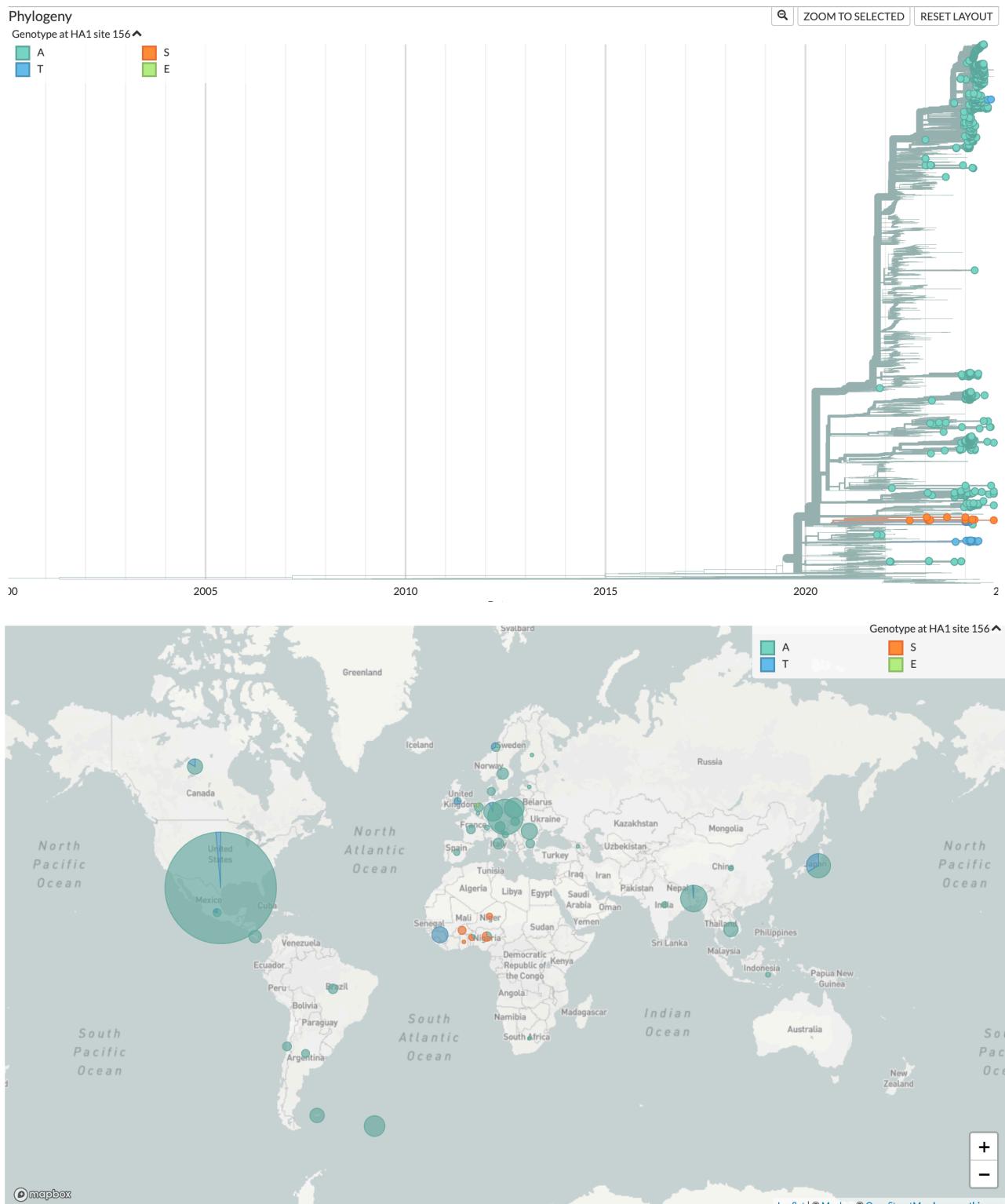
Figure 13: Avian H10 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with sequences and data downloaded from GISAID and shared with OFFLU. Sequences are coloured according to collection date. Sequences from humans are pink and CVVs are red.

