



# OFFLU AVIAN DATA PACKAGE FOR ZONOTIC INFLUENZA COMPONENT OF THE VACCINE COMPOSITION MEETING

September 2023 to February 2024

## SCOPE

In this document we present a summary of avian influenza A virus events reported from 1st September 2023 to 14th February 2024 and phylogenetic analysis of H3, H5, H7, H9 and H10 viruses.

## Contents

---

Introduction, data sources, methods and acknowledgements .....	3
Global avian influenza events in animals.....	4
Activity Table (H5) viruses.....	6
H5 Influenza A viruses .....	7
H5 Phylogenetic tree coloured by region .....	7
H5 2.3.4.4b phylogenetic tree .....	9
H5 2.3.4.4b subsampled tree .....	10
H5 2.3.4.4b Amino acid difference table .....	11
H5 2.3.4.4b Antigenic data.....	12
H5 2.3.2.1a phylogenetic tree .....	13
H5 2.3.2.1a amino acid difference table.....	14
H5 2.3.2.1a antigenic data .....	15
Avian H3 Influenza A viruses .....	16
H3 phylogenetic tree .....	16
H3 amino acid difference table.....	17
H3 antigenic data .....	18
H7 Influenza A viruses .....	19
H7 Phylogenetic analysis .....	19
H7 Amino acid difference table .....	20
H9 Influenza A viruses .....	21
H9 Y280 phylogenetic tree .....	21
H9 G1 phylogenetic tree.....	22
H9 G1 amino acid difference table.....	23
H9 G1 antigenic data.....	24
H10N5 .....	25
Annex.....	26

## 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: Australian Centre for Disease Preparedness (Australia), Agence National de Sécurité de Sanitaire (France), Animal and Plant Health Agency (UK), Animal and Plant Quarantine Agency (Rep. of Korea), Bangladesh Livestock Research Institute (Bangladesh), Brazilian Society for Virology (Brazil), Canadian Food Inspection Agency (Canada), Chittagong Veterinary and Animal Sciences University (Bangladesh), Federal Centre for Animal Health (Russia), Harbin Veterinary Research Institute (China), Istituto Zooprofilattico Sperimentale delle Venezie (Italy), Hokkaido University (Japan), The National Institute of Agricultural Technology (Argentina), National Institute of High Security Animal Diseases (India), National Lab of Veterinary Control on Poultry (Egypt), Laboratório Federal de Defesa Agropecuária (Brazil), United States Department of Agriculture National Veterinary Services Laboratories (USA) and 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 ACDP, APHA and 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 September 2023 and 29th January 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 (Kato 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.

# Global avian influenza events in animals

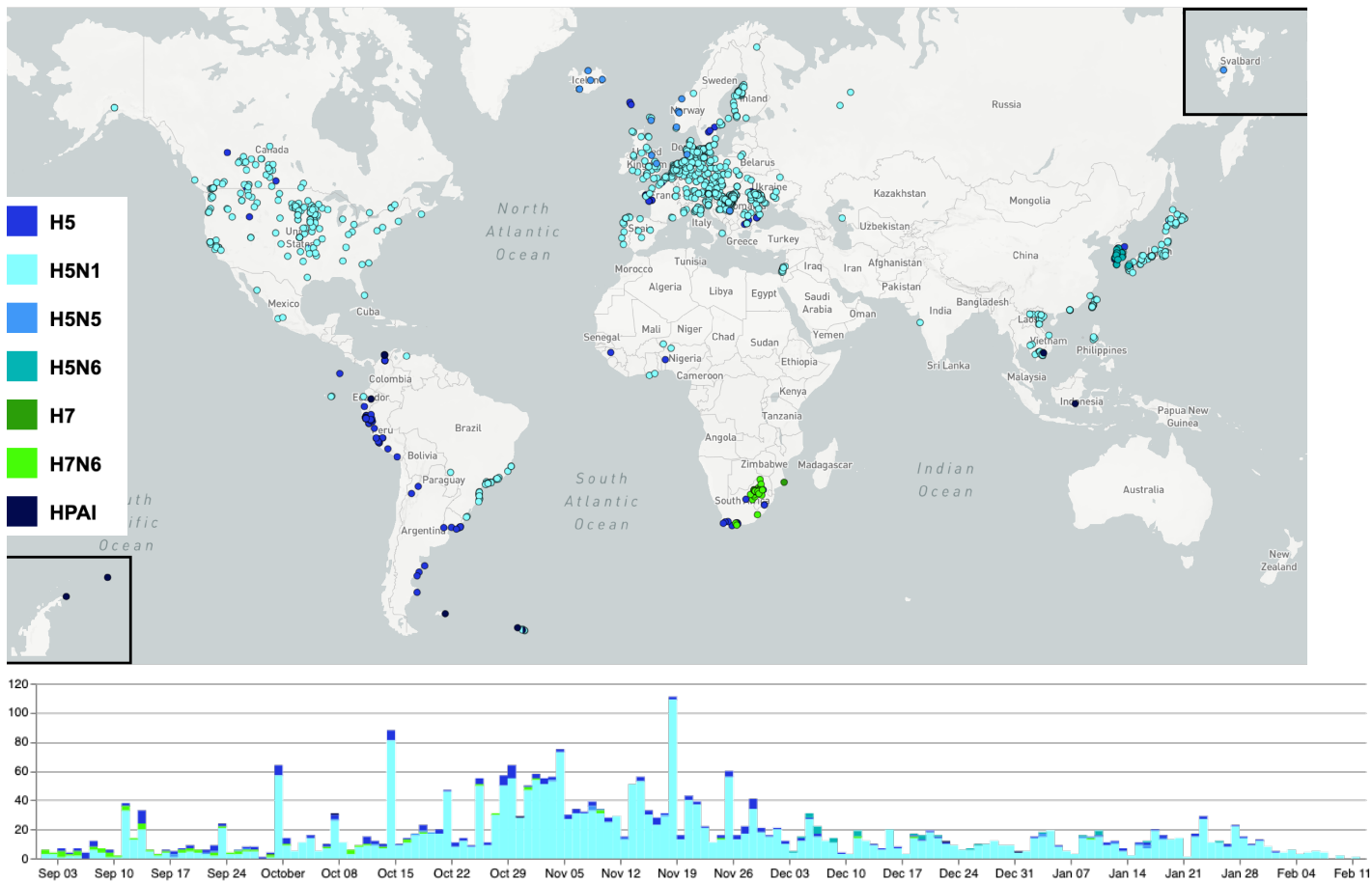


Figure 1: Microreact map showing the geographical and temporal distribution of Gs/Gd H5 and HPAI H7 avian influenza outbreaks reported to WOA via the WAHIS platform and to national authorities gathered through FAO EMPRES-i. Data shown falls within the reporting period of 1st September 2023 to present (last updated 14th February 2023). Points are coloured by subtype and the outbreak date is represented along the bottom timeline.

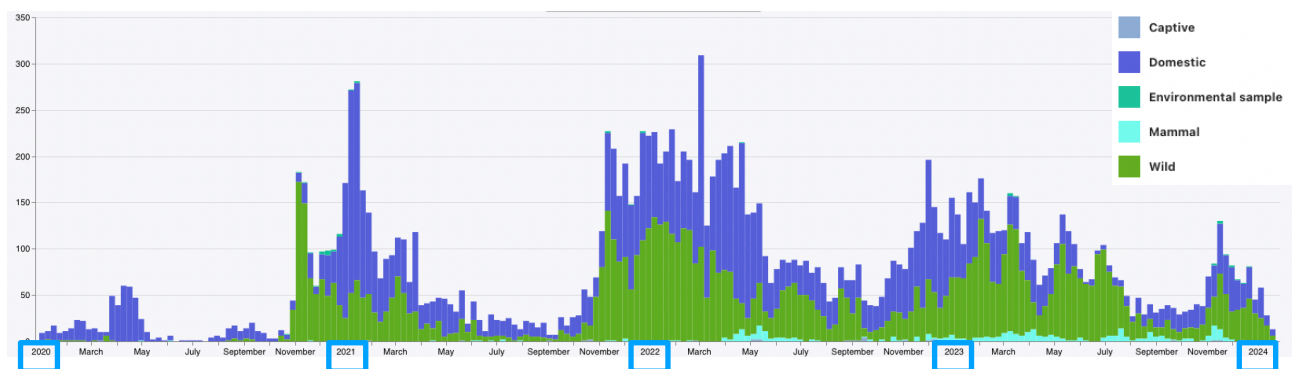


Figure 2: Timeline of number of HPAI outbreaks from 2020 to present coloured by type. Includes outbreaks reported to WOA via the WAHIS platform and to national authorities gathered by FAO EMPRES-i.

