



OFFLU AVIAN DATA PACKAGE FOR THE VCM

February 2023 to September 2023

SCOPE

In this document we present a summary of H5, H7 and H9 and Avian Influenza A virus events reported from 1st February 2023 to September 2023

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Avian Influenza A Viruses

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 WAHIS interface weekly disease information service 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, Agriculture Research Center Egypt, Agence National de Security de Sanitaire, Animal and Plant Health Agency, Canadian Food Inspection Agency, Centre of Excellence for Influenza Research and Response, Disease Investigation Centre Wales, Federal Centre for Animal Health, Harbin Veterinary Research Institute, Livestock Research, Institute National Institute for Veterinary Research, Chittagong Veterinary and Animal Sciences University, Hokkaido University, Ilia State University, Inocuidad y Calidad Agroalimentaria, Institute Pasteur Cambodia, National Institute of High Security Animal Diseases, Laboratório Federal de Defesa Agropecuária, South Eastern Poultry Research Laboratory, Wageningen Bioveterinary Research, and the RESUDIA network for sharing information, unpublished data and virus isolates via the OFFLU network.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, Francesco Bonefante and Nicola Lewis.

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, USDA-ARS NADC, Royal Veterinary College and the Francis Crick Institute for their expertise and sharing of data. Phylogenetic and sequence analyses were performed using the RVC and CEIRR pipeline. Avian influenza A virus haemagglutination inhibition (HI) assay antigenic data in this reporting period was generated using harmonised protocols by APHA, SEPRRL and IZSVe using ferret-origin reagents kindly provided by WHO CCs, CDC and the Crick institute. 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 2023 and 14th September 2023 were downloaded from GISAID and Genbank. These were added to data kindly provided through the OFFLU network and a reference dataset. 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.

Global zoonotic avian influenza events and 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 distinct clades. Some have become endemic in poultry populations in specific regions. Additionally, non Gs/Gd low pathogenicity H5Nx viruses, though not reportable, continue to be widely detected in wild birds and occasionally in poultry in some regions causing production loss and sporadic disease.

Since the autumn of 2021, H5N1 clade 2.3.4.4b viruses have dominated. These viruses have spread across Europe, Asia, Africa and the Americas, displacing other clade viruses and reassorting with local low pathogenic avian influenza viruses, resulting in a diverse range of genotypes which exhibit variable and species dependant pathogenicities. Despite this diversity, few genotypes prevail and predominate in geographically distinct areas and the HA of viruses generally remain genetically similar. These viruses have had significant impacts on ecologically important bird species, particularly seabirds, and mammals, as well causing devastating losses to the poultry sector.

As of the current reporting period, virus has not been observed in Antarctica nor Oceania, however, it is expected to affect the antarctic region in the upcoming season. Despite the predominance of H5N1 Gs/Gd lineage HPAI H5N1, H5N2, H5N4, H5N5 H5N6, H5N8 subtypes have all been sporadically detected in poultry or wild birds since October 2022.

Typically HPAI incidence in poultry in the northern hemisphere follows a distinctive pattern peaking in February and declining until October. Since the summer of 2021, a notable shift has been observed, with HPAI events remaining relatively elevated over the summer months, primarily affecting wild bird populations, specifically gulls, in well surveilled regions. In this reporting period, North America and Europe have seen a decrease in poultry outbreaks compared the same period in 2022. Outbreaks in poultry continue in undersurveilled regions such as South America and many parts of Asia and Africa.

Uncharacteristically for HPAI, but similarly to 2022, in 2023 the clade 2.3.4.4b H5N1 virus has persisted in wild bird populations, notably in *laridae sp*. This has been pronounced in well surveilled regions in Northern and Western Europe and has resulted in spillover into mammalian species and continued spillover into poultry. Europe has had return of H5N1 clade 2.3.4.4b from the 2023 spring migration of wild seabirds from Africa.

Africa has experienced multiple introductions of H5Nx clade 2.3.4.4b from Europe since 2020. These introductions have led to the maintenance of the virus in poultry populations, the replacement of previously circulating viruses and reassortment with locally circulating endemic viruses. Many of these viruses continue to circulate in geographically segregated regions in 2023 affecting wild sea birds and poultry.

Introductions into the Americas from Europe have established in wild bird populations leading to spillover and disease outbreaks in poultry. During the autumn migration in 2022 there were at least 3 separate introductions into Latin American Countries. Data from this region is limited but the virus appears to be establishing itself in poultry populations, as well as causing disease and mortality in marine mammals and birds. There are continued detections of movement of H5N1 virus between the North east of the Americas and East Asia since late 2021 however these viruses haven't been largely responsible for outbreaks in poultry.

In East Asia H5N6 clade 2.3.4.4b viruses continue to circulate in poultry populations. Additionally H5N1 viruses which were introduced in early 2021 and subsequently spread to South Asia, Eastern Russia and East Asia continue to circulate. Separate introductions of H5N1 viruses into South and South East Asia in 2021 appear to be establishing in poultry populations through spillover from local wild birds. They have undergone reassortment with other circulating viruses and maintain their presence in geographically segregated regions

Clade 2.3.4.4h viruses have been detected in environmental samples in China. Clade 2.3.2.1c and clade 2.3.2.1e viruses continue to circulate and diversify in poultry parts of South East Asia and clade 2.3.2.1a viruses continue to circulate and diversify in poultry in South Asia.

Notably, there has been a significant increase in the frequency of detections among non-avian species including both wild terrestrial and marine mammals, as well as farmed, captive and domestic mammals. While wild mammals often test positive incidentally, leading to limited clinical information, captive and domestic mammals have displayed a range of clinical symptoms. These symptoms vary from mortality, neurological and respiratory signs, to few or no clinical signs. Among affected wild mammals, scavengers and animals sharing their habitat with infected birds are the most affected species. Importantly, some domestic carnivores have become infected through contaminated meat in the food chain or consumption of infected poultry. There have been few reports of viral detection in non-carnivorous mammals. Beside outbreaks in farmed foxes in Finland and suspected events in sea mammals, there have been no confirmed cases of mammal to mammal transmission.

In Central and Latin America the region has faced significant challenges due to outbreaks of H5N1 clade 2.3.4.4b affecting poultry and seabirds. Notably, there has been a substantial increase in numbers of marine mammal deaths reported in countries such as Chile, Peru, Uruguay and Argentina, compared to previous years. Tens of thousands of sea lions and hundreds of thousands of seabirds have been affected. Whilst HPAI has been suspected it has only been clinically confirmed in few cases and testing capacity is very limited. Onset of marine mammal mortality coincided with significant seabird mortality along the West Coast some of which attributed to HPAI, however more recent reports from Argentina and Uruguay have not necessarily been accompanied by reports of mass sea bird die offs. It is unknown whether this is due to lack of surveillance and reporting. The exact aetiology of these cases is still unclear, as are routes of infection. Whether they represent confirmed HPAI cases resulting from mammalian transmission, environmental contamination, or transmission from birds remains questionable.

H3N8 viruses associated with zoonotic infections circulate in Chinese poultry. These viruses have not yet been detected outside China however surveillance data and reporting for this subtype is limited.

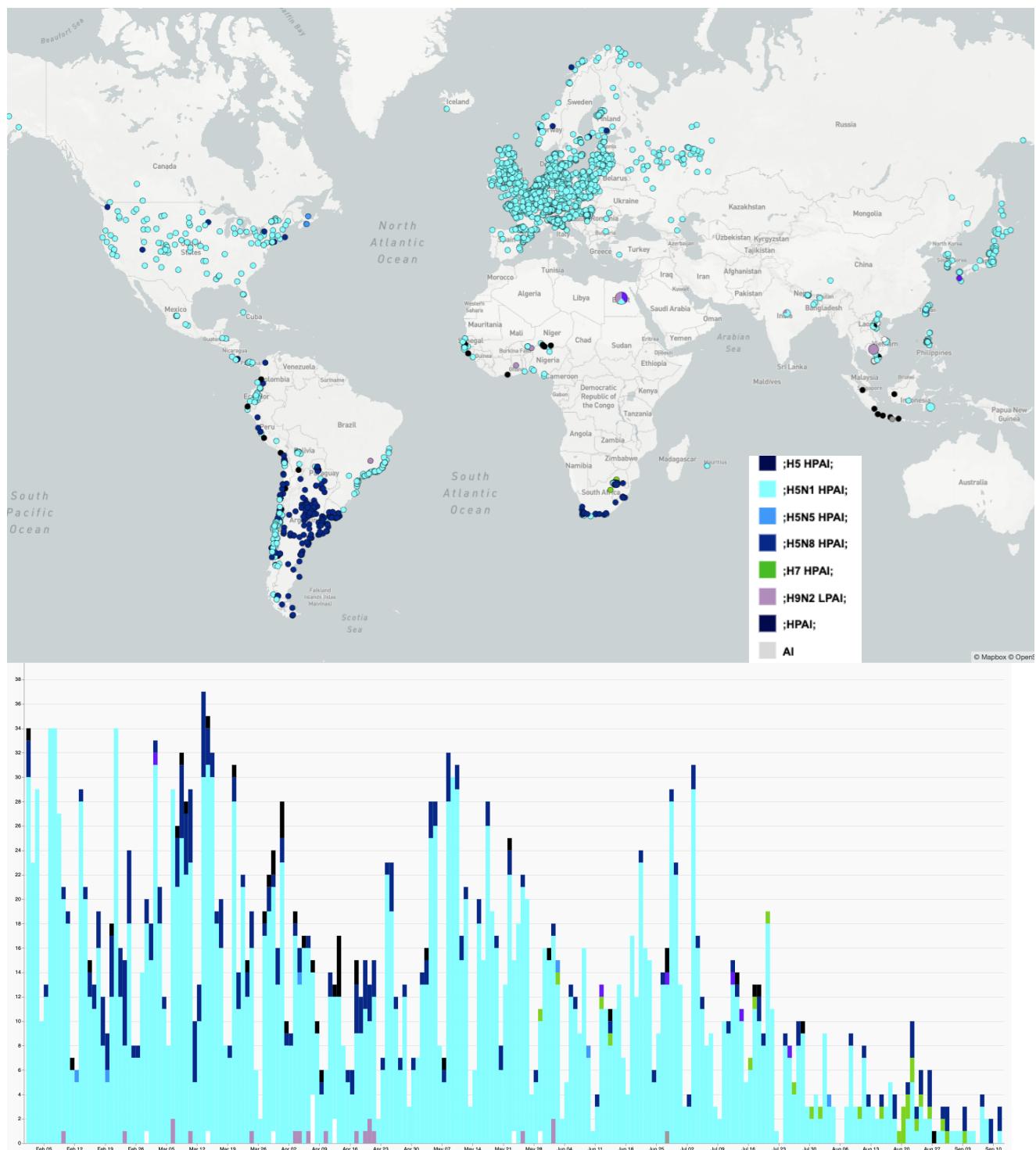
Low pathogenicity avian influenza (LPAI) H7 viruses have been detected in multiple countries in wild birds and environmental samples, occasionally in poultry. Low pathogenic H7N7 and H7N3 subtypes of unknown lineage were detected in environmental samples in Taiwan, Province of China. The UK reported H7N3 subtype in poultry in March 2023.

