OFFLU AVIAN INFLUENZA VCM REPORT

September 2021 to February 2022

SCOPE

In this document we present a summary of H5, H7 and H9 avian influenza A virus events reported from 1st September 2021 to 1st February 2022

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Avian Influenza A Viruses
Introduction, data sources and acknowledgements

The H5/H7/H9 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 (OIE). EMPRES-i is an information system designed to facilitate the compilation of animal disease data from different sources, such as the OIE, government Ministries of Agriculture and veterinary services and partner Non-Governmental Organizations (NGOs). Only data for confirmed reports in environmental samples, wild birds, captive wild birds and domestic birds were used; suspect cases were excluded where results were based solely on serology. Sequence data and viruses were shared by the OFFLU network and OIE/FAO members and we are very grateful for their collaboration.

We are very grateful to the OFFLU avian group for their expertise and for sharing data. We acknowledge APHA (United Kingdom), WUR (Netherlands), IZSVe (Italy), ARRIAH (Russia), QIA (Republic of Korea), NVL(Viet Nam), Institute Pasteur (Cambodia), CFIA (Canada), FLI (Germany), USDA (USA), AHRI (Egypt), HVRI (China), ANSES (France) and ICAR-NIHSAD (India) for sharing unpublished data and virus isolates via the OFFLU network.

We acknowledge and thank the OIE Reference laboratory and diagnostic laboratory teams for their expertise. Report analytical contributions were made from: APHA: Ian Brown, Nicola Lewis, Ashley Banyard, Joe James; RVC: Nicola Lewis, Sara Lopes; FAO/OFFLU: Amelia Coggon. Phylogenetic and sequence analyses were performed by OFFLU and RVC. Avian influenza A virus haemagglutination inhibition (HI) assay antigenic data in this reporting period was generated using harmonised protocols by APHA-Weybridge and IZSVe using WHO-CC and OFFLU-provided ferret-origin reagents kindly provided by the US-CDC, Crick Institute WHO CC World Influenza Centre and IZSVe EURL.

Analyses were conducted by subtype. Sequences collected between 1st September 2021 and 1st February 2022 were downloaded from GISAID, Genbank and NCBI on 7th February 2022. These were added to data kindly provided through the OFFLU network and a reference dataset. Sequences were aligned using MAFFT v7.475 (Katoh and Standley, 2013) using default settings. Alignments were manually inspected and trimmed to the start and stop codon of HA1. 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). HA1 sequences were compared against the most similar CVV using the NADC IAV bioinformatic toolkit (https://github.com/flu-crew) for amino acid differences. Molecular markers of phenotypic change were inferred using Suttie *et al.,* (2019) and the CDC H5N1 genetic changes inventory. 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.

Data for 859 HPAI H5, 67 LPAI H5, 6 H7 and 73 H9 sequences were contributed to OFFLU by animal health laboratories in countries representing Europe, Asia, Africa, Oceania, and the Americas to which 242 H5 and 23 H9 sequences from Genbank and GISAID were added and 46 H5 and 57 H9 sequences from WHO CCs. 2344b H5 sequences outwith the reporting period were removed, low pathogenic, duplicate or partial sequences were removed and 547 of the H5 and 24 of the H9 OFFLU contributions were included in final phylogenetic analyses.

Avian influenza A virus vaccination

In many AI endemic countries, H5 and other vaccination is employed as part of overall control efforts to limit disease. Recent H5 HPAI epizootic events have resulted in additional countries considering vaccination to control disease and this is expected to increase with the current burden of avian influenza.

Currently in China vaccination of poultry is mandatory in all provinces (including chickens, ducks, geese, quails, pigeons and other rare birds in captivity). China has had an extensive vaccination production system and their trivalent vaccine has recently been updated to include RE-13 an H5N6 2.3.4.4h, RE-14 an H5N8 2.3.4.4b and RE4 an H7N9 virus. A stamping out policy is followed in H5/H7 infected farms.

In Egypt vaccination has been in place since 2006 for the commercial sector and 2007 for backyard sector. In July 2009 vaccination in household poultry was suspended. Current understanding is that widely used vaccines include RG A/duck/Anhui/1/2006 an H5N1 (Re-5) clade 2.3.4 virus, A/chicken/Mexico/232/1994 an H5N2 classical virus and A/chicken/Vietnam/C58/2004, H5N3 clade 1 virus.

In Viet Nam it is understood that two commercial vaccines based on H5N1 viruses are used in poultry vaccination programs. The first contains a clade 1 antigen which is produced from inactivated split-virion A/Vietnam/1194/2004. The second is based on a clade 2.3.2 antigen which produced from inactivated rg A/duck/Guangdong/S1322/2010.

In Bangladesh H5 vaccination is implemented for commercial chickens in addition to stamping out disease in infected flocks. RE-6 inactivated vaccine (Merial, clade 2.3.2.1b) and Potsdam/1986 H5N2 inactivated vaccine (Nobilis®, Intervet, Potsdam/1986) are understood to be used in breeders and layers, and rHVT-H5 vectored vaccine (Vectormune®, Ceva, clade 2.2) used in day-old-chicks.

In Indonesia a vaccination program was implemented in 2004. Most recent information from 2020 indicates the currently used vaccine is based on an H5N1 clade 2.3.2.1 A/duck/Sukoharjo/BBVW-1428-9/2012.

Despite the risk of potential incursion of Asian lineage H7N9, H7 vaccination is also currently banned in Lao PDR, Viet Nam, Myanmar and Cambodia. Active surveillance for reportable H5 and H7 viruses occurs in poultry.

Global H5, H7 and H9 avian influenza events in animals

Epidemiology

Since the emergence of the A/Goose/Guangdong/1/96 (Gs/Gd) lineage of HPAI, H5Nx clades have been steadily evolving constituting an antigenically and genetically broad series of isolates. Since early 2020, H5N8 outbreaks have been reported across the European poultry sector. H5N8 outbreaks in Iraq, Russia and Kazakhstan occurred mid 2020 caused by a new H5 2.3.4.4b variant with closest relatives to 2.3.4.4b viruses detected in Israel and Iraq in 2019 and 2020 respectively. Other genetically distinct H5N8 2.3.4.4b events in Japan and South Korea occurred from October 2020 onwards. These outbreaks appear to be closely related to outbreaks seen in European Member States in early 2020.

In the summer of 2021 there was sustained HPAI circulation with an unprecedentedly broad range of genotypes. Viral reassortment occurred with low pathogenic subtypes and since autumn 2021 there is a dominance of H5N1 clade 2.3.4.4b virus with multiple but relatively stable genotypes. With the movement of migratory birds there has been a south westerly spread through Europe, Asia and Africa and more recently additionally north westerly spread to the Americas and Canada. This is the first time Gs/Gd lineage clade 2.3.4.4.b viruses have been detected in the Americas. This H5N1 virus has been found in a wider range of wild bird species than previously seen causing substantial die offs of wild bird species in certain areas and varied pathology, with this unusually high infection pressure leading to multiple spillover events into poultry.

The vast majority of outbreaks this reporting period where sequence data were available were caused by Gs/Gd H5N1 HPAI 2.3.4.4b. While not officially reportable, H9N2 LPAI viruses are known to be endemic in many countries across Asia, the Middle East, North and West Africa.

Avian Regional geographic summary and context

A(H5), A(H7), A(H9) activity

Figure 1: A. MicroReact map of H5, H7 and H9 events observed for the current reporting period 1st September 2021 to 1st February 2022 (downloaded 13th February 2022). Points are coloured by subtype. B. Collection data is represented along the timeline: the x axis represents time and y axis number of reports. C. Graph representing the frequency of species. Data was harvested from EMPRES-Plus (N=2160) and complimented with information shared via the OFFLU network (N=100). If no observation date was available a random date within the reporting period was allocated. A link to the online micro react is embedded within the title.