## Global AI epidemiology in animals

Since the emergence of the Gs/Gd lineage of high pathogenicity avian influenza (HPAI), H5 viruses have undergone significant evolutionary changes resulting in the emergence of various distinct clades, some of which have become endemic in poultry populations in certain regions. Globally, there has been dominance of the H5N1 clade 2.3.4.4b viruses since Autumn of 2021. Apart from this H5N2, H5N4, H5N5 H5N6 and H5N8 subtypes have all been sporadically detected in poultry or wild birds. H5N1 clade 2.3.4.4b viruses have spread through Europe, Asia, Africa and the Americas, displacing some other clade viruses and reassorting with local low pathogenic avian influenza viruses to produce a diverse range of genotypes which exhibit variable and species dependant pathogenicities.

Uncharacteristically, since 2021, HPAI events remained relatively elevated over the northern hemisphere's summer months although reduced detections were reported in the summer months prior to the 2023/2024 season, compared to the same period of 2022/2023. Since 2021 there has been an increase in the frequency of detections among non-avian species including both wild terrestrial and marine mammals, as well as farmed, captive and domestic animals. Among affected wild mammals, a prevalent pattern emerges among scavengers and those with potential proximity to infected birds. Since September 2023 there continues to be outbreaks in poultry in many countries, including in under-surveilled regions. In at least the USA and Canada, Europe and Korea there have been less outbreaks reported in poultry, wild birds and mammals than for the same period in the previous 2 years. The peak of reported outbreaks occurred in November. Of note, within the reporting period the first introductions of H5N1 clade 2.3.4.4b were reported in both the Galapagos and the Arctic in October 2023. Additionally, unusually large die offs of sea lions, fur seals and elephant seals were reported along the east coast of South America, with large losses of elephant seal pups. Die offs weren't necessarily concurrent with reports of extensive mortality in wild birds. The risk of AI incursion into other islands in the Antarctic Peninsula is considered high, and incursion into Oceania is possible.

In Canada and Alaska a few outbreaks have been caused by viruses introduced from the Pacific flyway in 2022 onwards. However, the majority of viruses detected in North America form a monophyletic group, introduced from the North Atlantic flyway in late 2021 and establishing themselves in wild bird populations. Outbreaks in wild birds and domestic poultry are reduced since the summer months compared with the same period in the previous year. In Central and South America HPAI was introduced from North America in mid 2022 and has spread south eastwardly. Since September 2023, outbreaks continue to be reported, particularly in backyard flocks. In Europe, outbreaks continue to be reported in poultry although at a lower rate compared to the same reporting period in 2022/2023. There has been a decrease in detections of AI in seabirds and increase in anseriformes accompanied by a change in the frequency of genotypes detected. H5N5 viruses have been detected in seabirds and scavenging birds in Northern Europe, Japan and Eastern Canada, clustering with older H5N5 viruses, suggesting potential maintenance in an under-surveilled population.

In Asia, countries continue to report outbreaks of H5N1 clade 2.3.4.4b which were introduced in 2020 and 2021, in addition to endemic circulation other clades including of the 2.3.2.1 lineages. Co circulating viruses of different subtypes and clades can be routinely found in Live Bird Markets in many countries in Asia. In Japan and South Korea, novel clade 2.3.4.4b H5N6 viruses have been detected in wild birds and caused outbreaks in poultry, albeit fewer than in previous years. The HA and NA are phylogenetically close to H5N6 clade 2.3.4.4b viruses detected previously in China however there is a paucity of information on this subtype and warrants further investigation to monitor spread. From available data the majority of viruses sequenced from Africa are derived from incursions from Europe in 2021 which have maintained in poultry and wild birds in parts of Sub Saharan Africa. In early 2024, further outbreaks have been reported in Sub Saharan Africa, possibly from new incursions from Europe. In North Africa endemic H5N8 continue to co circulate with H5N1.

H3N8 viruses which were found in poultry in markets China in 2022 have reassorted, giving rise to H3N3 viruses. H3N2 viruses of the 2010.1 and 2010.2 swine lineage have been detected in Turkeys in the USA.

LPAI H7 viruses have been detected in multiple countries in wild birds and environmental samples and occasionally in poultry, including in Taiwan and the Republic of Korea within this reporting period. Newly emerged HPAI from H7 viruses of Eurasian lineage have caused continuing outbreaks in domestic poultry in South Africa since June 2023 and spread to Mozambique in September 2023.

Outbreaks of AI in poultry caused by H9 viruses are not reportable to WOA. Zoonotic lineage H9 G1 or Y280 lineage viruses are known to have infected poultry in many countries including, but not limited to: Brazil, Cambodia, Egypt, Europe, Ghana, India, Indonesia, Morocco, Nepal, Niger, Nigeria, Pakistan, Russian Federation, Saudi Arabia, Taiwan Province of China, The People's Republic of China, The Republic of Korea, Viet Nam.

## Activity Table (H5) viruses

See Table 1 of [Genetic and antigenic characteristics of zoonotic influenza A viruses and development of candidate vaccine viruses for pandemic preparedness February 2024](#)

# H5 Influenza A viruses

H5 Phylogenetic tree coloured by region

Region ^

- |  |  |
|--|--|
| <span style="color: yellow;">■</span> Africa             | <span style="color: purple;">■</span> North America    |
| <span style="color: cyan;">■</span> Western Europe       | <span style="color: red;">■</span> East Asia           |
| <span style="color: darkgreen;">■</span> Central Europe  | <span style="color: orange;">■</span> South Asia       |
| <span style="color: purple;">■</span> South America      | <span style="color: darkred;">■</span> South East Asia |
| <span style="color: lightgreen;">■</span> Eastern Europe | <span style="color: purple;">■</span> Antarctica       |

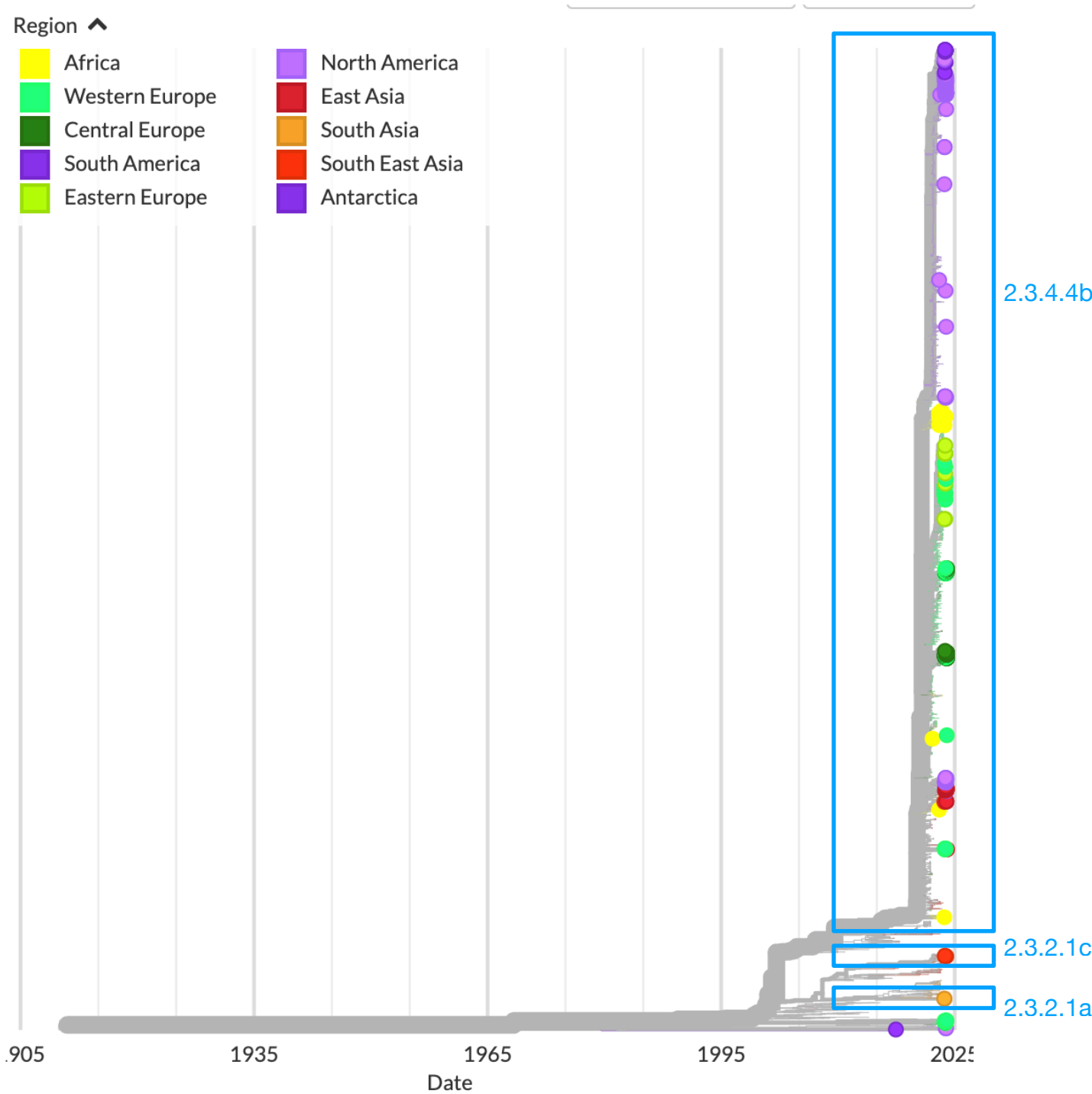


Figure 4: Avian H5 global summary time resolved phylogenetic tree. Analyses were conducted with reference sequences, sequences shared through the OFFLU network and data downloaded from GISAID (25th January 2024). Sequences from GISAID collected within the reporting period and submitted to OFFLU were coloured by geographic region.