Newly emerged HPAI from H7 viruses of Eurasian lineage have caused ongoing outbreaks in domestic poultry in South Africa since June 2023. HPAI H7N9 of the A/Guangdong/17SF003/2016 lineage have been reported detected in poultry and environmental samples in China in early 2023.

Outbreaks of AI in poultry caused by H9 viruses are not reportable to the World Organisation for Animal Health (WOAH). H9 viruses are known to have infected birds and mammals in many countries including but not limited to The Republic of Korea, Russian Federation, China, Morocco, Niger, Nigeria, Taiwan Province of China, Indonesia, Cambodia, Viet Nam, Côte D'Ivoire. Over the reporting period, H9 viruses have been reported in poultry in Brazil (unknown lineage), Egypt, Ghana, Niger, Indonesia, India and Cambodia (zoonotic G1 or Y280 lineages).

Global avian influenza events in animals

Figure 1: Microreact map showing the geographical and temporal distribution of Gs/Gd H5, HPAI H7 and zoonotic H9 lineage avian influenza outbreaks reported to WOAH via the WAHIS platform and to national authorities gathered through FAO EMPRES-i. Additional outbreaks inferred from sequence data gathered from GISAID or OFFLU or countries' AI dashboard's have been added. Data shown falls within the reporting period of 1st February 2023 to present (last updated 19 September 2023). Points are coloured by subtype and the outbreak date is represented along the bottom timeline.



Activity Table (H5) viruses

Table 2: 1st February 2023 to present (last updated 26th September 2023). A/goose/Guangdong/1/96 lineage viruses detected as summarised below. Data was collected through WOAH via the WAHIS system including unclosed reports, FAO through EMPRES-i and through OFFLU collaborators, within the reporting period.

Avian H3 phylogeny



Figure 2: Avian H3 Maximum likelihood phylogenetic tree. CVVs are coloured red. Human strains are coloured pink. Viruses shared within the previous reporting period are coloured light blue.

H3 Amino acid difference tables

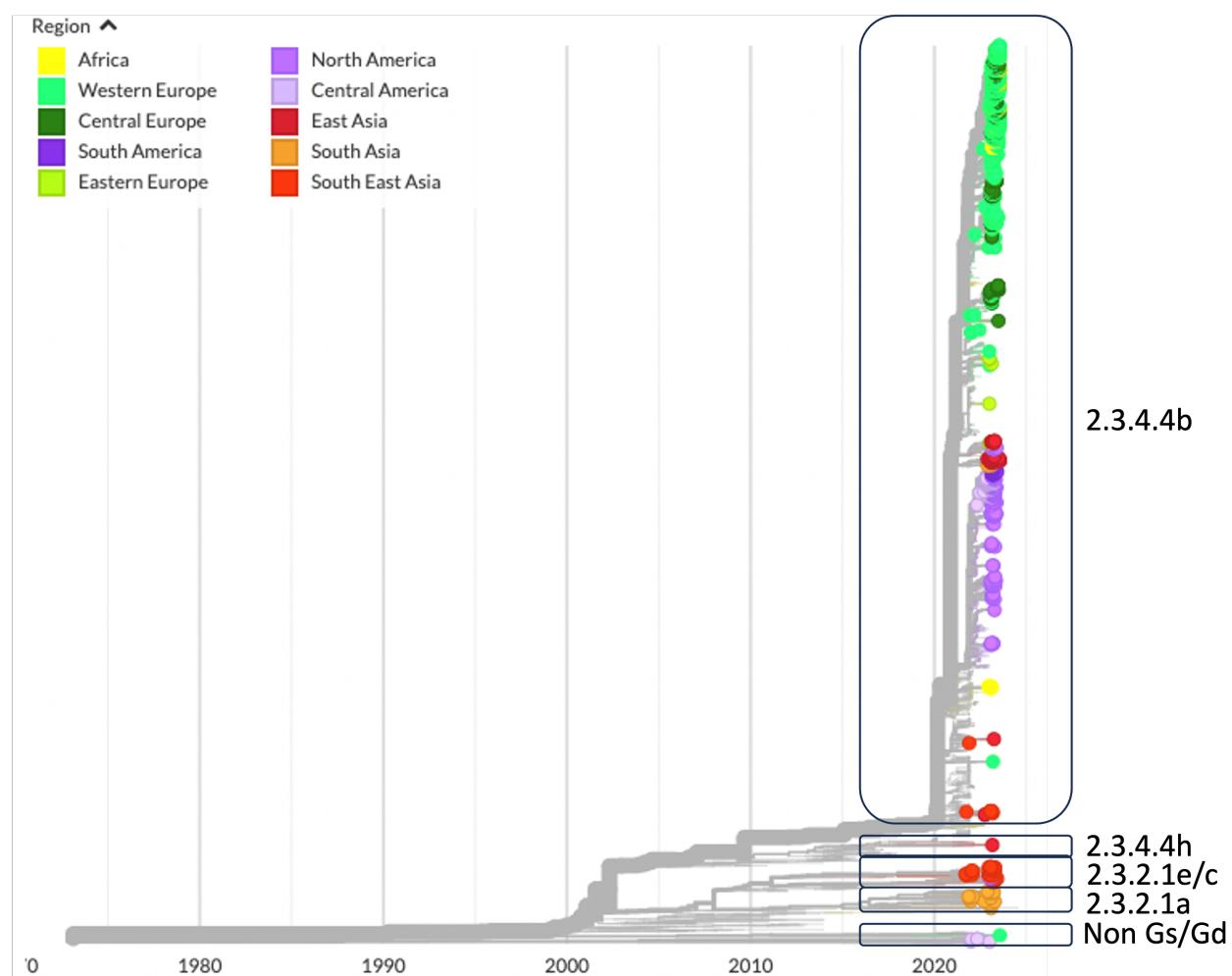
Table 3: Avian H3 sequence comparison to the within clade CVVs. Changes in putative antigenic sites are highlighted grey.

Ref A/Henan/4-14/CN/C/2023-235	GIS/AD1/A/Guangdong/ZS-235F/05/[2023-2023-01-1]
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/6058/2022/2022-01-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/6058/2022/2022-02-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/3758/2022/2022-01-22
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/6559/2022/2022-02-08
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/740/2022/2022-01-01
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/6485/2022/2022-01-29
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/1053/2022/2022-02-26
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/3711/2022/2022-01-22
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/12248/2022/2022-03-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/11230/2022/2022-03-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/1229/2022/2022-03-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/6541/2022/2022-02-08
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/6579/2022/2022-02-08
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/1174/2022/2022-03-05
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/1054/2022/2022-02-26
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/6591/2022/2022-02-26
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Jiangxi/1177/2022/2022-03-05
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/576/2022/2022-04-01
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/658/2022/2022-04-01
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/384/2022/2022-03-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/385/2022/2022-03-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/481/2022/2022-01-12
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/216/2022/2022-02-01
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/310/2022/2022-03-01
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/222/2022/2022-02-21
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/356/2022/2022-03-01
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/231/2022/2022-02-21
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/246/2022/2022-02-21
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/390/2022/2022-04-04
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/401/2022/2022-03-04
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/549/2022/2022-03-04
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/114/2022/2022-01-01
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/78/2022/2022-04-04
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/14/2022/2022-02-21
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/345/2022/2022-02-21
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/39/2022/2022-02-21
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/343/2022/2022-02-21
Ref A/Chengdu/1/00/0/2022	GIS/AD1/A/chicken/Guangdong/343/2022/2022-01-01
Total AA changes	7 4 5 6 4 6 6 6 6 7 7 7 7 7 6 6 8 6 7 7 5 3 3 3 3 4 3 5 2 3 3 3 3 3 3 5 3 5 4 4 3 3 3 8
In putative antigenic sites	4 0 1 2 1 0 2 1 1 2 2 2 2 1 1 1 2 1 1 1 1 1 4 2 0 0 1 1 1 1 1 1 0 1 0 0 1 1 2 1 2 1 0 0 5

H5 Influenza A viruses

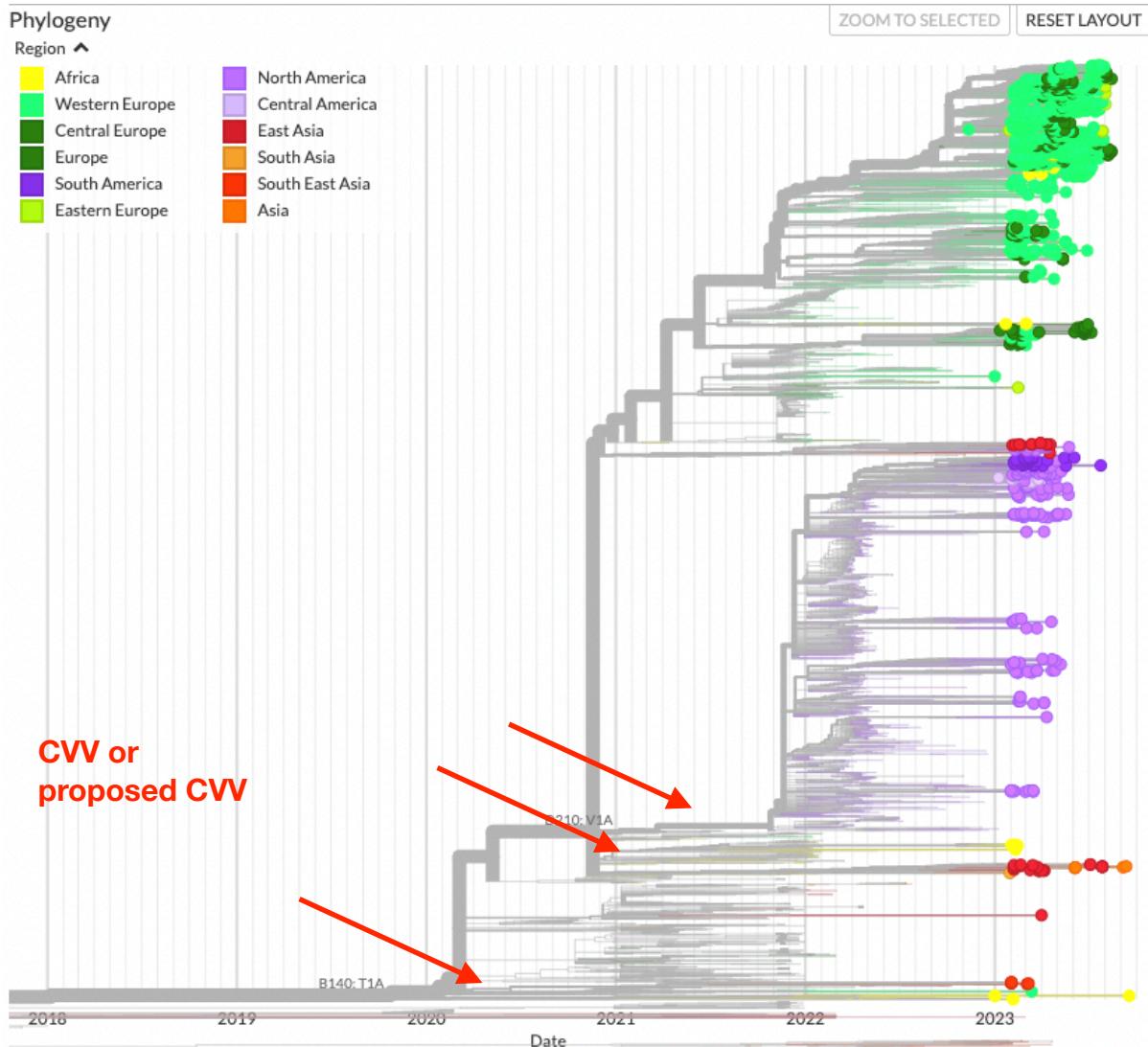
H5 Phylogenetic tree coloured by region

Figure 3: Avian H5 global summary time resolved maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences, sequences shared through the OFFLU network and data downloaded from GISAID (14th September 2023). Sequences within the reporting period were coloured by geographic region. Leaves with a collection date within the reporting period were coloured according to region.