Avian HPAI Activity within reporting period

Mammalian cases: Fox- Estonia (H5N1); Finland (H5N1). Seal -Denmark (H5N8).

EUROPE:

H5:

Czech republic, Russian Federation from September onwards,

Denmark, Norway and Ukraine from October onwards,

Belgium from November onwards,

UK and Netherlands from January onwards.

H5N1:

Faroe Islands, Finland, Russian Federation and Sweden from September onwards,

UK, Serbia, Germany, Ireland, Italy and Netherlands from October onwards,

Austria, Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Denmark, Estonia, France, Hungary, Luxembourg, Norway, Poland, Portugal, Romania and Switzerland from November onwards,

Greece, Slovakia, Slovenia and Spain from December onwards,

Moldova, Isle of Man and Latvia from January onwards.

H5N2:

Serbia from September onwards.

H5N3:

Germany and the Netherlands from December onwards.

H5N5:

Russian Federation from November onwards.

H5N8:

Estonia, France and Serbia from September onwards,

Sweden from October onwards,

Finland from November onwards,

Denmark and Netherlands from December onwards.

Asia:

H5:

Kazakhstan from September, Japan and Pakistan from November onwards.

H5N1:

Cambodia from September onwards,

China, Israel, Republic of Korea and Viet Nam from October onwards,

India and Japan from November onwards,

Hong Kong and Taiwan from December onwards,

Nepal from January onwards.

H5N2:

Taiwan from November onwards.

H5N5:

Taiwan from September onwards,

Iran from October onwards.

H5N6:

China from September onwards.

H5N8:

Viet Nam and Cambodia from September onwards,

India, Japan and Republic of Korea from November onwards.

Africa:

H5N1:

Botswana, Nigeria and South Africa, Egypt from September onwards,

Benin and Togo from October onwards,

Côte d'Ivoire from November onwards,

Niger and Ghana from December onwards,

Burkina Faso, Namibia, Cameroon and Senegal from January onwards.

H5N8:

Egypt from September onwards.

Americas:

H5N1:

Canada from November onwards,

USA from December onwards.

Activity Table (H5) viruses

Table 1: 1st September 2021- 1st February 2022. A/goose/Guangdong/1/96 lineage viruses detected as summarised below. Data was collected through EMPRES-i, OIE via the WAHIS system and through OFFLU collaborators and WHO CCs.

Country, area or territory	Host	Genetic clade
Austria	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
Bangladesh	Poultry	2.3.2.1a (H5N1)
Belgium	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
Benin	Poultry	2.3.4.4b (H5N1)
Bosnia and Herzegovina	Wild Birds	unknown (H5N1)
Botswana	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
Bulgaria	Poultry	unknown (H5)
Burkina Faso	Poultry	unknown (H5N1)
Cambodia	Poultry	2.3.2.1c (H5N1); 2.3.4.4b (H5N8)
Cameroon	Poultry	Unknown (H5N1)
Canada	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
China	Human (24) Poultry Wild Birds	2.3.4.4b (H5N6); unknown (H5N6) 2.3.4.4b (H5N6/8) unknown (H5N1)
Cote d'Ivoire	Poultry	unknown (H5N1)
Croatia	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
Czechia	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
Denmark	Poultry Wild Birds Mammal	unknown (H5N1/N8) unknown ((H5N1/N8) 2.3.4.4b (H5N8)
Egypt	Poultry	2.3.4.4b (H5N1/N8)
Estonia	Poultry Wild Birds Mammal	unknown (H5N8) 2.3.4.4b (H5N1/N8) 2.3.4.4b (H5N1)
Faroe Islands	Wild Birds	unknown (H5N1)
Finland	Wild Birds Mammal	2.3.4.4b (H5N1); unknown (H5N8) 2.3.4.4b (H5N1)
France	Poultry Wild Birds	2.3.4.4b (H5N1/N8) 2.3.4.4b (H5N1)
Germany	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1) unknown (H5N2/N3)
Ghana	Poultry	unknown (H5N1)
Greece	Wild Birds	unknown (H5N1)
China, Hong Kong SAR	Wild Birds	unknown (H5N1)
Hungary	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)

India	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1/N8)
Iran (The Islamic Republic of)	Poultry	unknown (H5N5)
Ireland	Poultry Wild Birds	unknown (H5N1) 2.3.4.4b (H5N1)
Isle of Man	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
Israel	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
Italy	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
Japan	Poultry Wild Birds	2.3.4.4b (H5N1/N8) 2.3.4.4b (H5N8)
Kazakhstan	Poultry	2.3.4.4b (H5N1)
Lao People's Democratic Republic	Poultry	2.3.2.1c (H5N1); 2.3.4.4b (H5N8)
Latvia	Wild Birds	unknown (H5N1)
Luxembourg	Poultry Wild Birds	unknown (H5N1) 2.3.4.4b (H5N8)
Mauritania	Wild Birds	unknown (H5N1)
Moldova	Poultry	unknown (H5N1)
Namibia	Wild Birds	unknown (H5N1)
Nepal	Poultry	unknown (H5N1)
Netherlands	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1/N3/8)
Niger	Poultry	unknown (H5N1)
Nigeria	Poultry	unknown (H5N1)
North Macedonia	Wild birds	unknown (H5N1)
Norway	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
Pakistan	Poultry	unknown (H5)
Poland	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
Portugal	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
Republic of Korea	Poultry Wild birds	2.3.4.4b (H5N1/N8) 2.3.4.4b (H5N1/N8)
Romania	Wild Birds	2.3.4.4b (H5N1)
Russian Federation	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1/N5)
Senegal	Wild Birds	Unknown (H5N1)
Serbia	Wild Birds	unknown (H5N1/N2/N8)
Slovakia	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
Slovenia	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
South Africa	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)
Spain	Poultry Wild Birds	unknown (H5N1) unknown (H5N1)

Sweden	Poultry Wild Birds Mammal	2.3.4.4b (H5N1) 2.3.4.4b (H5N1/N8) unknown (H5N1)
Switzerland	Poultry	unknown (H5N1)
Taiwan (Province of China)	Poultry Wild Birds	unknown (H5N2/N5) unknown (H5N1/N2)
Togo	Poultry	unknown (H5N1)
Ukraine	Poultry	unknown (H5)
United Kingdom of Great Britain and Northern Ireland	Human (1) Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1) 2.3.4.4b (H5N1)
United States of America	Poultry Wild Birds	2.3.4.4b (H5N1) 2.3.4.4b (H5N1)

Global H5, H7 and H9 avian influenza events in humans

Table 2: Data taken from Influenza at the human-animal interface summary and assessment ([October](), [December](), [January]()). Table contains data reported to the WHO within the reporting period of 1st September 2021- 1st February 2022. Where sequence data were available from GISAID, cases are marked by an asterisk* and annotated with clade. ★Information available through GISAID and not in summary and assessment updates.