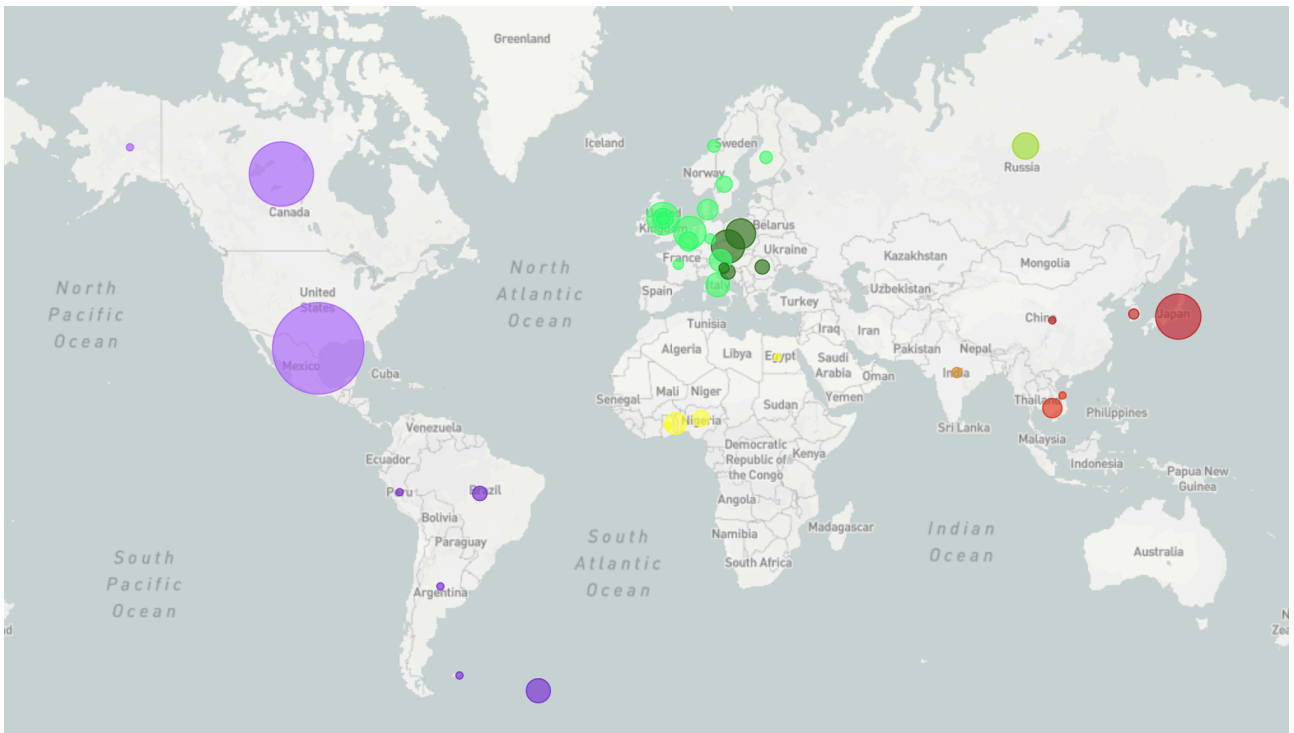


Figure 5: A map showing countries where H5 sequences collected within the reporting period were retrieved from GISAID, or countries which submitted sequences to OFFLU.



## H5 2.3.4.4b phylogenetic tree

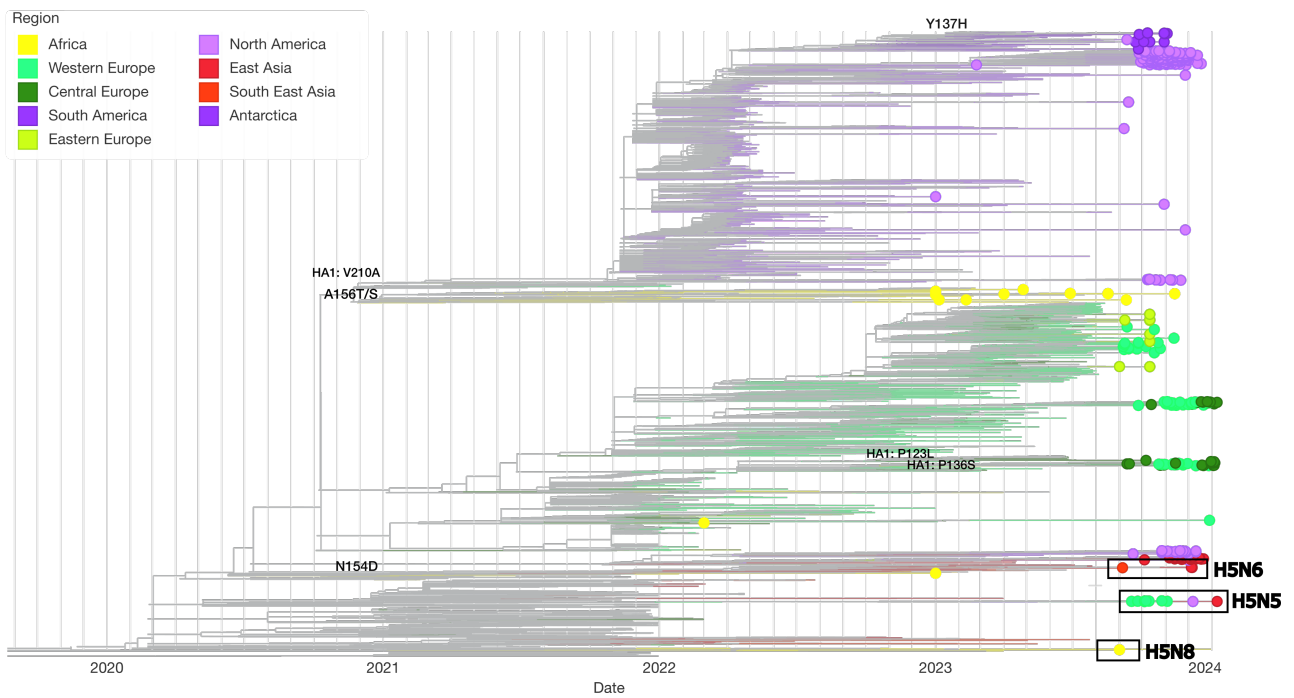


Figure 6: Avian H5 clade 2.3.4.4b time resolved phylogenetic tree. Analyses were conducted with reference sequences, sequences shared through the OFFLU network and data downloaded from GISAID (25th January 2024). Sequences from GISAID collected within the reporting period and submitted to OFFLU were coloured by geographic region. Amino acid changes in putative antigenic sites were annotated on the branches.