H5 2.3.4.4b phylogenetic tree

Figure 4: Avian H5 clade 2.3.4.4b maximum likelihood phylogenetic tree. Clade changes in putative antigenic sites are annotated on branches. Analyses were conducted with reference sequences, sequences shared through the OFFLU network and data downloaded from GISAID (14th September 2023). Sequences were coloured by geographic region. Leaves within the reporting period were coloured. Positioning of CVVs are denoted with red arrows.



H5 2.3.4.4b subsampled tree

Figure 5: H5 clade 2.3.4.4b maximum likelihood tree. Including CVVs, mammalian strains and strains antigenically characterised in previous reporting periods. Those antigenically characterised by OFFLU this reporting period are highlighted yellow.



H5 2.3.4.4b Amino Acid difference tables and summary

- Most viruses detected within the Americas fall into a sub-clade which is characterised by the change in putative antigenic site V210A compared to the Astrakhan CVV (but similar to the American Wigeon CVV). Some viruses detected in the US from other incursions post 2021 do not have this mutation.
- The motif 154D is found in a group of viruses which circulate in wild birds in Asia since 2021 (presumably circulating in wild birds in parts of Asia causing occasional spillover into poultry).
- The changes 156T/S (compared to the Astrakhan CVV but similar to the Ghana-like CVV) is found in viruses which continue to circulate in West African poultry since 2021. Other viruses without this change have since been introduced into West Africa.
- The change T188I compared to the Astrakhan CVV is found in a phylogenetic cluster of viruses introduced in South East Asia and East Asia in 2020 which continue to circulate.
- In Central and South America some viruses detected possess the motif 185K/R. Another cluster of viruses from possess the motif 136S and 214V.

Other sporadic changes in putative antigenic sites can be found in non phylogenically clustering and from a broad geographic range.

Some sporadic changes were found in mammalian or antigenically characterised viruses associated with increased adaption to mammals according to the CDC's H5N1 genetic changes inventory.

Table 3: Avian H5 2.3.4.4b clade sequence comparison to the within clade CVVs. Strains which were antigenically characterised in the previous report were included for reference. Sequences from this reporting period are blue, the previous reporting period light blue and the CVV red. Strains which have been antigenically characterised are coloured yellow and fold changes to the antigenically closest virus are indicated by colour. Changes in putative antigenic sites are highlighted grey and changes found in the CDC genetic changes inventory are annotated.

Putative antigenic site CDC genetic changes inventory													
site	31	D		N	X	V							
51 I	I			I	I	I							
69 V	V												
72 R	R	D	E	E	E	G	G	G	G	G	G	G	G
74 P	P												
83 A	A	V	V	S									
86 A	A	G	G	G	G	G	G	G	G	G	G	G	G
88 D	D												
84 E	E												
104 L	L	M											
114 I	I	T											
115 L	L												
118 P	P												
123 P	P												
128 S	S	T											
129 L	L												
130 G	G												
136 P	P	T	L										
140 A	A			S	S	S							
144 N	N												
148 V	V												
154 N	N	T	D										
155 D	D												
156 A	A			S	S	S							
163 S	S												
169 R	R			Q	Q	Q	X						
175 L	L												
188 T	T												
189 N	N			S									
195 T	T		I										
210 V	V	A	T										
213 I	I												
214 A	A												
219 V	V			I									
222 Q	Q												
226 M	M												
231 T	T												
236 D	D												
268 G	G												
282 V	V												
288 N	N												
294 I	I												
312 V	V												
AA changes	2	2	3	0	0	1	2	1	3	1	2	2	1
In antigenic sites	2	1	0	0	0	1	0	2	0	1	0	1	0

H5 2.3.4.4b Antigenic Data

Table 4: Antigenic characterisation of H5 clade 2.3.4.4b viruses. CVVs and proposed -like CVV are marked in red. Test antigens are coloured by region. Fold changes are colour coded. Amino acid differences compared to the closest CVV A/Astrakhan/3312/2020 are annotated and putative antigenic sites are highlighted bold.

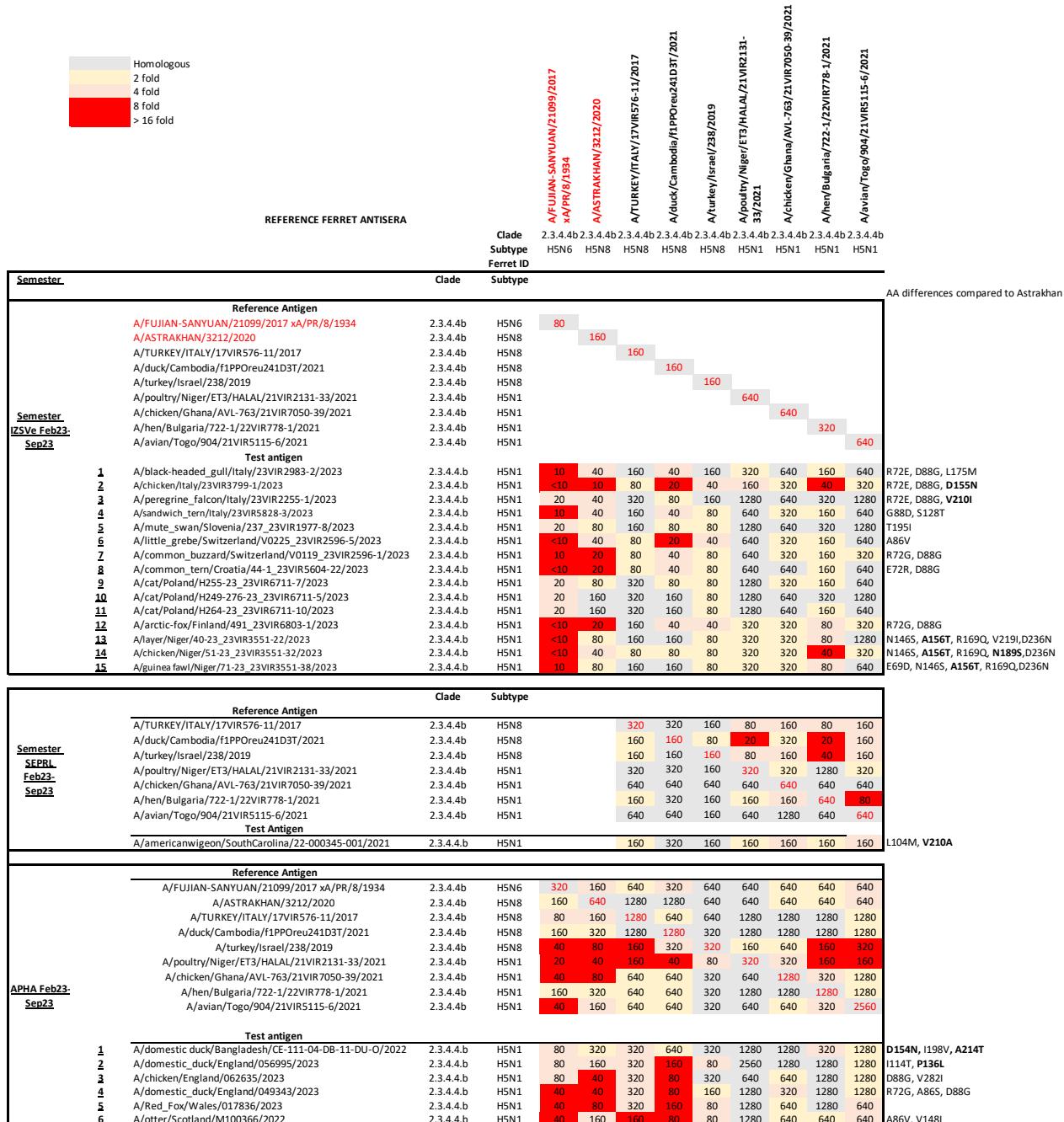
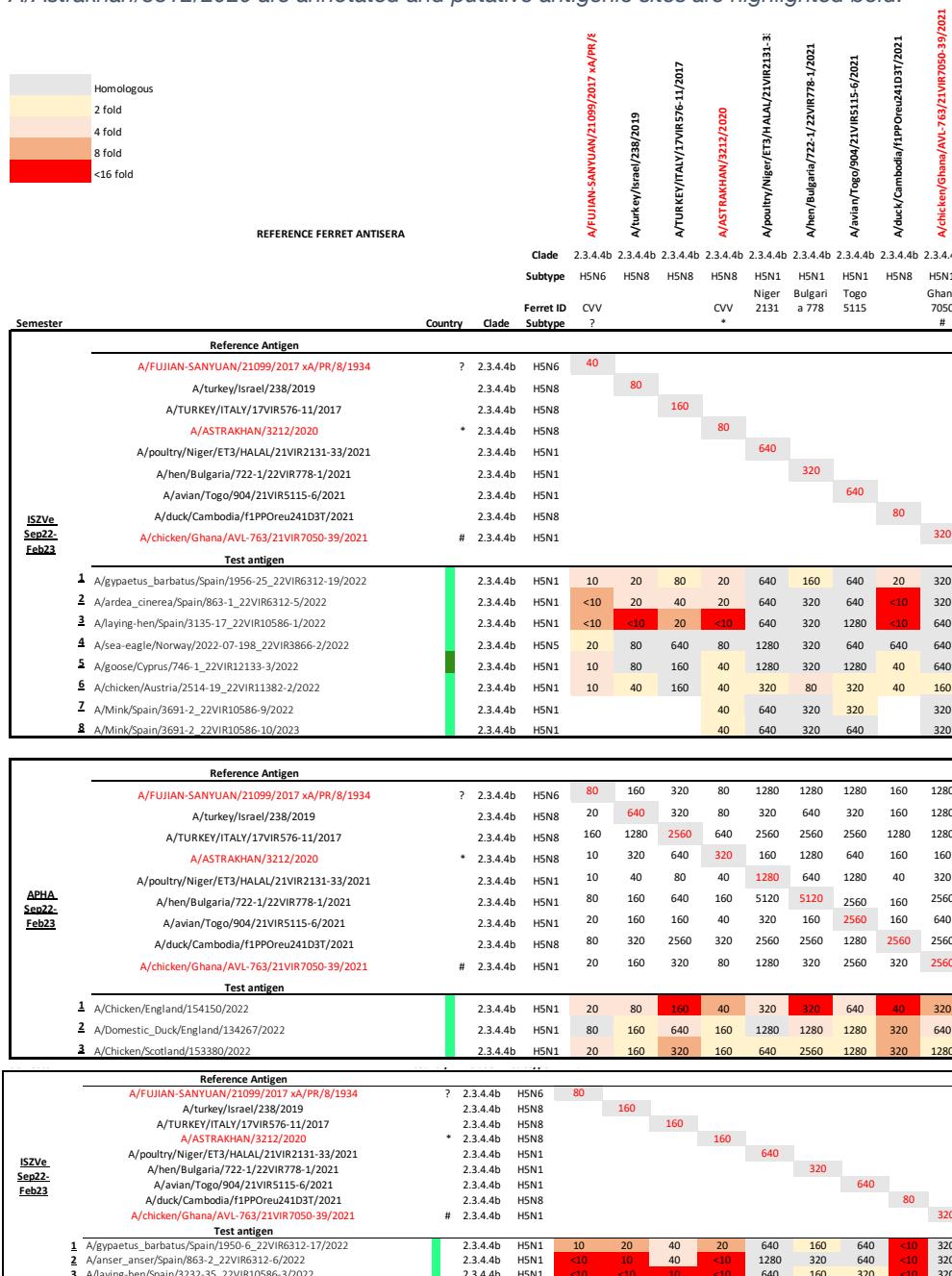