Reporting Date	State (Country)	Subtype	Age (Gender)	Further Information
3rd September 2021★	Hunan (China)*	H5N6 2.3.4.4b	58 (F)	Unknown
6th September 2021	Guizhou (China)	H9N2	11 (M)	Exposure to Poultry, detected in ILI surveillance
8th September 2021	Hunan, (China)*	H5N6 2.3.4.4b	40 (F)	Exposure to poultry, Severe disease, Fatal
13th September 2021	Guangdong (China)	H5N6	53 (M)	Exposure to poultry, Severe disease
16th September 2021	Chongqing (China)	H5N6	72 (M)	Exposure to poultry, Fatal
26th September 2021	Hunan (China)	H5N6	66 (M)	Exposure to poultry, Critical
26th September 2021	Guangdong (China)	H9N2	3 (F)	Exposure to poultry, mild
3rd October 2021	Hunan (China)	H5N6	60 (F)	Exposure to poultry, severe
20th October 2021	Guangdong (China)	H5N6	52 (M)	Exposure to poultry, Critical
29th October 2021	Guizhou (China)	H9N2	39 (M)	Exposure to poultry, Fatal, underlying medical conditions
13th November 2021	Anhui (China)	H9N2	5 (M)	Exposure to poultry, mild illness
15th November 2021	Guangxi (China)	H5N6	3 (M)	Exposure to poultry, severe
17th November 2021	Sichuan (China)	H5N6	54 (F)	Exposure to poultry, Fatal. Underlying health conditions.
22nd November 2021	Hunan (China)	H5N6	49 (M)	Exposure to poultry, severe
24th November 2021	Hunan (China)	H5N6	72 (M)	Exposure to poultry, severe
27th November 2021	Jiangsu (China)	H9N2	7 (F)	Exposure to poultry, mild illness
28th November 2021	Guangdong (China)	H9N2	7 (M)	Exposure to poultry, mild
1st December 2021	Sichuan (China)	H5N6	75 (M)	Deceased
3rd December 2021	Guangdong (China)	H5N6	68 (M)	Exposure to poultry, Critical
4th December 2021	Hunan (China)	H5N6	58 (M)	Exposure to poultry, mild
6th December 2021	Guangdong (China)	H9N2	7 (M)	Exposure to poultry, mild
7th December 2021	Hubei (China)	H9N2	3 (F)	Exposure to poultry, mild illness
8th December 2021	Sichuan (China)	H5N6	54 (M)	Deceased
9th December 2021	Guangxi (China)	H9N2	14 (F)	Exposure to poultry, mild illness
13th December 2021	Hubei (China)	H9N2	3 (M)	Exposure to poultry, mild
15th December 2021	Zhejiang (China)*	H5N6 2.3.4.4b	51 (F)	Critical
19th December 2021	Guangxi (China)	H5N6	53 (M)	Severe
23 December 2021	Guangxi (China)	H5N6	28 (M)	Critical
24th December 2021	England (United Kingdom) *	H5N1 2.3.4.4b	79 (M)	Asymptomatic
31st December 2021	Guangdong (China)	H5N6	43 (F)	Critical
3rd January 2021	Sichuan (China)	H5N6	68 (M)	Exposure to Poultry, Critical
6th January 2021	Zhejiang (China)	H5N6	55 (F)	Exposure to Poultry, Critical

H5 Influenza A viruses

H5 Phylogenetic tree coloured by geographic location (nucleotide)
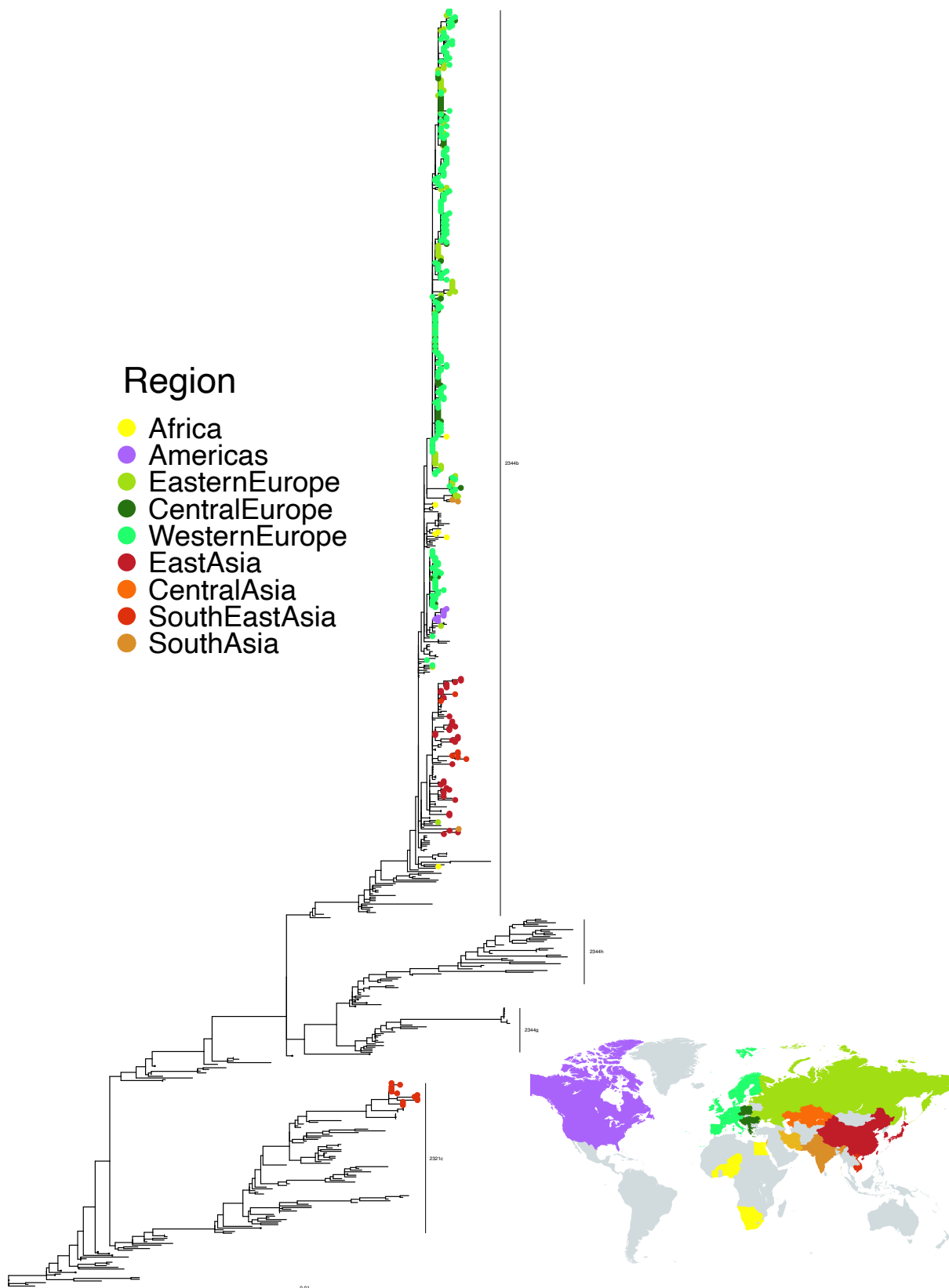
Figure 2: Avian H5 global summary maximum likelihood phylogenetic tree annotated by clades. Analyses were conducted with reference sequences, data downloaded from GISAID (1st September 2021 to 1st February 2022) and shared by the OFFLU network CNIC and St Judes. Sequences were coloured by geographic region. Only tips with sampling dates confirmed to be within the reporting period 1st September 2021 to 1st February 2022 were coloured. Clades were annotated where data was submitted this reporting period.