H5 2.3.4.4b subsampled tree

Sept2023-Feb2024  
 Feb2023-Sept2023  
 Human  
 CVV

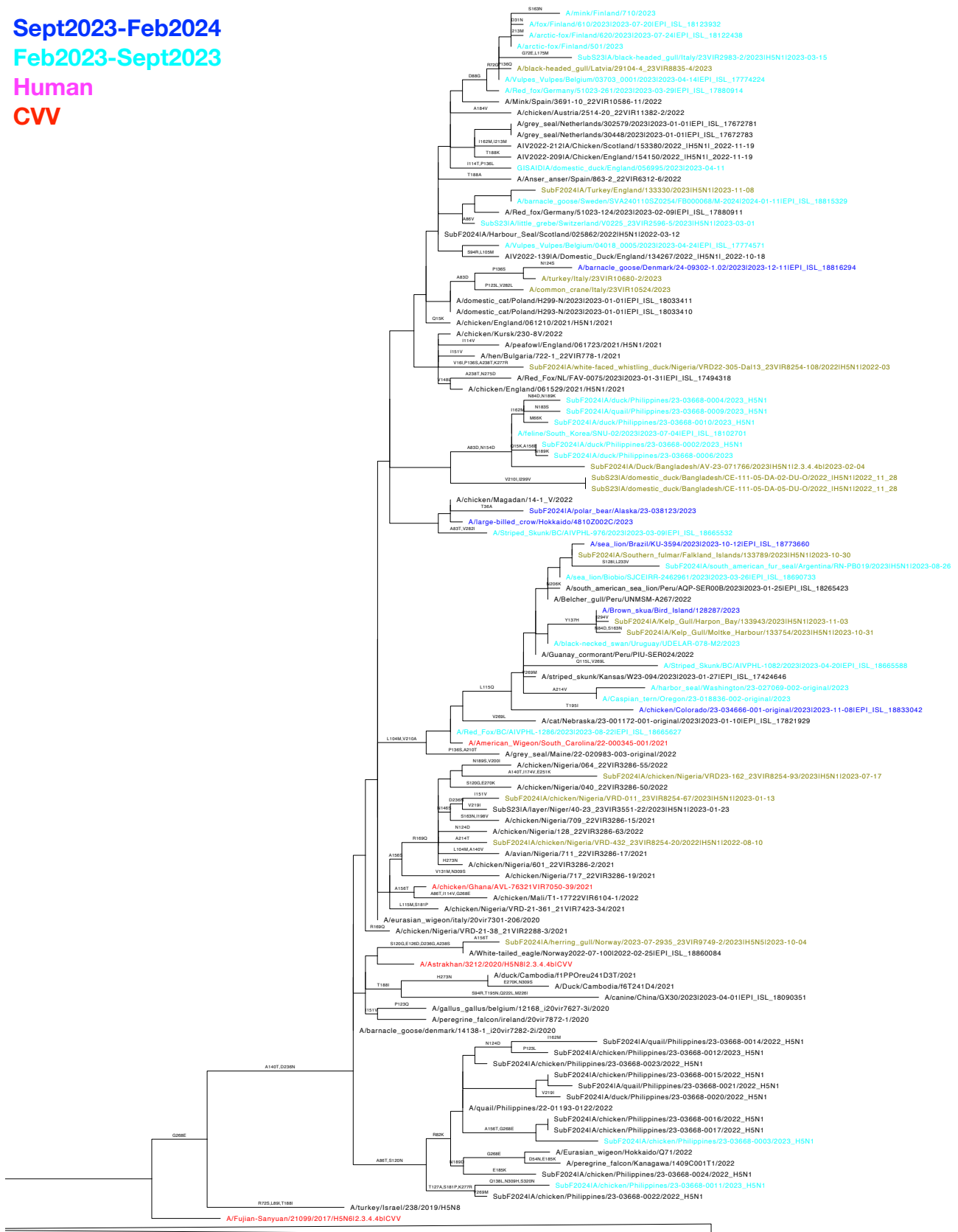


Figure 7: Avian H5 clade 2.3.4.4b subsampled time resolved phylogenetic tree. Mammalian strains, strains which have been antigenically characterised and CVVs were retained. The tree was downsized using PARNAS. Amino acid annotations are included along the branches.

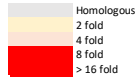
# H5 2.3.4.4b Amino acid difference table

Table 2: Avian H5 2.3.4.4b clade sequence comparison to the within clade CVVs of a subsection of sequences submitted to OFFLU for antigenic characterisation. Sequences antigenically characterised are yellow. Sequences collected within this reporting period are blue, the previous reporting period light blue and the CVV red. Changes in putative antigenic sites are highlighted grey. Genetic changes are annotated on the right hand side.

Site	Genetic Changes	Putative Antigenic Site
15	Q	
16	V	
66	M	
72	R	
82	A	
83	A	
84	N	Antigenic
86	A	
88	D	
104	L	
115	L	
120	S	
123	P	Antigenic
124	N	
126	E	
127	T	
136	P	Antigenic
137	Y	Antigenic
138	Q	Antigenic
140	A	Antigenic
146	N	
151	I	Antigenic
154	N	Antigenic
156	A	Antigenic
162	I	
163	S	
169	R	
174	I	
181	S	Antigenic
183	N	Antigenic
185	E	Antigenic
189	N	Antigenic
210	V	Antigenic
214	A	Antigenic
219	V	
236	D	
238	A	
251	E	
268	G	
269	V	
277	K	Antigenic
282	V	
294	I	
299	I	
309	N	
320	S	
Aadiffs	3 3 2 2 2 2 6 3 5 0 5 5 3 3 2 5 5 3 4 4 5 5 5 4 4 8 5 5 3 5 5 4 3 6 3 5	
Putative	1 3 1 1 1 1 3 1 2 0 1 1 1 1 1 2 2 2 2 2 2 1 3 3 2 1 4 1 0 0 1 1 0 0 3 0 2	

# H5 2.3.4.4b Antigenic data

Table 3: Avian H5 2.3.4.4b antigenic characterisation of viruses against CVV and reference post infection antisera. clade sequence comparison to the within clade CVVs of a subsection of sequences submitted to OFFLU for antigenic characterisation. Fold changes are coloured. Amino acid changes in test viruses compared to the Astrakhan CVV are annotated on the right hand side. Putative antigenic sites are in bold.