Table 5: Antigenic characterisation of H5 clade 2.3.4.4b viruses. CVVs and proposed -like CVV are marked in red. Test antigens are coloured by region. Fold changes are colour coded. Amino acid differences compared to the closest CVV A/Astrakhan/3312/2020 are annotated and putative antigenic sites are highlighted bold.



H5 2.3.2.1a phylogenetic tree

Feb2023-Sept2023

Sept2022-Feb2023

CVV

Human strain

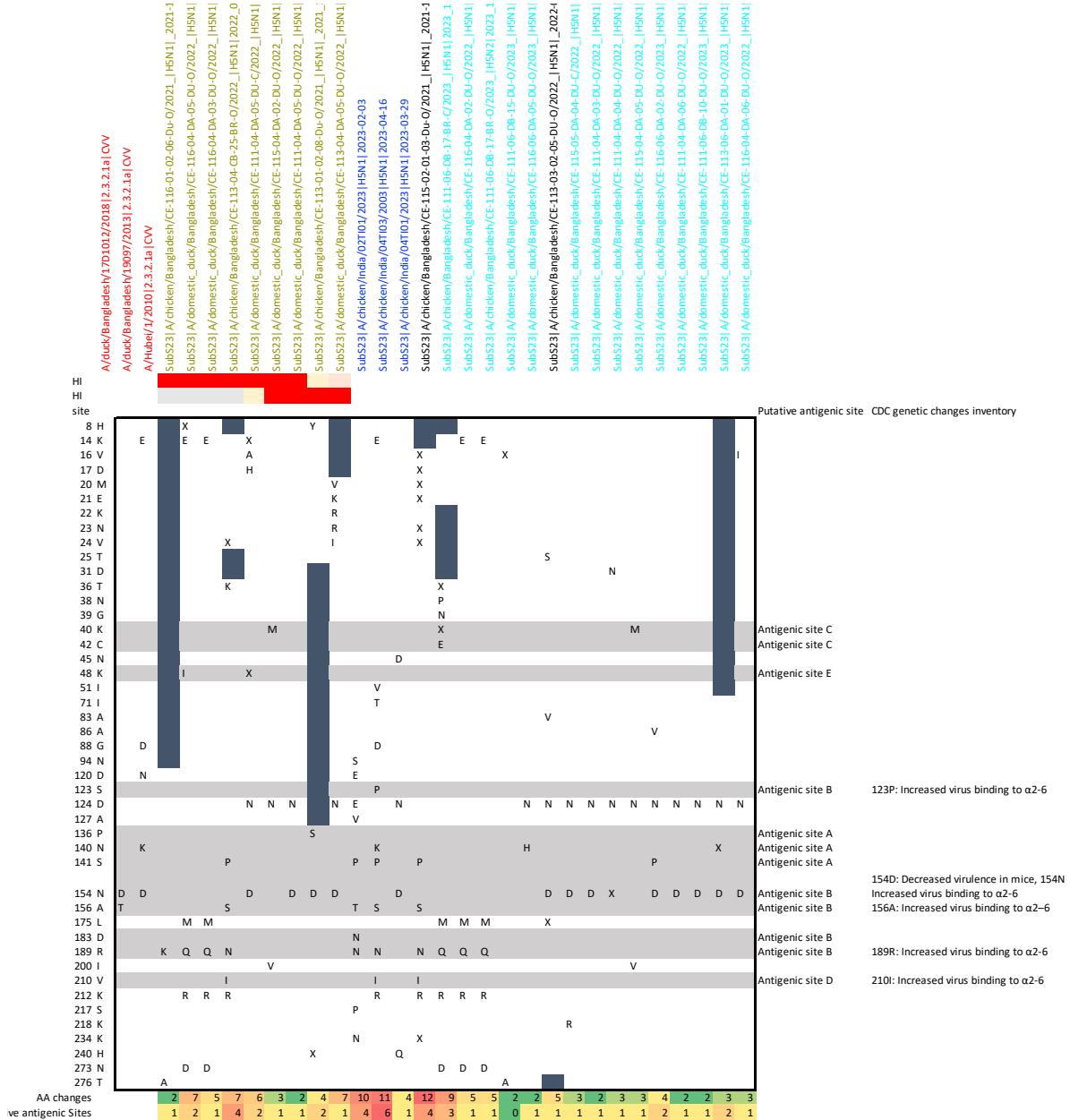
HI strain



Figure 6: Avian H5 2.3.2.1a Maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID, Genbank and kindly shared through the OFFLU network. Sequences within the reporting period (February 2023 to September 2023) are coloured dark blue, those from the previous reporting period light blue, CVVs in red and human sequences in pink. Viruses which have been antigenically characterised are yellow.

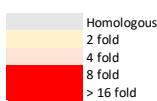
H5 2.3.2.1a amino acid difference table

Table 6: 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 CVV red. Strains which have been antigenically characterised are coloured yellow and fold changes to the antigenically closest virus are indicated by colour. Changes in putative antigenic sites are highlighted grey and changes found in the CDC genetic changes inventory are annotated.



H5 2.3.2.1a Antigenic data

Table 7: Antigenic characterisation of H5 2.3.2.1a viruses. CVVs are marked in red. Fold changes are colour coded. Amino acid differences compared to the closest CVV A/Duck/Bangladesh/17D1012/2018 are annotated and putative antigenic sites are highlighted bold.



Semester		REFERENCE FERRET ANTISERA		Clade	2.3.2.1a	2.3.2.1a	2.3.2.1a	A/HUBEI/1/2010 A/DUCK/BANGLADESH/19097/2013 A/DUCK/BANGLADESH/17D1012/2018					
		Clade	Subtype										
APHA Feb23-	Sep23	Test antigen											
		1 A/chicken/Bangladesh/CE-113-01-02-08-Du-O/2021_ H5N1 _2021_11_18	2.3.2.1a	HSN1	5120	2560	160	9HY, P136S, N154D					
		2 A/chicken/Bangladesh/CE-116-01-02-06-Du-O/2021_ H5N1 _2021_11_21	2.3.2.1a	HSN1	640	640	1280	R189K, T276A					
		3 A/chicken/Bangladesh/CE-113-04-CB-25-BR-O/2022_ H5N1 2022_09_18	2.3.2.1a	HSN1	640	640	640	T36K, S141P, A156S, R189N, V210I, K212R					
		4 A/domestic duck/Bangladesh/CE-115-04-DA-02-DU-O/2022_ H5N1 2022_09_15	2.3.2.1a	HSN1	80	320	40	K40M, D124N, I200V					
		5 A/domestic duck/Bangladesh/CE-111-04-DA-05-DU-O/2022_ H5N1 2022_09_17	2.3.2.1a	HSN1	80	40	80	D124N, N154D					
		6 A/domestic duck/Bangladesh/CE-111-04-DA-05-DU-C/2022_ H5N1 2022_09_17	2.3.2.1a	HSN1	160	40	320	V16A, D17H, D124N, N154D					
		7 A/domestic duck/Bangladesh/CE-113-04-DA-05-DU-O/2022_ H5N1 2022_09_18	2.3.2.1a	HSN1	1280	640	40	M20V, E21H, K22R, N23R, V24I, D124N, N154D					
		8 A/domestic duck/Bangladesh/CE-116-04-DA-03-DU-O/2022_ H5N1 2022_09_19	2.3.2.1a	HSN1	640	320	1280	K14E, L175M, R189Q,					
		9 A/domestic duck/Bangladesh/CE-116-04-DA-05-DU-O/2022_ H5N1 2022_09_19	2.3.2.1a	HSN1	640	640	1280	K14E, K48I, L175M, R189Q, K212R, N273D					