H5 Phylogenetic tree (Nucleotide) coloured by subtype, region and month
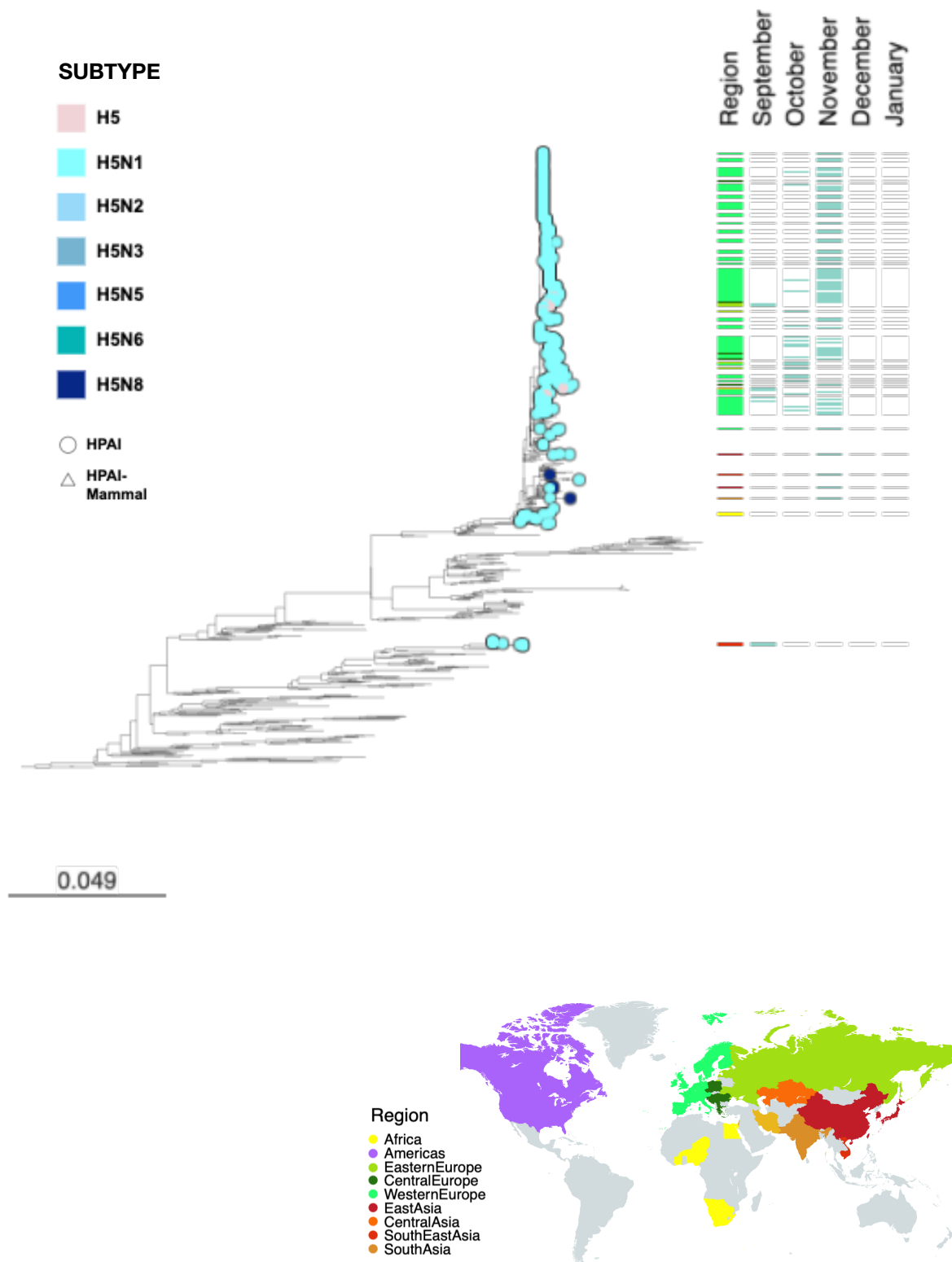
Figure 3: H5 tree to compliment the micro react map on page 6. Tips are coloured by subtype. Bars along the right hand side are coloured according to region as shown by map and month of sequence date is indicated on the right hand side. The tree is rooted by A/Goose/Guangdong/1/96.

H5 2.3.4.4b phylogenetic tree (amino acid) annotated with amino acid changes

Sept2021-Feb22
Feb21-Sept21
Outwith reporting period
CVV
Human
HI strain
@ - putative antigenic site
α - α2-6 receptor shift
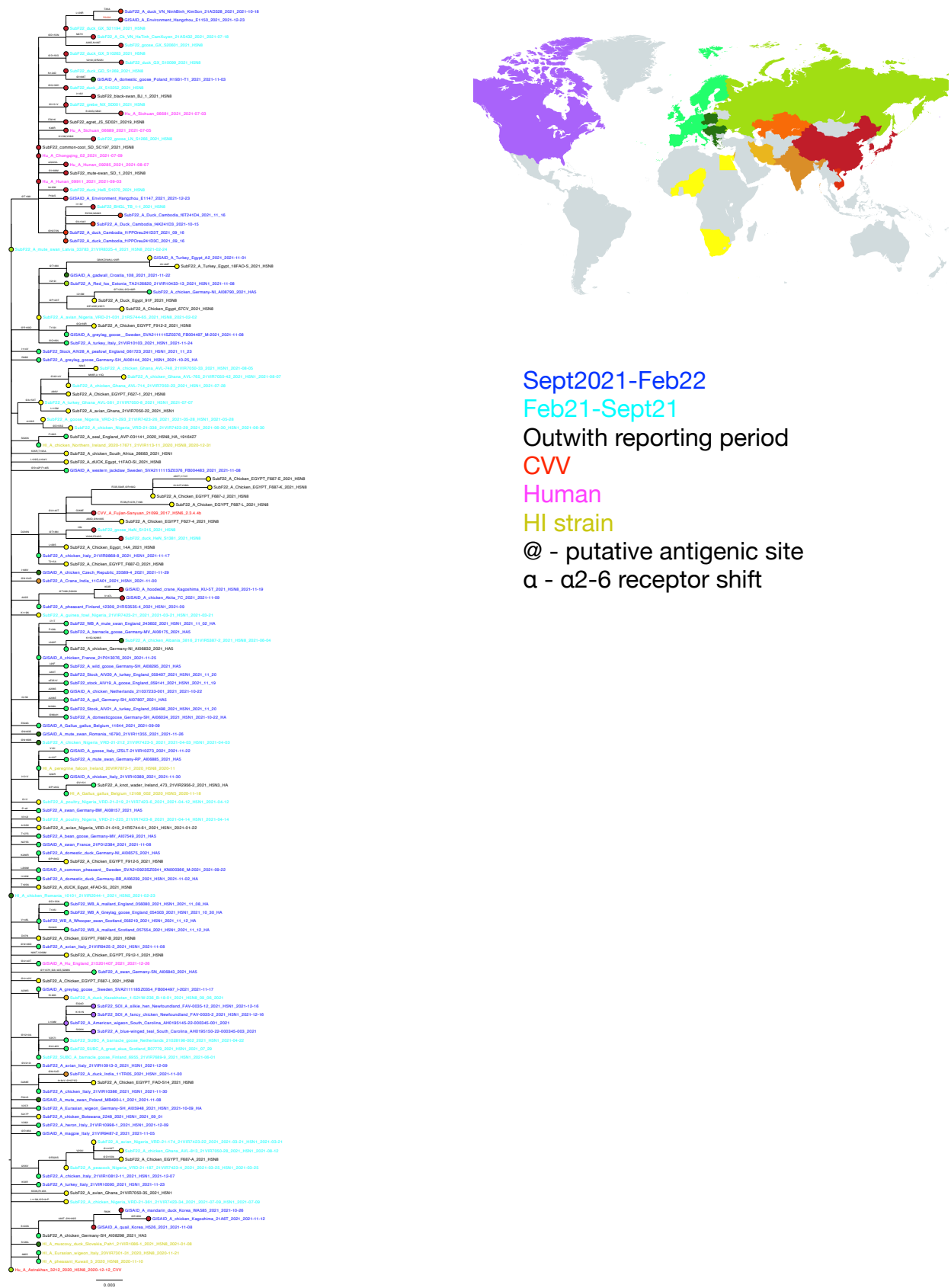
Figure 4: An amino acid phylogenetic tree inferred from HA1 amino acid sequences have been included to complement the HI table and amino acid difference table. Only unique sequences were included in this tree. Amino acid differences relative to proposed CVV A/Astrakhan/3212/2020/H5N8/2020-12-12 were annotated at the nodes and tips were coloured by geographic region.