Semester	Reference Antigen	Clade	Subtype	REFERENCE FERRET ANTISERA										AA changes from 3212 putative antigenic sites
				H5N6	H5N8	H5N8	H5N8	H5N8	H5N1	H5N1	H5N1	H5N1	H5N1	
APHA Sep23- Feb24	A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	2.3.4.4b	HSN6	160	80	320	80	80	320	320	640	640	20	
	A/ASTRAKHAN/3212/2020	2.3.4.4b	HSN8	10	160	320	160	320	20	320	320	20	640	
	A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	HSN8	20	40	640	320	320	160	640	160	320	320	
	A/duck/Cambodia/11PPPreu241D3T/2021	2.3.4.4b	HSN8	40	80	640	320	160	320	640	320	640	640	
	A/turkey/Israel/238/2019	2.3.4.4b	HSN8	20	40	160	80	320	160	640	160	160	160	
	A/poultry/Niger/ET3/HALAL/21VIR2131-33/2021	2.3.4.4b	HSN1	10	20	160	40	40	640	320	320	320	320	
	A/chicken/Ghana/AVL-763/21VIR7050-39/2021	2.3.4.4b	HSN1	0	20	320	320	160	320	640	80	640	640	
	A/hen/Bulgaria/722-1/22VIR778-1/2021	2.3.4.4b	HSN1	10	80	40	40	40	1280	640	640	640	640	
	A/avian/Togo/904/21VIR5115-6/2021	2.3.4.4b	HSN1	20	40	160	160	80	320	320	80	640	640	
	<b>Test antigen</b>													
1	A/Herring_gull/England/404234/2023	2.3.4.4b	HSN5	10	80	160	160	40	1280	320	160	640	5120G, E126D, A156T, D236N, A238S	
2	A/Duck/Bangladesh/CE-111-05-DA-05-DU-O/2022	2.3.4.4b	HSN1	20	80	160	80	80	640	640	320	1280	V210I, I299V	
3	A/Duck/Bangladesh/CE-111-05-DA-02-DU-O/2022	2.3.4.4b	HSN1	40	160	160	160	80	2560	1280	640	1280	V210I, I299V	
4	A/Kelp_Gull/Moltke_Harbour/133754/2023	2.3.4.4b	HSN1	8	8	80	16	16	320	320	320	1280	N84D, L104M, L115Q, Y137H, S163N, V210A	
5	A/Southern_Fulmar/Falkland_Islands/133789/2023	2.3.4.4b	HSN1	10	20	40	40	20	640	320	320	640	L104M, L115Q, V210A	
6	A/Kelp_Gull/Harpon_Bay/133943/2023	2.3.4.4b	HSN1	0	20	20	40	40	1280	640	640	1280	L104M, L115Q, Y137H, V210A, I294V	
7	A/Turkey/England/133330/2023	2.3.4.4b	HSN1	20	160	80	80	80	640	640	160	1280		
8	A/Duck/Bangladesh/AV-23-071766/2023	2.3.4.4b	HSN1	10	80	320	160	80	640	640	40	1280	A83D, N154D	
IZSVe Sep23- Feb24	A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	2.3.4.4b	HSN6	80	160	640	320	320	2560	1280	2560	2560	80	
	A/ASTRAKHAN/3212/2020	2.3.4.4b	HSN8	160	320	1280	640	320	5120	1280	1280	2560	320	
	A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	HSN8	20	80	320	160	160	640	640	640	1280	40	
	A/duck/Cambodia/11PPPreu241D3T/2021	2.3.4.4b	HSN8	40	80	640	320	320	1280	1280	1280	2560	40	
	A/turkey/Israel/238/2019	2.3.4.4b	HSN8	20	40	320	160	320	640	1280	640	1280	20	
	A/poultry/Niger/ET3/HALAL/21VIR2131-33/2021	2.3.4.4b	HSN1	20	80	160	80	80	1280	640	320	1280	80	
	A/chicken/Ghana/AVL-763/21VIR7050-39/2021	2.3.4.4b	HSN1	10	40	320	640	320	640	1280	160	5120	40	
	A/hen/Bulgaria/722-1/22VIR778-1/2021	2.3.4.4b	HSN1	<10	40	160	80	80	2560	2560	2560	2560	160	
	A/avian/Togo/904/21VIR5115-6/2021	2.3.4.4b	HSN1	10	80	320	320	160	640	640	80	2560	40	
	A/american_wigeon/South Carolina/22-000345-001/2021	2.3.4.4b	HSN1	80	320	2560	1280	640	10240	2560	2560	5120	160	
<b>Test antigen</b>														
1	A/chicken/Nigeria/VRD-011_23VIR8254-67/2023	2.3.4.4b	HSN1	<10	40	80	80	40	160	320	40	320	20	A146S, I15V, A156S, R169Q, D236N,
2	A/chicken/Nigeria/VRD23-162_23VIR8254-93/2023	2.3.4.4b	HSN1	<10	80	160	80	80	160	320	40	640	20	A140T, A156S, R169Q, I174V, E251K
3	A/chicken/Nigeria/VRD-432_23VIR8254-20/2022	2.3.4.4b	HSN1	18	80	640	640	320	640	1280	160	2560	40	A156S, R169Q, A214T
4	A/white-faced_whistling-duck/Nigeria/VRD23-305-Dal13_23VIR8254-10I	2.3.4.4b	HSN1	40	160	1280	640	320	5120	1280	1280	5120	80	V16I, P136S, A238T, K277R
5	A/black-headed_gull/Latvia/29104-4_23VIR8835-4/2023	2.3.4.4b	HSN1	40	80	640	160	320	1280	1280	640	2560	80	R72G, D88G, P163Q
6	A/common-crane/Italy/23VIR10524-3/2023	2.3.4.4b	HSN1	40	160	640	320	160	2560	1280	640	2560	40	A83D, P123L, V282L
7	A/turkey/Italy/23VIR10680-2/2023	2.3.4.4b	HSN1	<10	80	80	<10	40	1280	1280	640	1280	80	A83D, P123S
8	A/herring-gull/Norway/2023-07-2935_23VIR9749-2/2023	2.3.4.4b	HSN5	10	40	160	320	160	640	1280	160	1280	40	S120G, E126D, A156T, D236G, A238S
ACDP Sep23- Feb24	A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	2.3.4.4b	HSN6	160	80	160	320	40				80		
	A/ASTRAKHAN/3212/2020	2.3.4.4b	HSN8	<20	160	320	160	80				80		
	A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	HSN8	<20	40	80	320	20				80		
	A/duck/Cambodia/11PPPreu241D3T/2021	2.3.4.4b	HSN8	<20	80	80	160	40				80		
	A/turkey/Israel/238/2019	2.3.4.4b	HSN8	<20	20	80	320	80				40		
<b>Test antigen</b>														
1	A/duck/Philippines/23-03668-0006/2023	2.3.4.4b	HSN1	<20	<20	<20	40	20				<20	Q15K, A83D, N154D, A156E, N189K	
2	A/chicken/Philippines/23-03668-0011/2023	2.3.4.4b	HSN1	20	40	80	320	20				80	A86T, S120N, T127A, Q138L, S181P, K277R, N309H, S320N	
3	A/chicken/Philippines/23-03668-0012/2023	2.3.4.4b	HSN1	20	40	80	160	40				80	R82X, A86T, S120N, P123L, N124D	

# H5 2.3.2.1a phylogenetic tree

Sept2023-Feb2024  
 Feb2023-Sept2023  
 Human  
 CVV



Figure 8: Avian H5 2.3.2.1a maximum likelihood phylogenetic tree. Analyses were conducted with representative reference sequences downloaded from GISAID and downsampled using PARNAS, to which submissions to OFFLU were added. CVVs are coloured red, data within the reporting period dark blue, the previous reporting period light blue and human cases pink.

## H5 2.3.2.1a amino acid difference table

Table 4: Avian H5 2.3.2.1a clade sequence comparison to the within clade CVVs. Sequences from this reporting period are blue, the previous reporting period light blue and the CVVs red. Changes in putative antigenic sites are highlighted grey and changes found in the CDC genetic changes inventory are annotated.

Site	A/duck/Bangladesh/17D1012/2018   2.3.2.1a   CW	A/duck/Bangladesh/19097/2013   2.3.2.1a   CW	A/Hubei/1/2010   2.3.2.1a   CW	A/domesticduck/Bangladesh/CE-111-04-DA-05-DU-C/2022   H5N1	A/domesticduck/Bangladesh/CE-113-04-DA-05-DU-O/2022   H5N1	A/domesticduck/Bangladesh/CE-115-04-DA-02-DU-O/2022   H5N1	A/chicken/Bangladesh/CE-113-04-CB-25-BR-O/2022   H5N1   2022-C	A/chicken/Bangladesh/CE-113-01-02-08-DU-O/2021   H5N1   -2021	A/chicken/Bangladesh/CE-116-01-02-06-DU-O/2021   H5N1   -2021	A/domesticduck/Bangladesh/CE-111-04-DA-03-DU-O/2022   H5N1	A/domesticduck/Bangladesh/CE-111-04-DA-04-DU-O/2022   H5N1	A/domesticduck/Bangladesh/CE-116-04-DA-06-DU-O/2022   H5N1	A/chicken/Bangladesh/CE-113-03-02-05-DU-O/2022   H5N1   -2022	A/Bback_swami/India/09T01-03/2023   H5N1   2023-09-05	A/chicken/Bangladesh/CE-115-02-01-03-DU-O/2021   H5N1   -2021	A/Goose/Bangladesh/AV-23-071813/2023-02-05	A/Duck/Bangladesh/AV-23-071662/2023-01-01	A/Commercial_Layer/Bangladesh/AV-23-072159/2023-03-20	A/Duck/Bangladesh/AV-23-072171/2022-11-10	A/Duck/Bangladesh/AV-23-071982/2023-03-19	A/Duck/Bangladesh/AV-23-071928/2023-02-14	A/domesticduck/Bangladesh/CE-116-04-DA-02-DU-O/2022   H5N1	A/domesticduck/Bangladesh/CE-116-04-DA-05-DU-O/2022   H5N1	A/Duck/Bangladesh/AV-23-072326/2022-11-26	A/duck/Bangladesh/17D1012/2018   2.3.2.1a   CW	Site	Putative antigenic site		
8	H			Y																							H	8	
14	K	E		E															E	E	E						K	14	
16	V											I		X													V	16	
20	M													X													M	20	
21	E													X													E	21	
22	K													X													K	22	
23	N													X													N	23	
24	V													X													V	24	
25	T											S															T	25	
31	D									N																	D	31	
36	T																		A								T	36	
40	K				M																						K	40	Antigenic
42	C																										C	42	Antigenic
43	D												N														D	43	Antigenic
48	K																						I				K	48	Antigenic
83	A												V														A	83	
88	G	D																									G	88	
93	G																								A		G	93	
94	N													S						S					X		N	94	
114	I													M													I	114	
115	R	Q														Q											R	115	
120	D	N											E														D	120	
124	D	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	D	124	
127	A												V														A	127	Antigenic
136	P					S																					P	136	Antigenic
140	N	K																									N	140	Antigenic
141	S				P								P	P													S	141	Antigenic
154	N	D	D	D	D	D	D	D	X	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D		N	154	Antigenic
156	A	T				S							T	S													A	156	Antigenic
159	T																										T	159	
162	K																				R						K	162	
163	S	G																				N	N				S	163	
174	I	V				X																					I	174	
175	L											X										M	M				L	175	
183	D												N														D	183	Antigenic
184	E												D														E	184	Antigenic
188	T															A											T	188	Antigenic
189	R					N	K						N	N								Q	Q	Q			R	189	Antigenic
200	I					V																					I	200	
210	V																										V	210	Antigenic
212	K																										K	212	
215	T																										T	215	Antigenic
217	S																										S	217	
273	N																										N	273	Antigenic
276	T									A																	T	276	Antigenic
277	R																										R	277	Antigenic
309	N																										N	309	
	Aadiffs from CVV	2	8	2	2	3	6	4	3	2	3	3	5	14	10	3	2	3	4	5	6	8	3	5					
	Putative Antigenic Sites	2	2	1	1	1	4	2	2	1	1	1	1	8	4	1	1	2	1	2	2	3	1	1					

D: Decreased virulence in mice, transmissible among ferrets

I: Increased virus binding to α2-6.