AA differences compared to Bangladesh/17D1012/18

Semester		REFERENCE FERRET ANTISERA		Clade	2.3.2.1a	2.3.2.1a	2.3.2.1a	A/HUBEI/1/2010 A/DUCK/BANGLADESH/19097/2013 A/DUCK/BANGLADESH/17D1012/2018						
		Clade	Subtype											
Test antigen														
APHA Sep22- Feb23														
		1 A/Deshi/Chattogram/AV-22-080029/2021	2.3.2.1a	HSN1	160	160	320							
		2 A/Sonali/Chattogram/AV-22-080007/2021	2.3.2.1a	HSN1	640	640	20							
		3 A/broiler-chicken/Bangladesh/AV-22-079981/2022	2.3.2.1a	HSN1	640	640	640							
Reference Antigen														
APHA Sep22- Feb23														
		A/HUBEI/1/2010	2.3.2.1a	HSN1	5120	5120	80							
		A/DUCK/BANGLADESH/19097/2013	2.3.2.1a	HSN1	5120	5120	640							
		A/DUCK/BANGLADESH/17D1012/2018	*	2.3.2.1a	HSN1	1280	1280	2560						
Test antigen														
ACDP Feb22- Sep22														
		1 A/chicken/Nepal/22-02058-0001/2022	2.3.2.1a	HSN1	<20	<20	<20							
		2 A/chicken/Nepal/22-02058-0002/2022	2.3.2.1a	HSN1	20	40	<20							
		3 A/chicken/Nepal/22-02058-0007/2022	2.3.2.1a	HSN1	20	<20	<20							
Reference Antigen														
ACDP Feb22- Sep22														
		A/HUBEI/1/2010	2.3.2.1a	HSN1	640	640	<20							
		A/DUCK/BANGLADESH/19097/2013	2.3.2.1a	HSN1	320	320	<20							
		A/DUCK/BANGLADESH/17D1012/2018	*	2.3.2.1a	HSN1	160	160	1280						
Test antigen														
APHA Feb22- Sep22														
		A/DUCK/BANGLADESH/17D1012/2018	*	2.3.2.1a	HSN1	1280								
		Test antigen												
		1 A/Sonali-chicken/Bangladesh/AV-22-079911/2022	2.3.2.1a	HSN1	2560									

AADiffs compared to closest CVV *

H180P, R189K, T276A
H8Y, N154D, Y164C

K14E, Q15K, D43N, I51V, S141P, A156S, R189N, V210I, K212R
D129N, N154D, D309N
K14E, D43N, L129Q, I51V, S141P, A156S, R189N, V210I, K212R

K35N, L233I

H5 2.3.2.1e and 2.3.2.1c phylogenetic tree

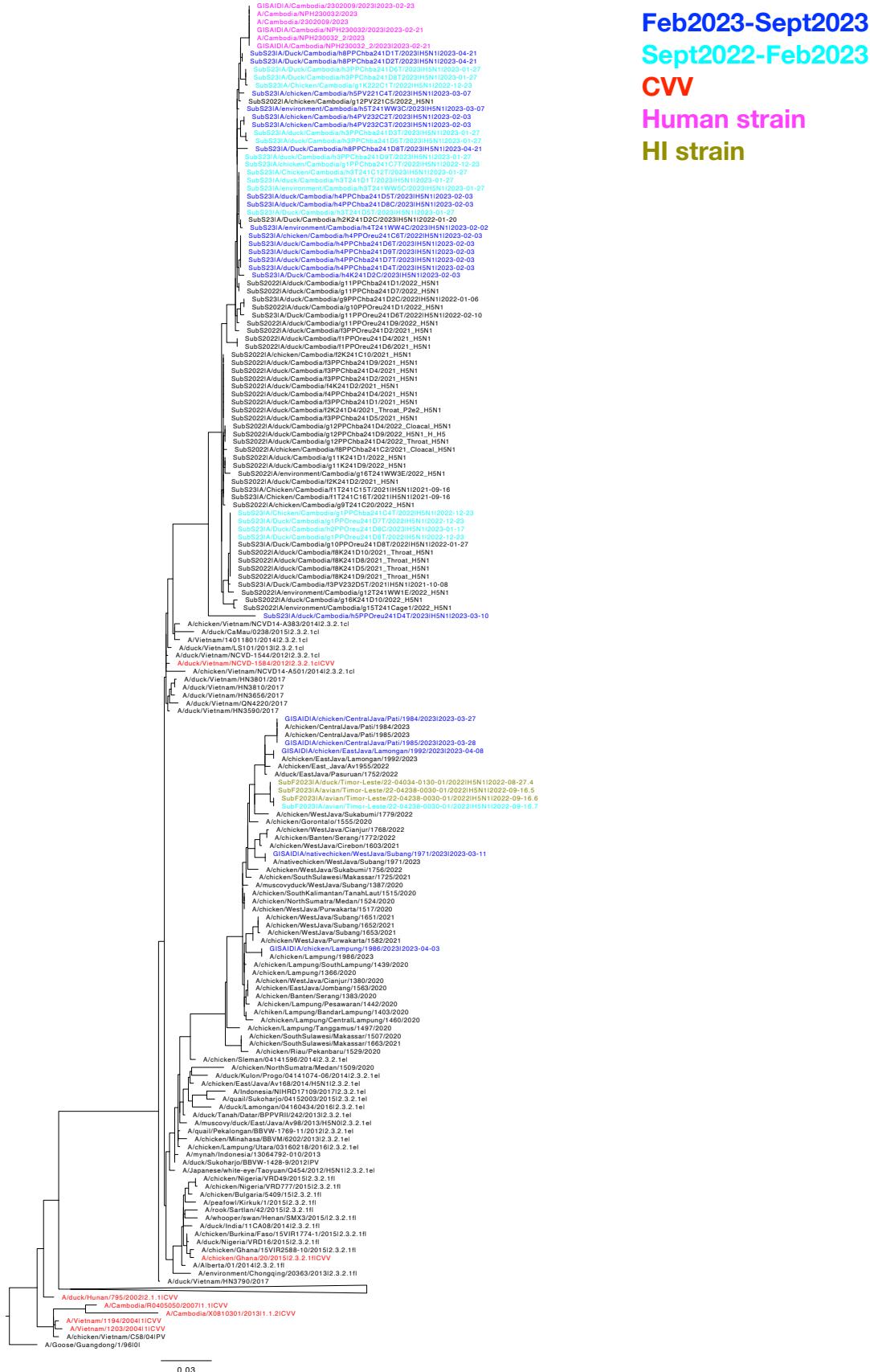


Figure 7: Avian H5 2.3.2.1e and c Maximum likelihood phylogenetic tree. CVVs are coloured red, strains selected for antigenic characterisation are coloured yellow, strains collected in the previous reporting period are coloured light blue and from this reporting period dark blue.

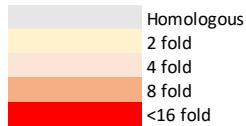
H5 clade 2.3.2.1e Amino Acid Difference Table

Table 8: Avian H5 2.3.2.1e clade sequence comparison to the within clade CVVs. Sequences from this reporting period are blue, the previous reporting period light blue and the CVV red. Strains which have been antigenically characterised are coloured yellow and fold changes to the antigenically closest virus are indicated by colour. Changes in putative antigenic sites are highlighted grey and changes found in the CDC genetic changes inventory are annotated.

site	A/chicken/Ghana/20/2015 2.3.2.1f CVV		A/duck/Vietnam/NCVO-1584/2012 2.3.2.1c CVV		GISaid A/chicken/CentralJava/Pati/1984/2023 2023-03-27		GISaid A/chicken/EastJava/Lamongan/1992/2023 2023-04-08		Subf/2023 A/duck/Timor-Leste/22-04034-0130-01/2022 HSN1 2022-08-27.		Subf/2023 A/avian/Timor-Leste/22-040238-0030-01/2022 HSN1 2022-09-16		GISaid A/chicken/Lampung/1986/2023 2023-04-03		GISaid A/chicken/East-Java/Av1955/2022	
2	H	Q	Q	Q	Q											
28	H															
30	Q															
36	T															
43	N	D	D	D	D	D	D	D	D	D	D	D	A	A		
66	L	S											D	D		
86	A								T							
94	N												S			
115	Q					R							T			
121	S						F									
123	S	P														
124	D	E	E	E	E	E	E	E	E	E	E	E	E	E		
127	A	E	E	E	E	E	E	E	E	E	E	E	V	V		
136	S	L	L	L	L	L	L	L	L	L	L	L	L	L		
154	G	D											S	S		Putative Antigenic site
155	N												D	D		Putative Antigenic site
156	A								T				T			Putative Antigenic site
162	K		R		R	R										
163	G	S	S	S	S	S	S	S	S	S	S	S	S	S		
174	I												S	S		
183	D								N				V			
185	A									T						
188	T			I	I											188I: Increased pseudovirus binding to α 2-6
189	K	R							N							
195	T	S	S	S	S	S	S	S	S	S	F	S	S	S		
198	I	V	V	V	V	V	V	V	V	V	V	V	V	V		
208	R					K										
210	V								I							
213	I								V							
221	G						E									
236	N			H												
249	A	V	V	V	V	V	V	V	V	V	V	V	V	V		
257	I				S	S										
272	G				V	V							S			
285	I			L												
288	S					N										
292	H											T				
293	N											T				
299	I				V											
320	S					N										
	4	10	9	8	10	10	11	9	7	8	8	10	7	9	9	
	2	0	0	0	0	1	1	0	0	0	0	0	3	0	1	
	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	
	1	1	0	0	0	0	0	0	0	0	0	0	2	2	2	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	8	8	9	10	11	11	11	11	11	11	11	11	11	11	11	

H5 2.3.2.1e antigenic analysis

Table 9: Antigenic characterisation of H5 2.3.2.1e viruses from previous reporting period. CVVs are marked in red. Fold changes are colour coded.



Semester	Reference Ferret Antisera	Country	Clade	Ferret ID				
				Clade	2.3.2.1a	2.3.2.1a	2.3.2.1c	
				Subtype	H5N1	H5N1	H5N1	
REFERENCE FERRET ANTISERA								
<u>ACDP</u> <u>Sep22-</u> <u>Feb23</u>	A/HUBEI/1/2010 A/DUCK/BANGLADESH/17D1012/2018 A/duck/Vietnam/NCVD-1584/2012			2.3.2.1a	H5N1	1280	20	1280
				2.3.2.1a	H5N1	80	2560	640
				2.3.2.1c	H5N1	640	20	640
Test antigen								
	<u>1</u> A/duck/Timor-Leste/22-04034-0130-01/2022			2.3.2.1e	H5N1	20	20	80
	<u>2</u> A/avian/Timor-Leste/22-04238-0030-01/2022			2.3.2.1e	H5N1	20	20	80
	<u>3</u> A/avian/Timor-Leste/22-04238-0038-01/2022			2.3.2.1e	H5N1	20	20	80

H5 2.3.2.1c amino acid difference table

Table 10: Avian H5 2.3.2.1c clade sequence comparison to the within clade CVVs. Sequences from this reporting period are blue, the previous reporting period light blue and the CVV red. Strains which have been antigenically characterised are coloured yellow and fold changes to the antigenically closest virus are indicated by colour. Changes in putative antigenic sites are highlighted grey and fold changes to the antigenically closest virus are indicated by colour. Changes found in the CDC genetic changes inventory are annotated.