H5 2.3.4.4b comparative amino acid substitutions to clade CVV

Table 3: Avian H5 2.3.4.4b clade sequence comparison to the CVV A/Astrakhan/3212/2020. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering. Putative receptor sites were coloured light grey for specificity, grey for antigenic and dark grey for antigenic and specificity sites. Human strains are coloured pink, CVVs coloured red, HI strains coloured yellow, sequences sampled between September 2021 and February 2022 were shown in dark blue, February 2021 to September 2021 light blue and unknown dates or pre February 2021 are coloured black. A heat map showing the total number of amino acid substitutions, putative antigenic site substitutions and receptor specificity site substitutions are shown below each strain name. Geographical region is indicated by colours below strain name.
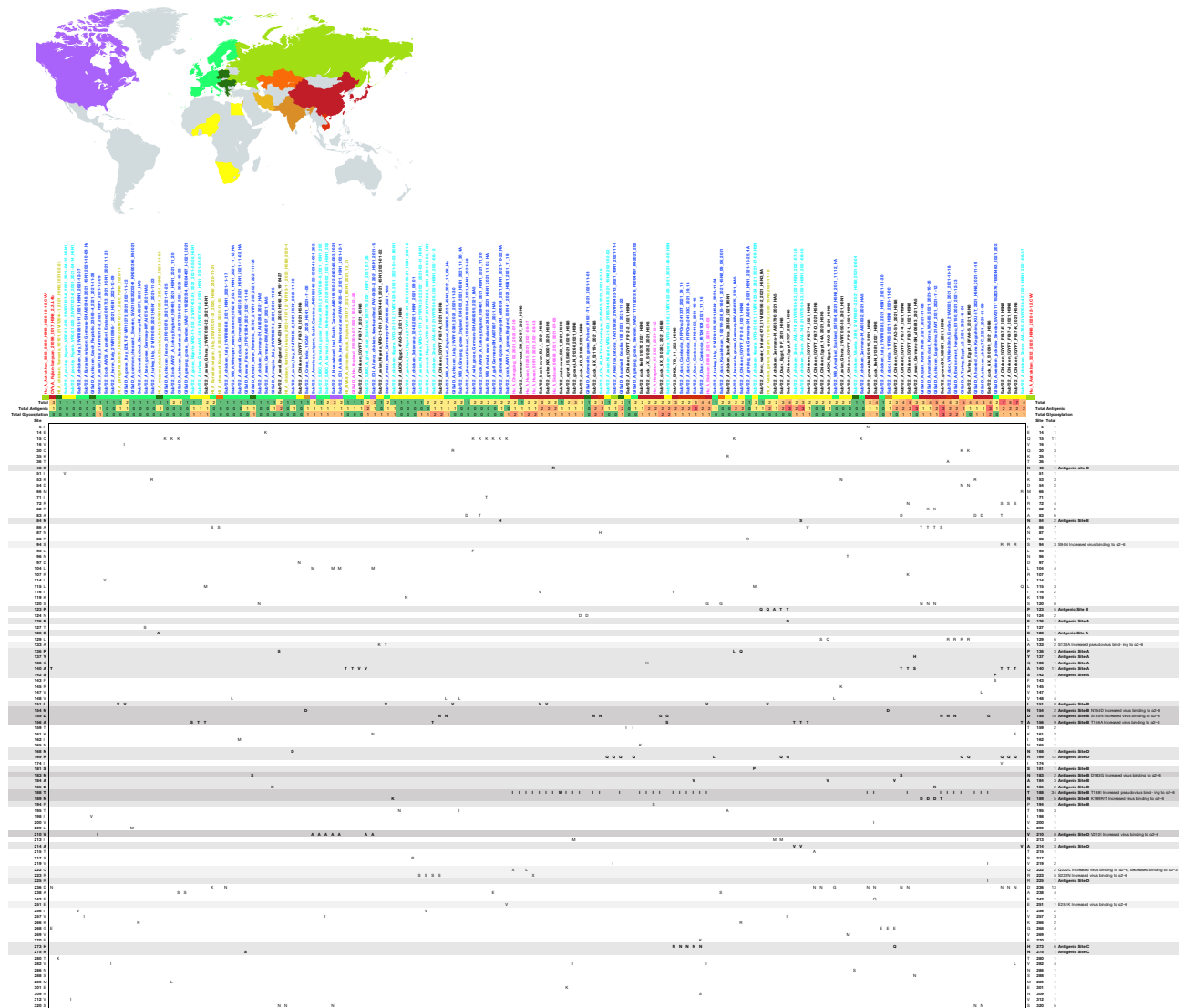
H5 2.3.4.4b HI Strains comparative amino acid substitutions to clade CVV

Table 4: Avian H5 2.3.4.4b clade sequence comparison to the CVV A/Astrakhan/3212/2020. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering. Putative receptor sites were coloured light grey for specificity, grey for antigenic and dark grey for antigenic and specificity sites. HI strains are coloured yellow and CVVs coloured red. The strain marked by a star is linked to the human case A/England/215201407/2021.

Site	Hu_A_Astrakhan_3212_2020_H5N8_2020-12-12_CVV	CVV_A_Fujian-Sanyuan_21099_2017_H5N6_2.3.4.4b	SubF22_HI_A_chicken_Ghana_AVL-714_21VIR7050-23_2021	SubF22_HI_A_chicken_Ghana_AVL-748_21VIR7050-33_2021	SubF22_HI_A_chicken_Nigeria_VRD-21-338_21VIR7423-29_2021	SubF22_HI_A_chicken_Nigeria_VRD-21-361_21VIR7423-34_2021	SubF22_HI_A_turkey_Italy_21VIR10737-1_2021	SubF22_HI_A_avian_Italy_21VIR10913-3_2021	SubF22_HI_A_white-tailed_eagle_Finland_6984_21VIR7689-10_2021	SubF22_HI_AIV24_A_chicken_England_061210_2021	SubF22_HI_AIV19_A_goose_England_059141_2021	SubF22_HI_AIV21_A_turkey_England_059498_2021	SubF22_HI_AIV20_A_turkey_England_059407_2021	SubF22_HI_AIV28_A_peafowl_England_061723_2021	SubF22_HI_AIV26_A_chicken_England_061529_2021	SubF22_HI_A_turkey_England_057679_2021	SubF22_HI_SUBC_A_great_skua_Scotland_B077779_2021	SubF22_AIV69_HI_A_domestic_duck_England_074477_2021 ★	SubF22_AIV07_HI_A_chicken_England_053485_2021	Hu_A_Astrakhan_3212_2020_H5N8_2020-12-12_CVV	Site	
15	Q									K	K	K	K	K						Q	15	
83	A												T							A	83	
84	N		S																	N	84	**Antigenic Site E**
114	I												V							I	114	
115	L				M															L	115	
140	A	T															V	T	T	A	140	**Antigenic Site A**
148	V														L					V	148	
155	D			G																D	155	**Antigenic Site B**, Glycosylation site
156	A			T	T	S														A	156	**Antigenic Site B**, Glycosylation site
169	R						L													R	169	**Antigenic Site D**
181	S					P														S	181	**Antigenic Site B**
210	V							I	A								A			V	210	**Antigenic Site D**, Glycosylation site
214	A		V	V																A	214	**Antigenic Site D**
251	E										V									E	251	Glycosylation site
289	M											L								M	289	
		1	2	3	2	2	1	1	1	1	2	2	2	1	1	0	2	1	1			