## H5 2.3.2.1a antigenic data

Table 5: Avian H5 2.3.2.1a antigenic characterisation of viruses against CVVs. Fold changes are coloured. Amino acid changes in test viruses compared to the closest CVV are annotated on the right hand side. Putative antigenic sites are in bold.

Grey	Homologous
Yellow	2 fold
Orange	4 fold
Red	8 fold
Dark Red	> 16 fold

REFERENCE FERRET ANTISERA		Clade	Subtype	A/HUBEI/1/2010	A/DUCK/BANGLADESH/19097/2013	A/DUCK/BANGLADESH/17D1012/2018	
		2.3.2.1a	H5N1	H5N1	H5N1	H5N1	
		Ferret ID					
Semester	Reference Antigen	Clade	Subtype				
	A/HUBEI/1/2010	2.3.2.1a	H5N1	2560	320	80	
	A/DUCK/BANGLADESH/19097/2013	2.3.2.1a	H5N1	1280	320	80	
	A/DUCK/BANGLADESH/17D1012/2018	2.3.2.1a	H5N2	160	80	320	
	Test antigen						
	1 A/domesticduck/Bangladesh/CE-113-04-DA-05-DU-O/2022	2.3.2.1a	H5N1	320	40	20	D124N, <b>N154D</b>
	2 A/chicken/Bangladesh/CE-113-04-CB-25-BR-O/2022	2.3.2.1a	H5N1	0	0	20	K14E, <b>S141P, A156S, R189N, V210I, K212R</b>
	3 A/domesticduck/Bangladesh/CE-111-04-DA-05-DU-O/2022	2.3.2.1a	H5N1	640	80	40	D124N, <b>N154D</b>
	4 A/domesticduck/Bangladesh/CE-111-04-DA-05-DU-C/2022	2.3.2.1a	H5N1	640	80	40	D124N, <b>N154D</b>
	5 A/domesticduck/Bangladesh/CE-111-04-DA-03-DU-O/2022	2.3.2.1a	H5N1	640	80	40	D124N, <b>N154D</b>
	6 A/domesticduck/Bangladesh/CE-111-04-DA-04-DU-O/2022	2.3.2.1a	H5N1	160	40	40	D31N, D124N
	7 A/domesticduck/Bangladesh/CE-115-04-DA-02-DU-O/2022	2.3.2.1a	H5N1	40	0	40	<b>K40M</b> , D124N, I200V
	8 A/chicken/Bangladesh/CE-113-01-02-08-DU-O/2021	2.3.2.1a	H5N1	1280	320	80	8HY, <b>D124N, P136S, N154D</b>
	9 A/domesticduck/Bangladesh/CE-116-04-DA-06-DU-O/2022	2.3.2.1a	H5N1	640	80	80	V16I, D124N, <b>N154D</b>
	10 A/chicken/Bangladesh/CE-111-06-DB-17-BR-C/2023	2.3.2.1a	H5N1	20	20	160	Q30P, D31N, S163N, L175M, <b>R189Q, K187R, N248D</b>
	11 A/chicken/Bangladesh/CE-116-01-02-06-DU-O/2021	2.3.2.1a	H5N1	160	10	160	<b>R189K, T276A</b>

AA changes compared to 17D1012  
putative antigenic sites

# Avian H3 Influenza A viruses

## H3 phylogenetic tree

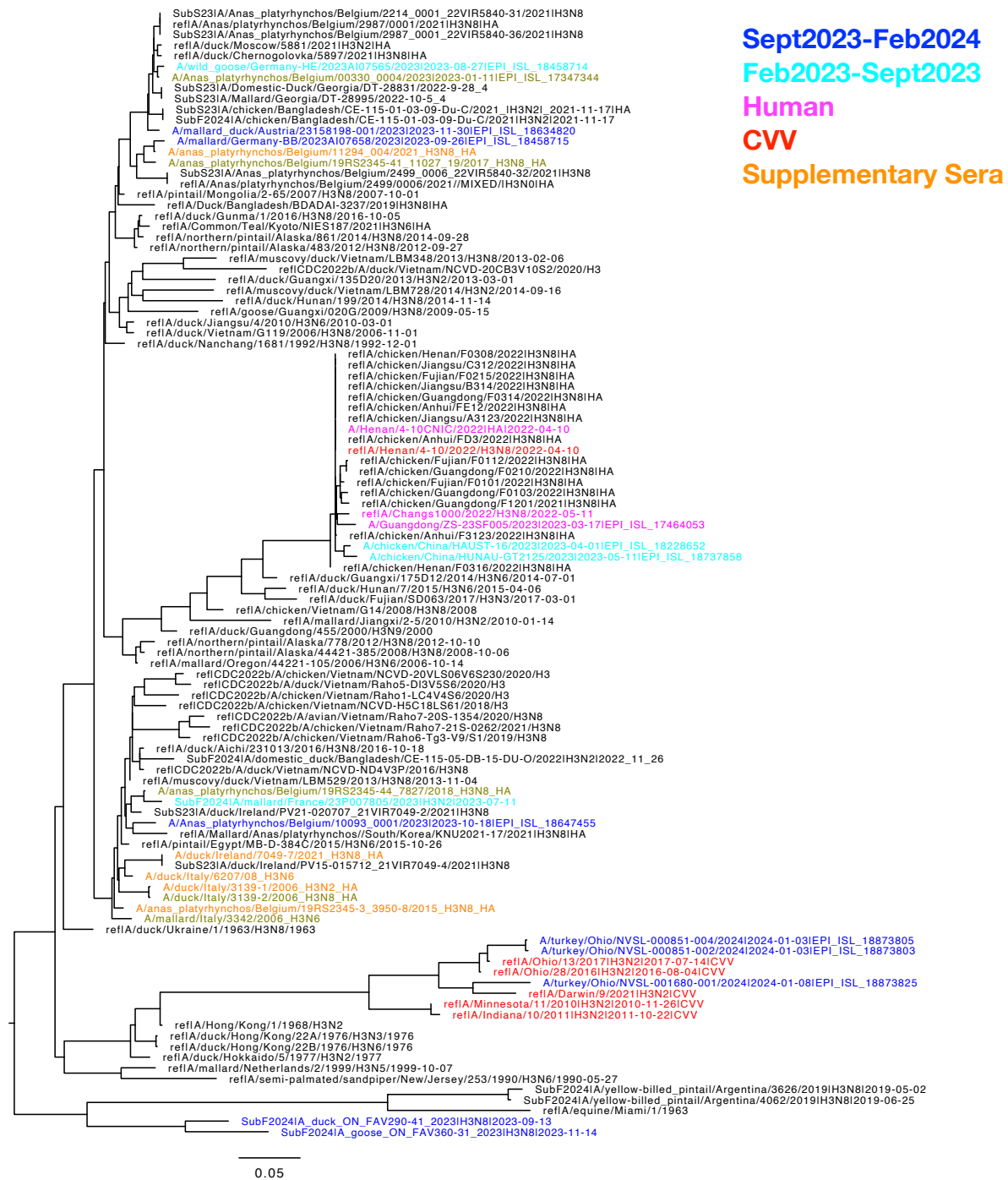


Figure 9: Avian H3 maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences kindly shared by HKU, sequences shared through the OFFLU network and data downloaded from GISAID (12th February 2024).



### H3 amino acid difference table

Table 6: Avian H3 clade sequence comparison to the CVV. Sequences from this reporting period are blue, the previous reporting period light blue and the CVVs red. Changes in putative antigenic sites are highlighted grey.