site	A/duck/Vietnam/NCD-1584/2012/2.3.2.1c CVN																										
15	GSA(D) A/Cambodia/NH423/003/2/2023/2023-02-21																										
28	GSAD(D) A/Cambodia/NH423/003/2/2023/2023-02-21																										
53	SubS23 A/Chicken/Cambodia/g1K22C1T/2022/H5N1/2022-12-23																										
71	SubS23 A/Duck/Cambodia/h3PClba24ID57/2023/H5N1/2023-02-27																										
84	SubS23 A/Chicken/Cambodia/h3PClba24ID57/2023/H5N1/2023-02-03																										
86	SubS23 A/Chicken/Cambodia/h3PClba24ID57/2023/H5N1/2023-02-03																										
94	SubS23 A/duck/Cambodia/g3PClba24ID57/2023/H5N1/2023-01-27																										
120	SubS23 A/duck/Cambodia/g3PClba24ID57/2023/H5N1/2023-01-06																										
124	SubS2022 A/duck/Cambodia/g10PPOr/eu24ID57/2021/H5N1/2022-02-10																										
134	SubS2022 A/duck/Cambodia/g10PPOr/eu24ID57/2021/H5N1/2022-02-10																										
137	SubS2022 A/Chicken/Cambodia/g12PV/221/CS/2022/H5N1																										
151	SubS2022 A/duck/Cambodia/g3PPOr/eu24ID57/2022/H5N1																										
154	SubS2022 A/Chicken/Cambodia/f1724C15T/2022/H5N1/2021-09-16																										
155	SubS2022 A/duck/Cambodia/g12PPChba24ID57/2022/Clearal_H5N1																										
156	SubS2022 A/duck/Cambodia/g12PPChba24ID57/2022/Clearal_H5N1																										
159	SubS2022 A/duck/Cambodia/g3PPOr/eu24ID57/2022/H5N1																										
163	SubS2022 A/duck/Cambodia/g3PPOr/eu24ID57/2022/H5N1																										
174	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
175	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
183	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
185	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
188	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
189	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
204	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
212	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
217	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
257	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
269	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
270	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
273	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
309	SubS2022 A/duck/Cambodia/g11PPOr/eu24ID57/2022/H5N1																										
	13	13	11	10	11	10	10	10	12	13	10	10	10	10	10	10	9	8	8	9	9	9	9	11	8	9	10
	1	1	1	1	2	0	1	1	2	3	2	1	1	1	2	1	1	1	2	2	1	1	3	0	2	2	

H7 Phylogenetic analysis

Feb2023-
Sept2023
Sept2022-
Feb2023
CVV

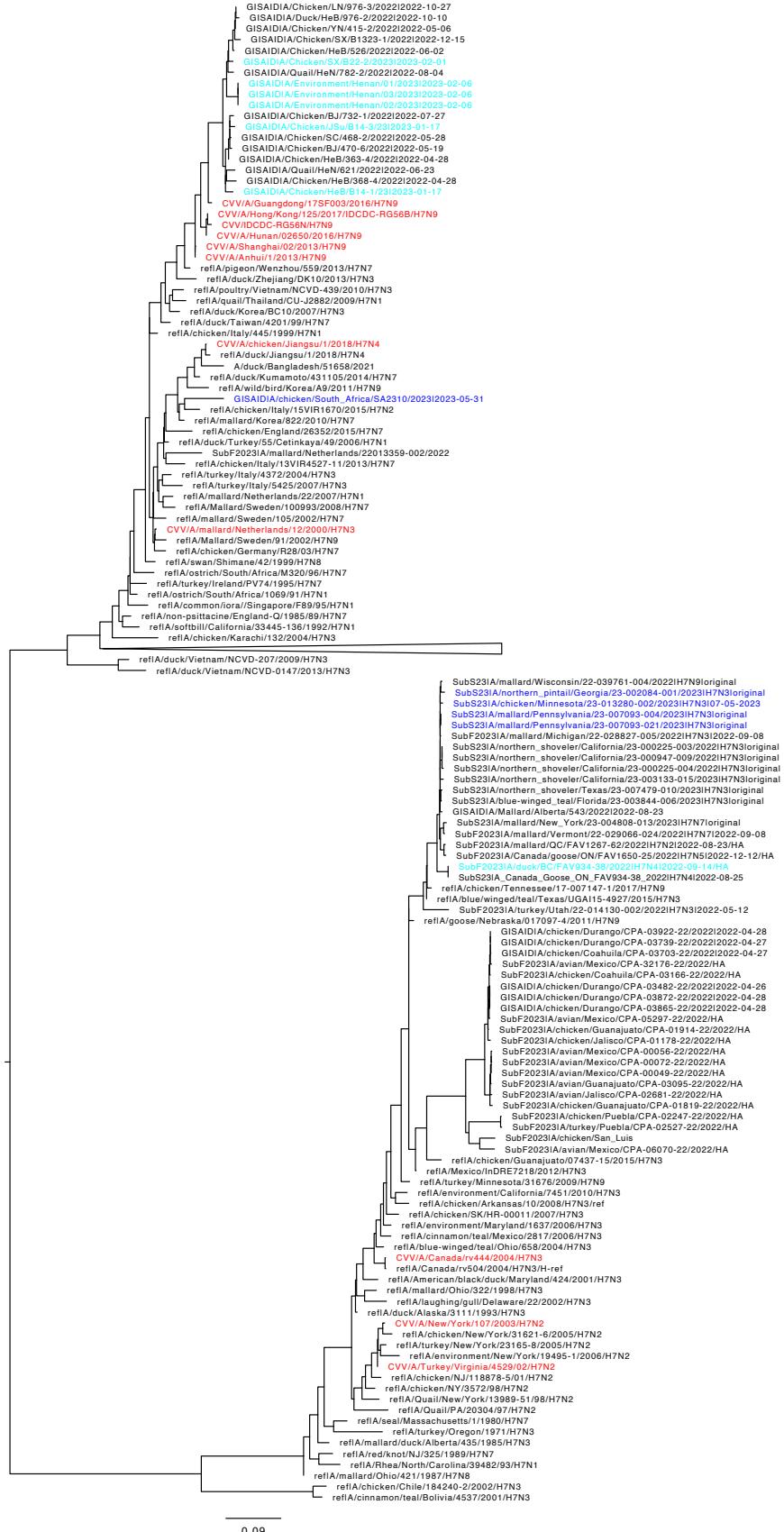


Figure 8: Avian H7 maximum likelihood phylogenetic tree. CVVs are coloured red, strains selected for antigenic characterisation are coloured yellow, strains collected in the previous reporting period are coloured light blue and from this reporting period dark blue.

H7 Amino Acid Difference Tables

Table 11: Avian H7 Amino acid difference tables compared to the CVV. Sequences from this reporting period are blue, the previous and the CVVs red. Changes in putative antigenic sites are highlighted grey.

site	CVV/A/Guangdong/17SF003/2016/H7N9																				
	GISaid A/Environment/Henan/01/2023/2023/2023-02-0	GISaid A/Environment/Henan/02/2023/2023/2023-02-0	GISaid A/Environment/Henan/03/2023/2023/2023-02-0	GISaid A/Chicken/HeB/363-4/2022/2022-04-28	GISaid A/Chicken/SC/468-2/2022/2022-05-28	GISaid A/Chicken/Bj/470-6/2022/2022-05-19	GISaid A/Chicken/Bj/732-1/2022/2022-07-27	GISaid A/Chicken/Jsu/B14-3/23/2023-01-17	GISaid A/Chicken/HeB/B14-1/23/2023-01-17	GISaid A/Quail/HeN/621/2022/2022-06-23	GISaid A/Chicken/HeB/368-4/2022/2022-04-28	GISaid A/Chicken/YN/415-2/2022/2022-05-06	GISaid A/Chicken/LN/976-3/2022/2022-10-27	GISaid A/Chicken/HeB/526/2022/2022-06-02	GISaid A/Duck/HeB/976-2/2022/2022-10-10	GISaid A/Chicken/SX/B1323-1/2022/2022-12-15	GISaid A/Quail/HeN/782-2/2022/2022-08-04	GISaid A/Chicken/SX/B22-2/2023/2023-02-01			
9 A	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
12 N																					
20 T																					
22 R	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	
37 N	T	T	T																		
47 R	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	
48 T																		R			
49 V																		A			
53 Q																		R			
68 Q																		K	K	K	K
71 E	K	K	K	K	K	K	K	K	K	T	K	K	K	K	K	K	K	K	K	K	
74 A																		H			
78 I	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
82 E																		G			
84 S		N	N	N	N	D	N														
93 V														M	M	M	M	M	M	M	M
111 E		K	K	K	K	K	K	K	K	K	K	K	K								
112 P	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
116 T	I	I	I	K	R	R	R	R	R	K	N	E	E	E	E	E	E	E	K	E	
118 N	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	E	D	D	
119 E	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	
123 N	T	T	T	T	T	T	T	T	T	T	T	T	D	D	D	D	D	D	D	D	
125 V	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	
127 S	R	R	R										R	R	R	R	R	R	R	R	R
130 R		M	M	M	M	M	M	M	M	M	M	M									
132 S	A	A	A										T	T	T	T	T	T	T	T	T
133 G	E	E	E	R	R	R	R	R	R	R	R	R	E	E	E	E	E	E	E	E	
141 K		R	R	R	R	R	R	R	R	R	R	R									
146 N	K	K	K										T					K			
147 T													K					A			
151 A	T	T	T	T	T	T	T	T	T	P	T	T	T	T	T	T	T	T	T	T	
157 K																		R			
160 K																		R			
162 T													I								
163 K	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
164 E													G	D				G			
169 I	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
177 V	I	I	I																		
179 T	I	I	I																		
190 N				D	D	D	D	D	D				D								
196 G	E	E	E	E	E	E	E	E	E				E	E	E	E	E	E	E	E	E
205 V													I								
213 Q													K								
227 I	V	V	V																		
237 S				N	N	N	N	N	N				N	N	N	N	N	N	N	N	
241 A																		V			
261 R	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	
264 V																		I			
266 A													P	P	P	P	P	P			
271 D																		N			

Table 12: Avian H7 Amino acid difference tables compared to the CVV. Changes in putative antigenic sites are highlighted grey.

site	CVV/A/mallard/Netherlands/12/2000/H7N3		GISaid A/chicken/South Africa/SA2310/2023 2023-05-3
60	I	V	
78	I	V	Putative antigenic site
84	S	R	
95	E	D	
112	T	S	Putative antigenic site
130	R	T	Putative antigenic site
228	I	M	
265	V	I	
268	N	S	Putative antigenic site
270	E	D	
276	S	N	
294	V	I	
307	L	M	