H5 2.3.4.4b Antigenic Analysis

Table 5: H5 2.3.4.4b Antigenic Analysis. Fold changes are indicated by colour. Amino acid substitutions are annotated to the right of each strain, relative to CVV A/Astrakhan/3212/2020. Antigenic sites are marked by an @ and glycosylation sites by an α. The strain marked by a star is linked to the human case A/England/215201407/2021.
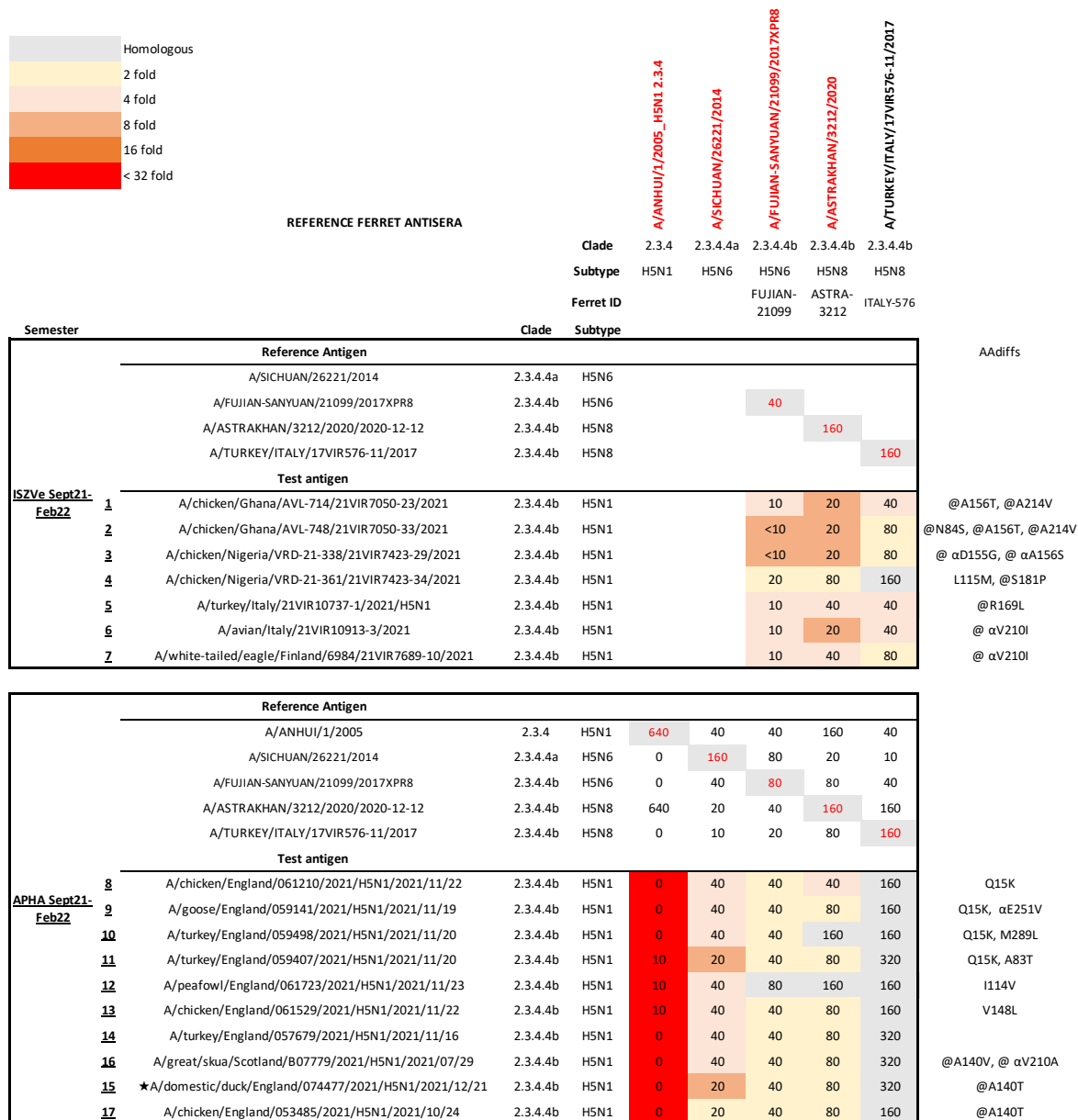
Colour key:
- Homologous
- 2 fold
- 4 fold
- 8 fold
- 16 fold
- < 32 fold

REFERENCE FERRET ANTISERA			A/ANHUI/1/2005_H5N1 2.3.4	A/SICHUAN/26221/2014	A/FUJIAN-SANYUAN/21099/2017XPR8	A/ASTRAKHAN/3212/2020	A/TURKEY/ITALY/17VIR576-11/2017	
Clade			2.3.4	2.3.4.4a	2.3.4.4b	2.3.4.4b	2.3.4.4b	
Subtype			H5N1	H5N6	H5N6	H5N8	H5N8	
Ferret ID					FUJIAN-21099	ASTRA-3212	ITALY-576	
Semester		Clade	Subtype					AAdiffs
	Reference Antigen							
	A/SICHUAN/26221/2014	2.3.4.4a	H5N6					
	A/FUJIAN-SANYUAN/21099/2017XPR8	2.3.4.4b	H5N6			40		
	A/ASTRAKHAN/3212/2020/2020-12-12	2.3.4.4b	H5N8				160	
	A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	H5N8					160
	Test antigen							
ISZVe Sept21-Feb22	1	A/chicken/Ghana/AVL-714/21VIR7050-23/2021	2.3.4.4b	H5N1			10	20	40	@A156T, @A214V
	2	A/chicken/Ghana/AVL-748/21VIR7050-33/2021	2.3.4.4b	H5N1			<10	20	80	@N84S, @A156T, @A214V
	3	A/chicken/Nigeria/VRD-21-338/21VIR7423-29/2021	2.3.4.4b	H5N1			<10	20	80	@ αD155G, @ αA156S
	4	A/chicken/Nigeria/VRD-21-361/21VIR7423-34/2021	2.3.4.4b	H5N1			20	80	160	L115M, @S181P
	5	A/turkey/Italy/21VIR10737-1/2021/H5N1	2.3.4.4b	H5N1			10	40	40	@R169L
	6	A/avian/Italy/21VIR10913-3/2021	2.3.4.4b	H5N1			10	20	40	@ αV210I
	7	A/white-tailed/eagle/Finland/6984/21VIR7689-10/2021	2.3.4.4b	H5N1			10	40	80	@ αV210I

	Reference Antigen							
	A/ANHUI/1/2005	2.3.4	H5N1	640	40	40	160	40	
	A/SICHUAN/26221/2014	2.3.4.4a	H5N6	0	160	80	20	10	
	A/FUJIAN-SANYUAN/21099/2017XPR8	2.3.4.4b	H5N6	0	40	80	80	40	
	A/ASTRAKHAN/3212/2020/2020-12-12	2.3.4.4b	H5N8	640	20	40	160	160	
	A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	H5N8	0	10	20	80	160	
	Test antigen								
APHA Sept21-Feb22	8	A/chicken/England/061210/2021/H5N1/2021/11/22	2.3.4.4b	H5N1	0	40	40	40	160	Q15K
	9	A/goose/England/059141/2021/H5N1/2021/11/19	2.3.4.4b	H5N1	0	40	40	80	160	Q15K, αE251V
	10	A/turkey/England/059498/2021/H5N1/2021/11/20	2.3.4.4b	H5N1	0	40	40	160	160	Q15K, M289L
	11	A/turkey/England/059407/2021/H5N1/2021/11/20	2.3.4.4b	H5N1	10	20	40	80	320	Q15K, A83T
	12	A/peafowl/England/061723/2021/H5N1/2021/11/23	2.3.4.4b	H5N1	10	40	80	160	160	I114V
	13	A/chicken/England/061529/2021/H5N1/2021/11/22	2.3.4.4b	H5N1	10	40	40	80	160	V148L
	14	A/turkey/England/057679/2021/H5N1/2021/11/16	2.3.4.4b	H5N1	0	40	40	80	320	
	16	A/great/skua/Scotland/B07779/2021/H5N1/2021/07/29	2.3.4.4b	H5N1	0	40	40	80	320	@A140V, @ αV210A
	15	★A/domestic/duck/England/074477/2021/H5N1/2021/12/21	2.3.4.4b	H5N1	0	20	40	80	320	@A140T
	17	A/chicken/England/053485/2021/H5N1/2021/10/24	2.3.4.4b	H5N1	0	20	40	80	160	@A140T

Table 6: For reference Antigenic analysis from previous VCM.