Site	A/Henan/4-10CNIC/2022 HAI 2022-04-10	A/chicken/China/HAUUST-16/2023 2023-04-01 EPI_ISL_18228652	A/chicken/China/HUNAU-GT2125/2023 2023-05-11 EPI_ISL_1873785€	A/duck/Italy/3169-1/2006_H3N2_HA	A/duck/Italy/6207/06 H3N6	A/Anas platyrhynchos/Belgium/11294_004/2021_H3N8_HA	A/Anas platyrhynchos/Belgium/19RS2345-3_3950-8/2015_H3N8_HA	A/duck/Ireland/7049-7/2021_H3N8_HA	A/mallard/Italy/3342/2006 H3N6	A/Anas platyrhynchos/Belgium/19RS2345-44_7827/2018_H3N8_HA	A/duck/Italy/3169-2/2006_H3N8_HA	A/Anas platyrhynchos/Belgium/19RS2345-41_11027_19/2017_H3N8_H/	A/Anas platyrhynchos/Belgium/00330_0004/2023 EPI_ISL_17347344 †
8	S	N	N	N	N	N	N	N	N	N	N	N	N
19	S	A	A	A	A	A	A	A	A	A	A	A	A
34	I												V
45	N	S	S	S	S	S	S	S	S	S	S	S	S
47	S	L								L			
57	K		R	R	R	R	R	R	R	R	R	R	R
58	V	I	I	I	I	I	I	I	I	I	I	I	I
63	D	G											
70	M	L	L	L	L	L	L	L	L	L	L	L	L
78	V		A										
83	K		T	T	T	T	T	T	T	T	T	T	T
92	S	G	N	N	N	N	N	N	N	N	N	N	N
94	F		Y							Y			
110	S		A							A			
112	I		V	V	V	V	V	V	V	V	V	V	V
114	S		T							T			
119	D		E	E	E	E	E	E	E	E	E	E	E
124	S		G	G	G	G	G	G	G	G	G	G	G
126	T		I										
128	A		N	T	T	T	T	T	T	N	T	T	T
131	S		T	T	T	T	T	T	T	T	T	T	T
137	S		G	G	N	N	G	N	G	G	N	N	N
145	N		S	S	S			S		S	S	S	S
159	S		X	N	N					N	N	N	N
160	S		A	A	T	A	T	T	A	A	A	A	A
163	L		V	V	V	V	V	V	V	V	V	V	V
171	N		S							S			
172	Y		D	D	D	D	D	D	D	D	D	D	D
173	N									G			
192	T					X							
193	N		S			S		S					
198	A	T											
205	S								X				
208	R		K							K			
214	V		I	I	I	I	I	I	I	I	I	I	I
223	V		I										
228	X	G	G	G	G	G	G	G	G	G	G	G	G
237	V								A				
242	V									I			
244	V	A											
257	F		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
260	I		M	M	M	M	M	M	M	M	M	M	M
264	R		K	K	K	K	K	K	K	K	K	K	K
273	P				S								
275	E		D	D	D	D	D	D	D	D	D	D	D
278	I					V							
288	I					V							
312	N										S		
328	T	S											

Aadiffs from CW	5	6	28	27	26	26	25	25	27	28	28	25
Putative antigenic sites	1	3	15	15	15	15	13	13	14	15	15	14

## H3 antigenic data

Table 7: Avian H3 antigenic characterisation of viruses against CVVs. Fold changes are coloured. Amino acid changes in test viruses compared to the closest CVV are annotated on the right hand side. Putative antigenic sites are in bold.

		REFERENCE FERRET ANTISERA					
		Clade	Zoo	EA	EA	EA	EA
		Subtype	H3N8	H3N2	H3N6	H3N8	H3N8
		Ferret ID	A/Henan/4-10CNIC/2022	A/duck/Italy/3139-1/2006	A/duck/Italy/6207/2008	A/Anas platyrhynchos/Belgium/11294_004/2021	A/Anas platyrhynchos/Belgium/19RS2345-3_3950-8/2015
Semester	Reference Antigen	Subtype					
<u>IZSve</u> <u>Sept23-</u> <u>Feb24</u>	A/Henan/4-10CNIC/2022	H3N8	<b>640</b>	160	80	160	1280
	A/duck/Italy/3139-1/2006	H3N2	<10	<b>160</b>	20	40	<10
	A/duck/Italy/6207/2008	H3N6	<10	80	<b>160</b>	640	40
	A/Anas platyrhynchos/Belgium/11294_004/2021	H3N8	<10	<10	20	<b>320</b>	80
	A/Anas platyrhynchos/Belgium/19RS2345-3_3950-8/2015	H3N8	40	<10	<10	10	<b>160</b>
	A/duck/Ireland/7049-7/2021	H3N8	40	20	40	40	<b>160</b>
	<b>Test antigen</b>						
<u>1</u>	A/mallard/Italy/3342/2006	H3N6	40	<10	20	20	160
<u>2</u>	A/Anas platyrhynchos/Belgium/19RS2345-44_7827/2018	H3N8	20	<10	20	20	160
<u>3</u>	A/duck/Italy/3139-2/2006	H3N8	<10	40	40	640	<10
<u>4</u>	A/Anas platyrhynchos/Belgium/19RS2345-41_11027_19/2017	H3N8	<10	<10	10	320	80
<u>5</u>	A/Anas platyrhynchos/Belgium/00330_0004/2023	H3N8	<10	<10	<10	80	40

# H7 Influenza A viruses

## H7 Phylogenetic analysis

Sept2023-Feb2024  
Feb2023-Sept2023  
CVV



Figure 10: Avian 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 within the reporting period (September 2023 to February 2024) are coloured dark blue, those from the previous reporting period light blue, CVVs in red. Clade branches of recently detected HPAI are coloured red.

## H7 Amino acid difference table

Table 8: Avian H7 sequence comparison to the CVVs. Sequences from this reporting period are blue, the previous reporting period light blue and the CVVs red. Changes in putative antigenic sites are highlighted grey.

Site	CVV/A/mallard/Netherlands/12/2000/H7N3	CVV/A/Anhui/1/2013/H7N9	SubF2024 A/avian/Mozambique/857-P3-1_23VIR11699-9/202	GISAD A/chicken/South_Africa/SA2310/2023 2023-05-31	Putative Antigenic Site
38	V	I			
60	I	V	V		
78	I	V	V		
84	S	R	R		
95	E	D	D		
112	T	A	S	S	Antigenic
130	R	T	T		Antigenic
165	D	S			
170	I	V			
177	G	V			
180	T	A			
193	I	V			
217	Q	L			
227	I	M	M	M	
264	V	I	I		
267	N	S	S		Antigenic
269	E	D	D		
275	S	N	N		
289	N	D			
293	V	I	I		
303	E	R			
306	L	M	M		
317	I	P	P		
Aadiffs from CVV	11	14	14		
Putative antigenic sites	1	3	3		

# H9 Influenza A viruses

## H9 Y280 phylogenetic tree

Sept2023-Feb2024  
 Feb2023-Sept2023  
 CVV  
 Human



Figure 11: Avian H9 maximum likelihood phylogenetic. Analyses were conducted with reference sequences and data downloaded from GISAID, Genbank and kindly shared from HKU and the OFFLU network. Sequences within the reporting period (September 2023 to February 2024) are coloured dark blue, those from the previous reporting period light blue, CVVs in red and human strains pink.

# H9 G1 phylogenetic tree

Sept2023-Feb2024  
 Feb2023-Sept2023  
 Human  
 HI strain  
 CVV



Figure 12: Avian H9 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data downloaded from GISAID on 13th January 2024, and kindly shared through the OFFLU network.

# H9 G1 amino acid difference table

Table 9: Avian H9 G1 clade sequence comparison to CVV. Antigenic sites have been coloured in light grey. Sequences within the reporting period (September 2023 to February 2024) are coloured dark blue, the CVV red and sequences antigenically characterised are yellow.