H9 Influenza A viruses

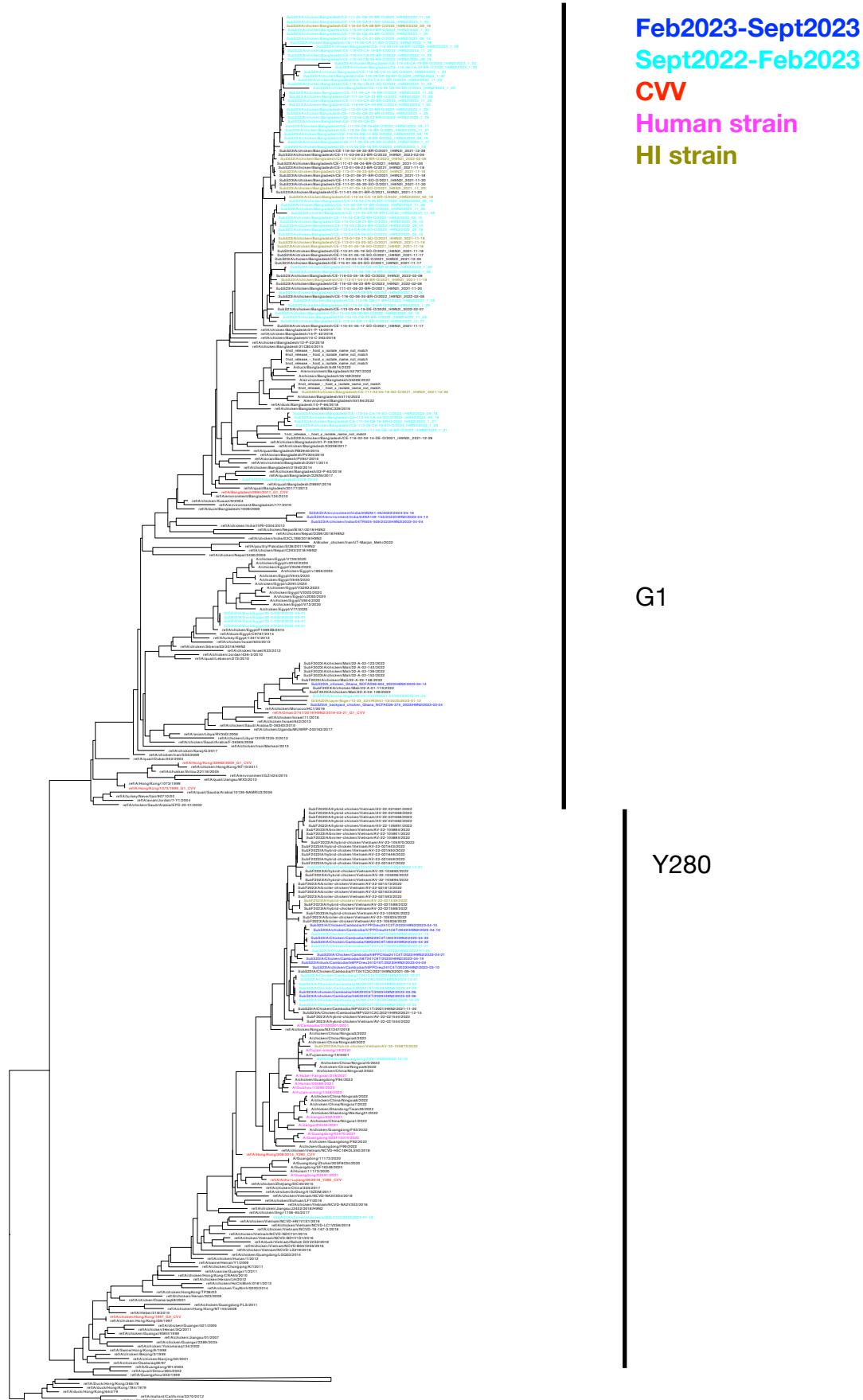


Figure 9: Avian H9 time resolved Maximum likelihood phylogenetic tree. Leaves are coloured by clade. CVVs are coloured red, strains selected for antigenic characterisation are coloured yellow, strains collected in the previous reporting period are coloured light blue and from this reporting period dark blue.

H9 Y280 phylogenetic tree

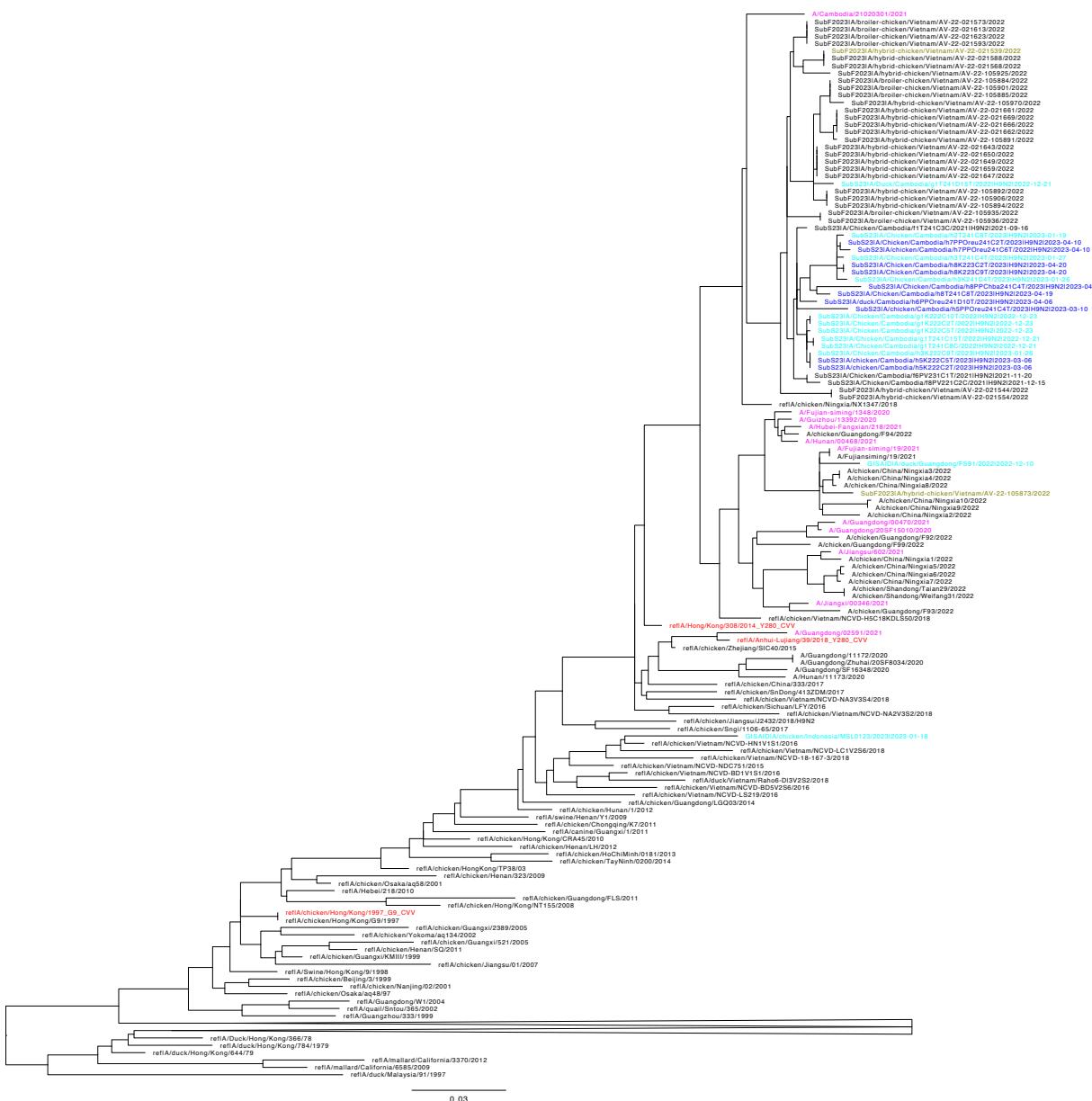
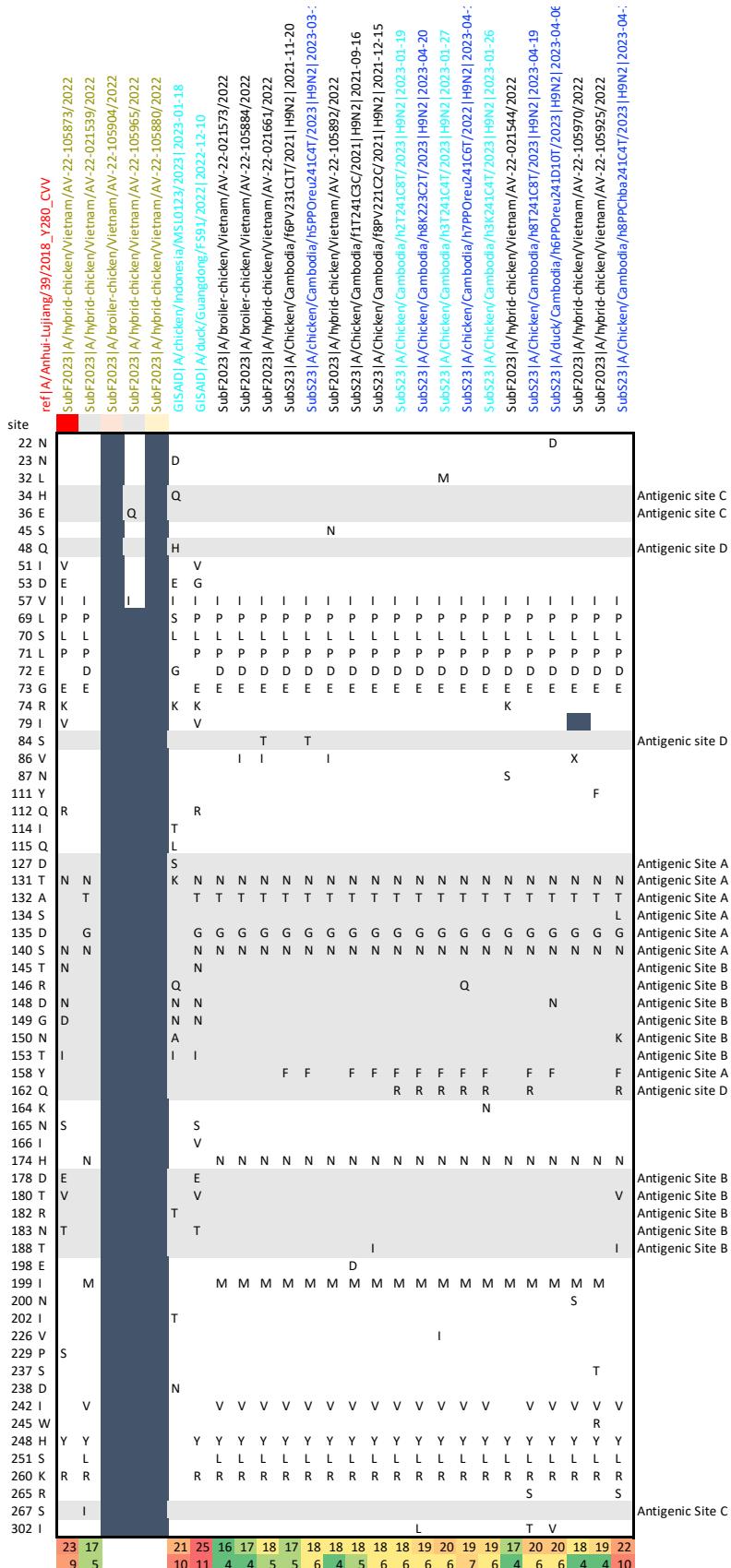


Figure 10: Avian H9 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data downloaded from GISAID, Genbank and kindly shared through the OFFLU network. Sequences within the reporting period (September 2022 to February 2023) are coloured dark blue, those from the previous reporting period light blue, CVVs in red and human sequences in pink.