Legend:
- Homologous
- 2 fold
- 4 fold
- 8 fold
- 16 fold
- < 32 fold

REFERENCE FERRET ANTISERA

| Lab	Semester	#	Antigen	Clade	Ferret ID Subtype	A/ANHUI/1/2005_H5N1 2.3.4 (2.3.4, H5N1)	A/SICHUAN/26221/2014 (2.3.4.4a, H5N6)	A/FUJIAN-SANYUAN/21099/2017XPR8 (2.3.4.4b, H5N6)	A/TURKEY/ITALY/17VIR576-11/2017 (2.3.4.4b, H5N8)
IZSVe	Feb21-Sept21		**Reference Antigen**						
			A/SICHUAN/26221/2014	2.3.4.4a	H5N6		320		
			A/FUJIAN-SANYUAN/21099/2017XPR8	2.3.4.4b	H5N6			160	
			A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	H5N8				640
			Test antigen						
		1	A/chicken/Romania/10101_21VIR2044-1/2021	2.3.4.4b	H5N5		160	160	1280
		2	A/poultry/Niger/ET3_HALAL_21VIR2131-33/2021	2.3.4.4b	H5N1		80	80	640
		3	A/chicken/Italy/21VIR1151-2/2021	2.3.4.4b	H5N8		80	40	320
		4	A/chicken/Austria/21052483_21VIR3291-1/2021	2.3.4.4b	H5N8		40	40	160
		5	A/chicken/Lithuania/4323_21VIR3369-1/2021	2.3.4.4b	H5N8		80	40	320
		6	A/chicken/Kosovo/82_21VIR5162-1/2021	2.3.4.4b	H5N8		40	40	160
		7	A/knot_wader/Ireland/472_21VIR2956-1/2021	2.3.4.4b	H5N3		320	160	1280
		8	A/yellow-legged_gull/Switzerland/15-0039_21VIR3035/2021	2.3.4.4b	H5N4		<10	<20	160
		9	A/chicken/Northern_Ireland/2020-17671_21VIR113-11/2020	2.3.4.4b	H5N8		80	40	320
		10	A/muscovy_duck/Slovakia/Pah1_21VIR1086-1/2021	2.3.4.4b	H5N8		160	80	640
	Feb21-Sept21		**Reference Antigen**						
			A/SICHUAN/26221/2014	2.3.4.4a	H5N6		320		
			A/FUJIAN-SANYUAN/21099/2017XPR8	2.3.4.4b	H5N6			80	
			A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	H5N8				320
			Test antigen						
		11	A/chicken/Nigeria/VRD21-100_21VIR2370-423/2021	2.3.4.4b	H5N3		40	20	160
	Sept20-Feb21		**Reference Antigen**						
			A/SICHUAN/26221/2014	2.3.4.4a	H5N6		320		
			A/FUJIAN-SANYUAN/21099/2017XPR8	2.3.4.4b	H5N6			160	
			A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	H5N8				640
			Test antigen						
		12	A/eurasian_wigeon/italy/20vir7301-206/2020	2.3.4.4b	H5N1		20	10	40
		13	A/peregrine_falcon/denmark/13776-1_i20vir7282-1i/2020	2.3.4.4b	H5N5		40	40	320
		14	A/mute_swan/netherlands/20015931-001/20	2.3.4.4b	H5N8		40	40	320
		15	A/barnacle_goose/denmark/14138-1_i20vir7282-2i/2020	2.3.4.4b	H5N8		40	40	160
		16	A/mallard/italy/20vir7139-73/2020	2.3.4.4b	H5N8		40	20	160
		17	A/eurasian_wigeon/italy/20vir7301-31/2020	2.3.4.4b	H5N8		40	40	160
		18	A/peregrine_falcon/ireland/20vir7872-1/2020	2.3.4.4b	H5N8		40	20	160
		19	A/gallus_gallus/belgium/12168_i20vir7627-3i/2020	2.3.4.4b	H5N5		20	20	80
APHA	Feb21-Sept21		**Reference antigen**						
			A/ANHUI/1/2005	2.3.4	H5N1	640	<10	<10	10
			A/SICHUAN/26221/2014	2.3.4.4a	H5N6	<10	80	80	20
			A/FUJIAN-SANYUAN/21099/2017XPR8	2.3.4.4b	H5N6	<10	40	80	160
			A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	H5N8	<10	10	20	320
			A/DUCK/ENGLAND/36254/2014	2.3.4.4c	H5N8	<10	40	160	1280
			A/DUCK/HYOGO/1/2016	2.3.4.4e	H5N6	<10	40	80	40
			Test antigen						
		20	A/chicken/Ukraine/1/2020	2.3.4.4b	H5N8	<10	10	40	160
		21	A/turkey/Norway/FU496/2020	2.3.4.4b	H5N8	<10	10	20	80
		22	A/chicken/England/030786/2020	2.3.4.4b	H5N8	<10	20	40	320
		23	A/pheasant/Kuwait/5/2020	2.3.4.4b	H5N8	<10	20	40	160
		24	A/chicken/England/034547/2020	2.3.4.4b	H5N8	<10	10	20	160

H5 2.3.2.1c phylogenetic tree (Nucleotide)
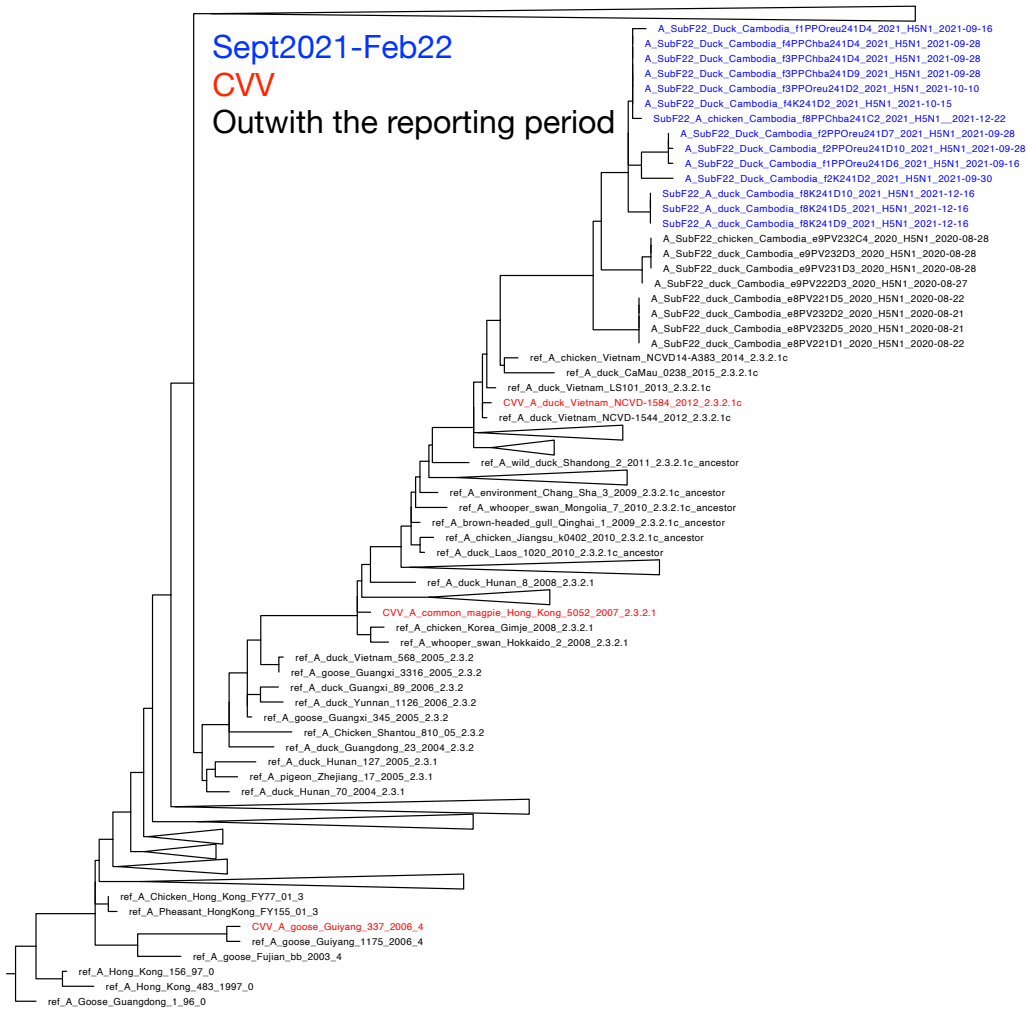
Figure 5: Maximum likelihood phylogenetic tree showing the 2.3.2.1c clades. Data included from OFFLU and downloaded from GISAID. Rooted by A/Goose/Guangdong/1/96. CVVs are shown in red and strains from this reporting period in dark blue. Other clades are collapsed.