Annex

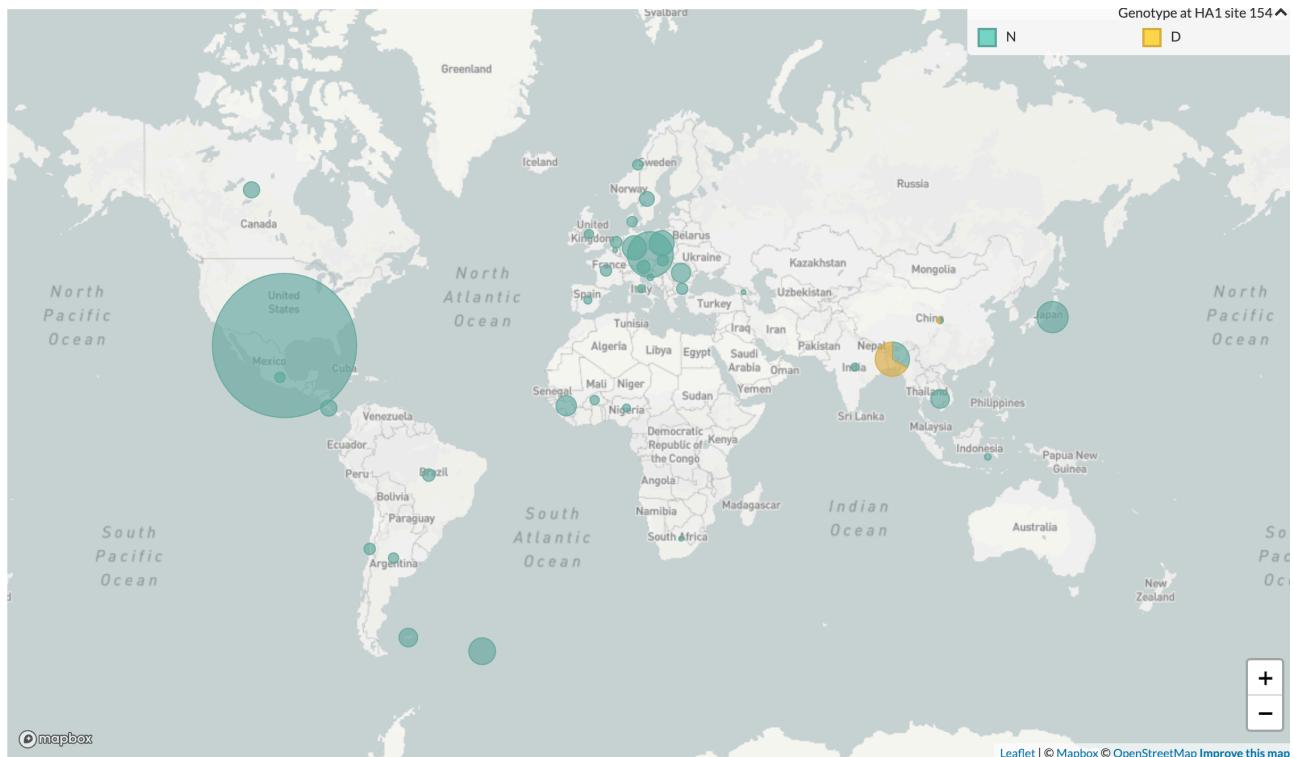
2.3.4.4b: HA1 site 136 - sequences from this reporting period and submitted to OFFLU



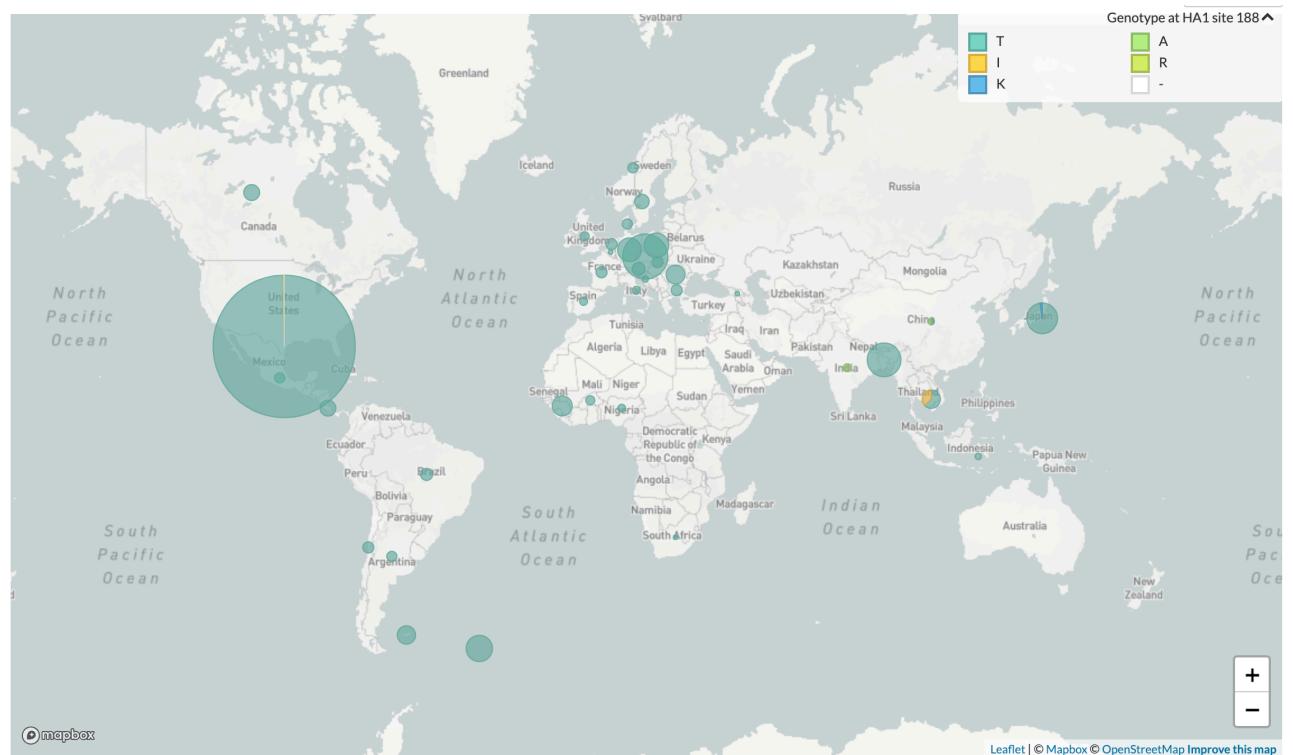
2.3.4.4b: HA1 site 156 - sequences from this reporting period and submitted to OFFLU