Site	ref A/Oman/2747/2019/H9N2/2019-03-21_G1_CW	ref A/Bangladesh/06994/2011_G1_CW	SubF2024 A/chicken/Egypt/CV16G/2023 H9N2 2023	SubF2024 A/chicken/Egypt/F404/2023 H9N2 2023	SubF2024 A/chicken/Egypt/F537/2023 H9N2 2023	SubF2024 A/chicken/Egypt/FAO-SG3/2023 H9N2 2023	SubF2024 A/chicken/Egypt/FAO/S18/2023 H9N2 2023	SubF2024 A/chicken/India/10TR323-327/2023 H9N2 2023-10-00	SubF2024 A/environment/India/10NA124-127/2023 H9N2 2023-09-29	SubS23 A/chicken/Bangladesh/CE-116-03-06-24-BR-O/2022 H9N2 2022-02-1	SubF2024 A/chicken/Bangladesh/CE-111-02-04-16-DE-O/2021 H9N2 2021-1	SubS23 A/chicken/Bangladesh/CE-113-04-CA-19-SO-O/2022 H9N2 2022-09	SubS23 A/chicken/Bangladesh/CE-111-06-DB-16-BR-O/2023 H9N2 2023-1-1	Putative Antigenic Site
17	D													N N
22	T							N S	N S	N N				N N
23	N		G G	G G	G G									
28	Q	H H	H H	H H	H H	H H	H H	H H	H H	H H				H H
36	E	D						D D	D D	D D				Antigenic
40	M		K K	K K	K K	K K								Antigenic
43	A									S S				Antigenic
48	R	H N	N N	N N	N N	N N		H Q	Q H	H H				Antigenic
56	T	N								N N	N N			N N
57	I	V V	V V	V V	V V	V V				V V	V V			V V
60	L								X					
69	M	L L	L L	L L	L L	L L		L L	L L	S S				S S
74	R						K							
95	V							I						
103	T	I I	I I	I I	I I	I I								Antigenic
112	Q							K K						
114	V	I I	I I	I I	I I	I I				I I				I I
115	Q									L L				Antigenic
116	L	I M	M M	M M	M M	M M	I I	I I	I I	I I				Antigenic
120	S	T T	T T	T T	T T	T T	T T	T T	T T	T T				T T
127	T	S S	S S	S S	S S	S S								Antigenic
132	S	A A												
135	D							G						Antigenic
148	N							G						Antigenic
149	G									S S				Antigenic
150	G	L N	N N	N N	N N	N N	A A	L Q	Q L	L L				Antigenic
152	P						T							Antigenic
153	I	V V	V V	V V	V V	V V	V V	V V	V V	V V				Antigenic
158	Y	F					F	F	F	F				F F
161	N	T T	T T	T T	T T	T T								Antigenic
162	R						Q	Q	Q					Antigenic
163	G						K							Antigenic
165	D	N N	N N	N N	N N	N N								
169	V						M							
176	P							S						Antigenic
177	T							S S						Antigenic
179	T							D						Antigenic
180	A							Q						Antigenic
184	L							X						Antigenic
187	X	R R	R R	R R	R R	R R	R R	R R	R R	R R				Antigenic
194	V	I I	I I	I I	I I	I I								
198	T	N S	S S	S S	S S	S S	N N	N N	N N					
216	L		Q Q				R							Antigenic
217	I	A A												
222	Y							F						
226	V				I			I						
246	F	Y						Y Y	Y Y	Y Y				Y Y
249	V							I						
260	R	K K	K K	K K	K K	K K	K K	K K	K K	K K				K K
262	D	N						N N	N N	S S				S S
264	S	N N	N N	N N	N N	N N	K	N N	N N	N N				Antigenic
265	S	N						N						
267	N							D						Antigenic
269	V							L		I I				Antigenic
276	K									R R				
283	M	L L	L L	L L	L L	L L	L L	L L	L L	L L				L L
285	F		S											
288	I							V V	V V					
295	T	N N	N N	N N	N N	N N	N N	N N	N N	N N				N N
303	K	X												
315	H	P P	P P	P P	P P	P P	P P			P P				P P
317	R	K						K	K	K K				K K
Aadiffs from CW	23	23	27	26	24	23	25	32	26	24	28	28		
Putative antigenic sites	7	9	10	10	9	9	10	15	9	8	11	11		

## H9 G1 antigenic data

Table 10: Avian H9 antigenic characterisation of viruses against CVVs. Fold changes are coloured. Amino acid changes in test viruses compared to the closest CVV are annotated on the right hand side. Putative antigenic sites are in bold.

		REFERENCE FERRET ANTISERA		A/Oman/2747/2019	A/Bangladesh/0994/2011
		Clade	Subtype	G1	G1
		Ferret ID	Subtype	H9N2	H9N2
Semester		Clade	Subtype		
		<b>Reference Antigen</b>			
				640	40
				320	1280
		<b>Test antigen</b>			
<b>APHA Sep23-</b>				320	40
<b>Feb24</b>	<u>1</u>			320	40
	<u>2</u>			160	20
	<u>3</u>			160	80
	<u>4</u>			320	40



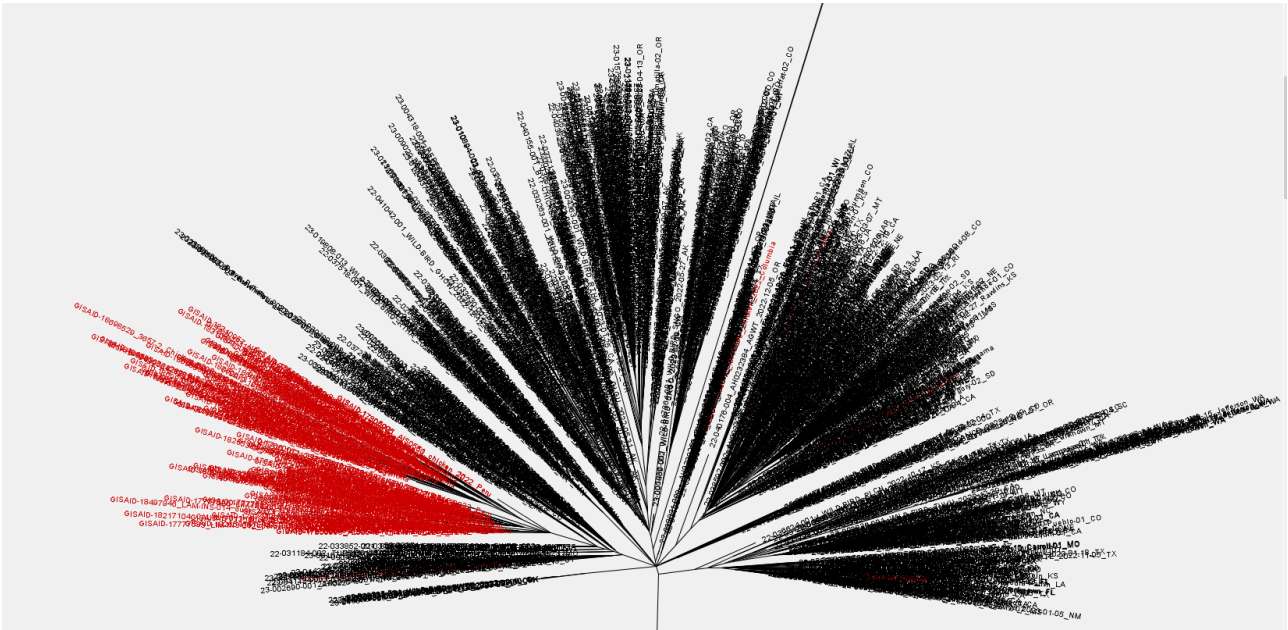
# H10N5

Feb2023-Sept2023  
Human  
CVV



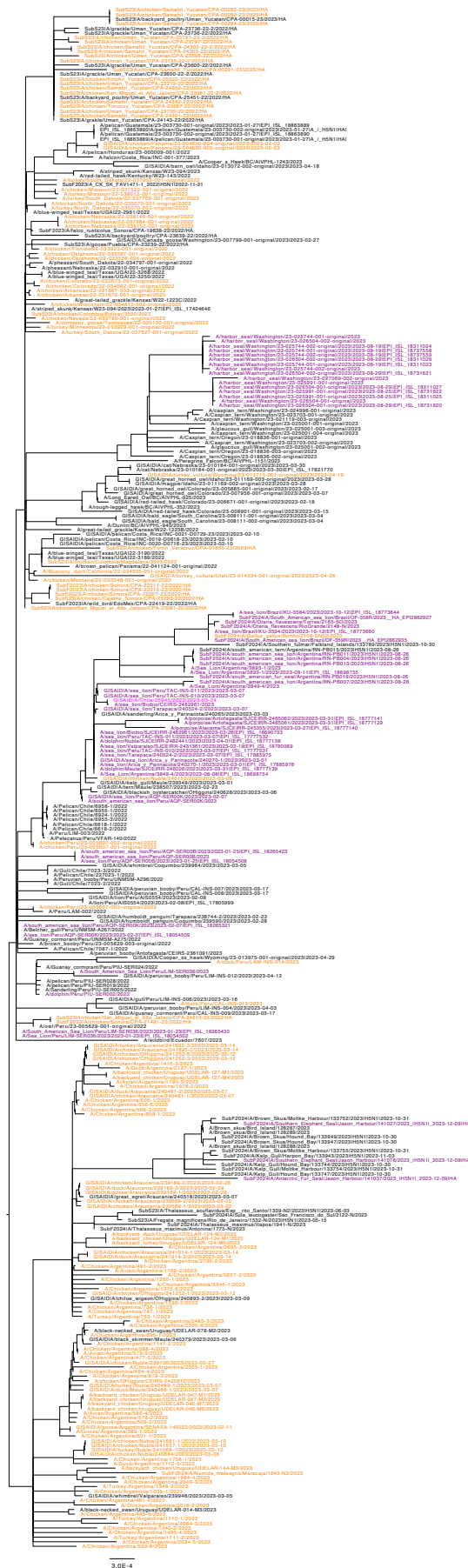
Figure 12: Avian H10 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with sequences and data downloaded from GISAID.

# Annex



vSNP tree of genotype B3.2: Red are available sequences from Central/South America, and Antarctica. No US origin viruses fall within the two major distinct branches of the B3.2 tree represented by these viruses.

Pinnipeds  
Human  
Poultry



H5N1 clade 2.3.4.4b time resolved subsampled tree focusing on infections in pinnipeds