H9 Y280 amino acid difference table

Table 12: Avian H9 Y280 clade sequence comparison to the closest within clade CVV. Antigenic sites have been coloured in light grey, absence of sequence is coloured dark blue. Sequences within the reporting period (February 2023 to September 2023) are coloured dark blue, those from the previous reporting period light blue and the CVV red. Strains which have been antigenically characterised are coloured yellow and fold changes compared to the closest CVV are indicated by colour underneath the strain name. A navy box indicates no sequence was available at this position.



H9 Y280 Antigenic Data

Table 13: Antigenic characterisation of H9 Y280 lineage viruses. CVVs are coloured in red. Fold changes are colour coded. Amino acid differences compared to the closest CVV are annotated.

Semester	Reference Antigen	REFERENCE FERRET ANTISERA			Clade	Subtype	Ferret ID	Clade	Subtype	A/chicken/Hong/Kong/1997	A/Hong/Kong/308/2014	A/Hong/Kong/39/2018
		Clade	Y280	Y280								
<u>APHA Feb23-</u>	A/chicken/Hong/Kong/1997	Y280	H9N2	320	10	20						
<u>Sep23</u>	A/Hong/Kong/308/2014	Y280	H9N2	80	5120	640						
	A/Anhui-Lujiang/39/2018	Y280	H9N2	80	640	2560						
	Test antigen											
<u>1</u>	A/hybrid-chicken/Vietnam/AV-22-021539/2022	Y280	H9N2	80	640	2560						
<u>2</u>	A/hybrid-chicken/Vietnam/AV-22-105880/2022	Y280	H9N2	40	320	1280						
<u>3</u>	A/hybrid-chicken/Vietnam/AV-22-105965/2022	Y280	H9N2	160	640	2560						
<u>4</u>	A/broiler-chicken/Vietnam/AV-22-105904/2022	Y280	H9N2	20	160	640						
<u>5</u>	A/hybrid-chicken/Vietnam/AV-22-105873/2022	Y280	H9N2	40	80	160						

H9 G1 phylogenetic tree (nucleotide)

Feb2023-Sept2023

Sept2022-Feb2023

CVV

H1 strain

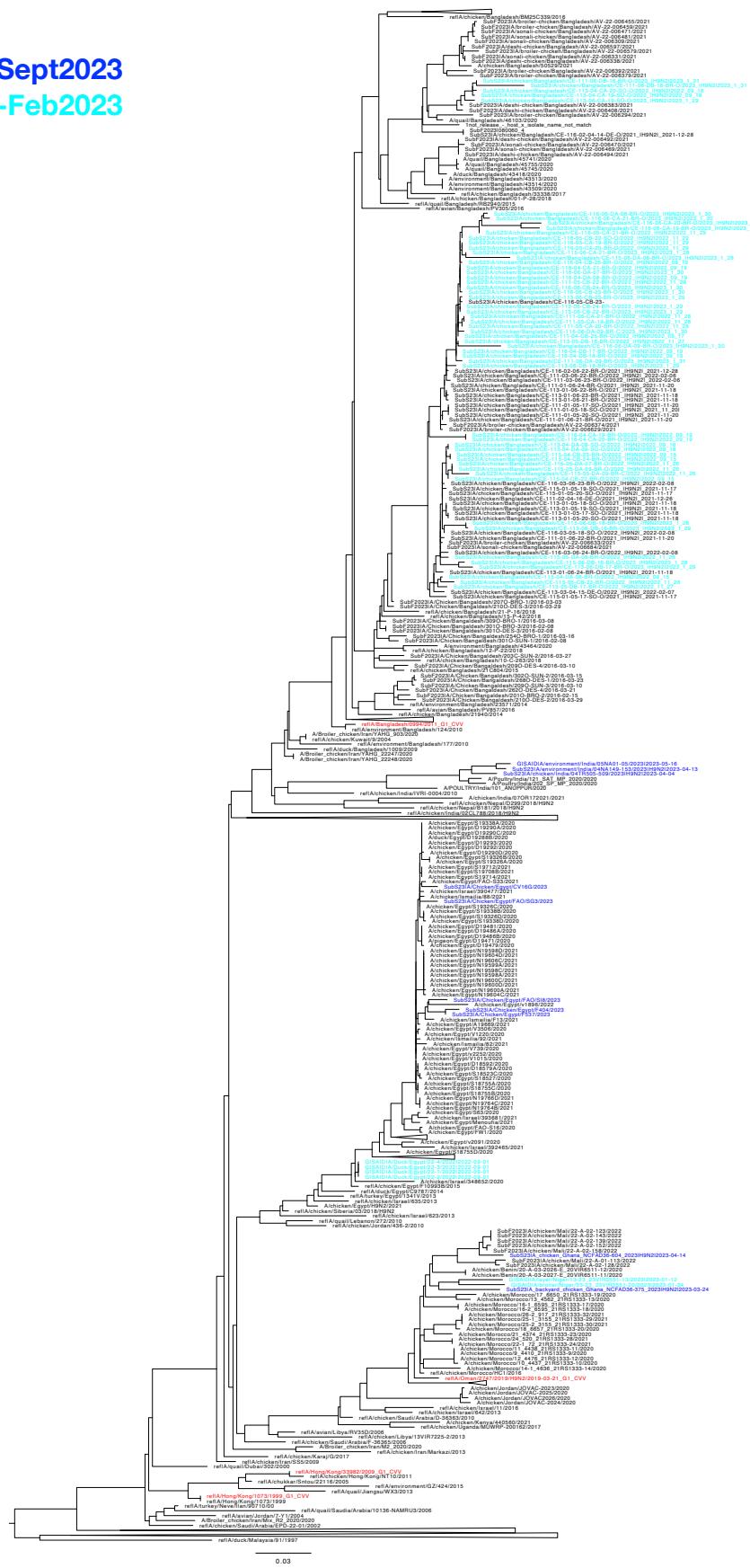


Figure 11: Avian H9 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data downloaded from GISAID, Genbank and kindly shared through the OFFLU network.

H9 G1 Amino acid difference table

Table 15: Avian H9 G1 clade sequence comparison to the closest CVV. Antigenic sites have been coloured in light grey.

site	ref A/Bangladesh/0994/2011_G1_CVV	SubS23 A/Chicken/Egypt/FAO/SG3/202	SubS23 A/Chicken/Egypt/CV16G/2023	SubS23 A/Chicken/Egypt/FAO/S18/2023	SubS23 A/Chicken/Egypt/F404/2023	SubS23 A/Chicken/Egypt/F537/2023
23	N	G	G	G	G	G
36	D	E	E	E	E	E
40	M	K	K	K	K	K
48	H	N	N	N	N	N
56	N	T	T	T	T	T
103	T	I	I	I	I	I
114	I		V			
116	I	M	M	M	M	M
128	T	S	S	S	S	S
133	S			A	A	
152	L	N	N	N	N	N
160	F	Y	Y	Y	Y	Y
163	N	T	T	T	T	T
167	D	N	N	N	N	N
196	V	I	I	I	I	I
200	N	S	S	S	S	S
218	L			Q	Q	
219	I			A	A	
228	V	I				
248	Y	F	F	F	F	F
264	N	D	D	D	D	D
267	S		N			
287	F			S		
AA diff	17	18	16	20	19	
	8	8	8	10	10	

H9 G1 Antigenic Data

Table 16: Antigenic characterisation of H9 G1 lineage viruses. CVVs are coloured in red. Fold changes are colour coded. HI tables from the previous reporting period are included.

		REFERENCE FERRET ANTISERA		Clade	G1 H9N2	G1 H9N2	A/Oman/2747/2019	A/Bangladesh/0994/2011
Semester	Reference Antigen	Clade	Subtype					
APHA Feb23-								
Sep23	A/chicken/Bangladesh/CE-113-01-05-20-SO-O/2021_ H9N2 _2021_11_18	G1	H9N2	1280	320			
	A/Bangladesh/0994/2011	G1	H9N2	640	2560			
	Test antigen							
1	A/chicken/Bangladesh/CE-113-01-05-18-SO-O/2021_ H9N2 _2021_11_18	G1	H9N2	1280	320	T225, H48Q, Q112K, D135E, L150Q, R162Q		
2	A/chicken/Bangladesh/CE-113-01-05-18-SO-O/2021_ H9N2 _2021_11_18	G1	H9N2	2560	320	T225, H48Q, Q112K, I114V, D135E, L150Q, R162Q, I288V, P315H		
3	A/chicken/Bangladesh/CE-113-01-06-24-BR-O/2021_ H9N2 _2021_11_18	G1	H9N2	1280	640	T225, H48Q, Q112K, D135E, L150Q, R162Q, I288V		
4	A/chicken/Bangladesh/CE-113-01-05-17-SO-O/2021_ H9N2 _2021_11_18	G1	H9N2	2560	320	T225, H48Q, Q112K, D135E, L150Q, R162Q, I288V		
5	A/chicken/Bangladesh/CE-113-01-06-23-BR-O/2021_ H9N2 _2021_11_18	G1	H9N2	1280	320	T225, H48Q, S109R, Q112K, G149S, L150Q, R162Q		
6	A/chicken/Bangladesh/CE-111-01-05-18-SO-O/2021_ H9N2 _2021_11_20	G1	H9N2	640	320	T225, H48Q, S109R, Q112K, L150Q, R162Q, I288V		
7	A/chicken/Bangladesh/CE-111-02-05-18-SO-O/2021_ H9N2 _2021_12_26	G1	H9N2	640	160	M40K, T54K, G149S, N198D, V226I, I288V		
8	A/chicken/Bangladesh/CE-116-04-DA-08-BR-O/2022_ H9N2 2022_09_19	G1	H9N2	2560	320	T225, H48Q, S109R, Q112K, G149S, L150Q, R162Q, I288V		
9	A/chicken/Bangladesh/CE-116-04-CA-19-BR-O/2022_ H9N2 2022_09_19	G1	H9N2	640	160	H48Q, Q112K, L150Q, R162Q, K276R, I288V		
10	A/chicken/Bangladesh/CE-111-03-06-23-BR-O/2022_ H9N2 _2022_02_06	G1	H9N2	640	160	H48Q, S109R, Q112K, G149S, L150Q, R162Q, I288V		
Sep23								
	Reference Antigen							
	A/Oman/2747/2019	G1	H9N2	1280				
	A/Bangladesh/0994/2011	G1	H9N2		320			
	Test antigen							
1	A/layer/Niger/13-23_23VIR3551-13/2023	G1	H9N2	1280	80	N45K, S77A, L104F, T127S, G150N, X187R, D262N, S265I, R317K		