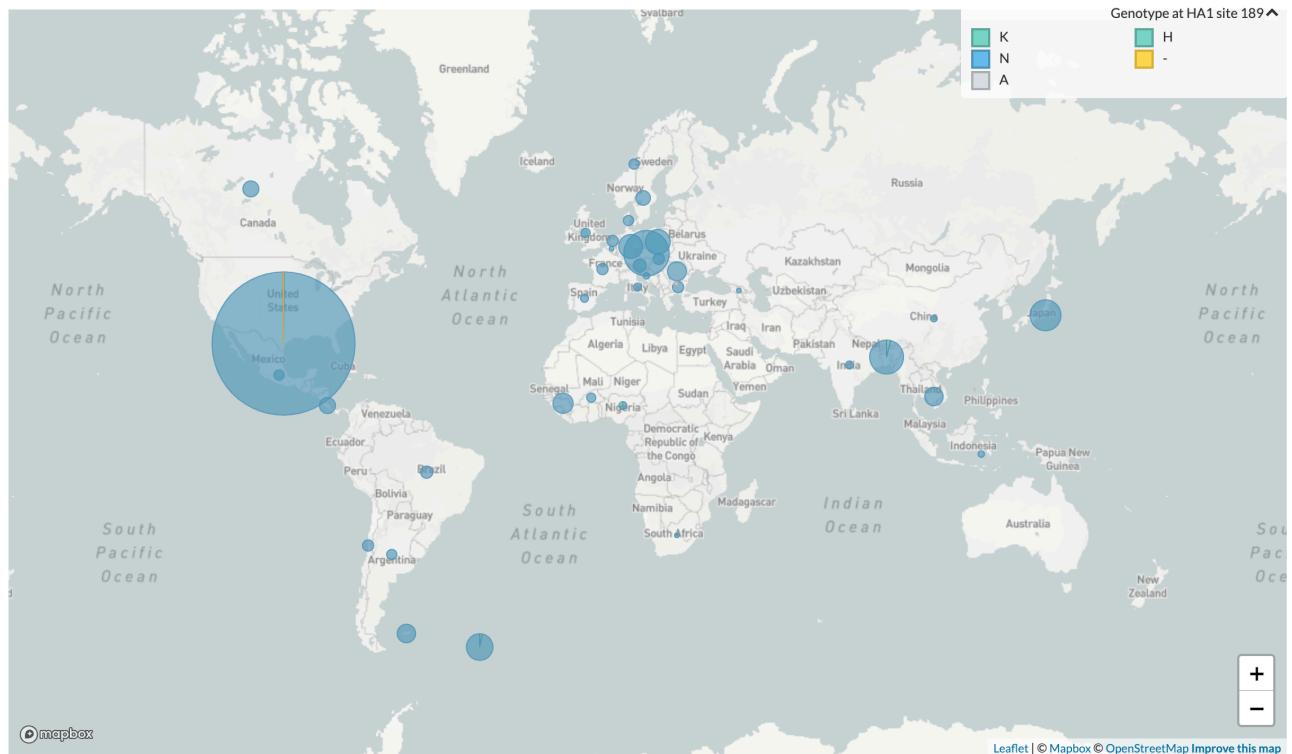
2.3.4.4b: HA1 site 154 - sequences from this reporting period and submitted to OFFLU



2.3.4.4b: HA1 site 188 - sequences from this reporting period and submitted to OFFLU



2.3.4.4b: HA1 site 189 - sequences from this reporting period and submitted to OFFLU



2.3.4.4b: HA1 site 189 - sequences since 2020