H5 2.3.2.1c amino acid difference table

Table 7: Avian H5 2.3.2.1c clade sequence comparison to the CVV which is shown in red. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering. Putative antigenic sites were coloured grey and Putative antigenic and glycosylation sites coloured dark grey.

site	CVV_A_duck_Vietnam_NCVD-1584_2012_2.3.2.1c	SubF22_A_duck_Cambodia_f8K241D10_2021	SubF22_A_chicken_Cambodia_f8PPChba241C2_2021	A_SubF22_Duck_Cambodia_f4K241D2_2021	A_SubF22_Duck_Cambodia_f1PPOreu241D4_2021	A_SubF22_Duck_Cambodia_f1PPOreu241D6_2021	A_SubF22_Duck_Cambodia_f2PPOreu241D10_2021	A_SubF22_Duck_Cambodia_f2K241D2_2021	A_SubF22_duck_Cambodia_e9PV222D3_2020	A_SubF22_duck_Cambodia_e8PV221D1_2020		
15	Q								K			
28	H	Q	Q	Q	Q	Q	Q	Q	Q	Q		
71	T								I			
120	D	N	N	N	N	N	N	N	N	N		
154	D		N	N	N	N	N	N		N	**Antigenic Site B**	Glycosylation site
155	N		D					S		S	**Antigenic Site B**	Glycosylation site
156	A	V									**Antigenic Site E**	
163	G	D	D	D	D	D	D	D	D	D	**Antigenic Site B**	Glycosylation site
188	T					M	M	V			**Antigenic Site B**	Glycosylation site
189	R	K				K						
212	K	R	R	R	R	R	R	R	R			
217	S	P	P	P	P	P	P	P	P	P		
226	I				M							
270	E	K	K	K	K	K	K	K	K	Q		
309	N	S	S	S	S	S	S	S	S	S		
Total		9	9	8	9	10	9	9	9	8		
…igenic		2	3	2	2	3	3	3	2	3		
…lation		1	3	2	2	3	3	3	2	3		

H5N1 Tanglegram
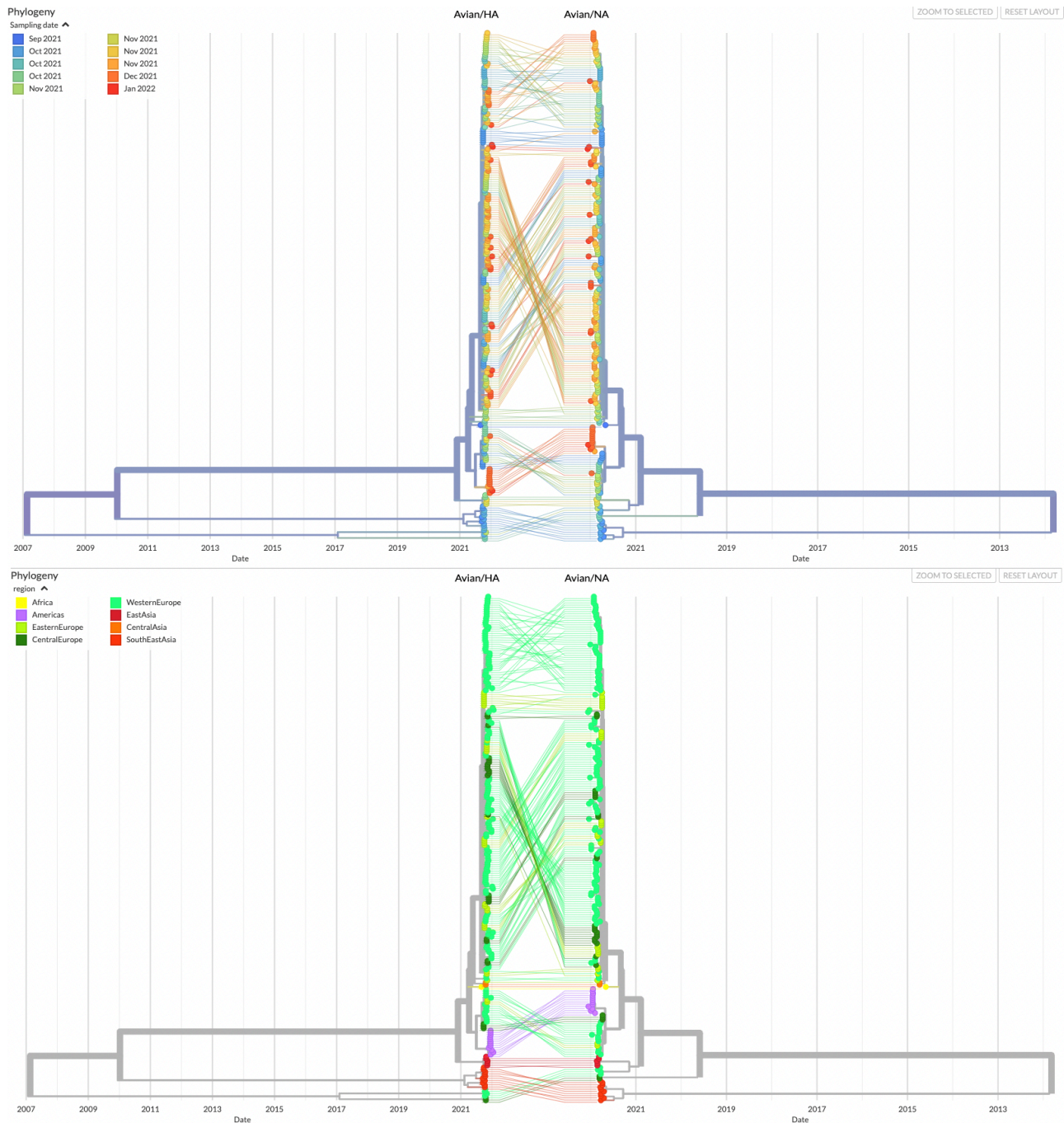
H5N1 time resolved tangle tree (Nucleotide)

Figure 6: Analyses conducted using the NEXSTRAIN augur pipeline and auspice for visualisation. Whole avian HPAI and LPAI H5N1 HA and NA sequences were downloaded from GISAID on 7th February 2022. OFFLU sequences were subsequently added to both datasets. Nodes were coloured by country or by sampling date. (2.3.2.1c and 2.3.4.4b clades included).
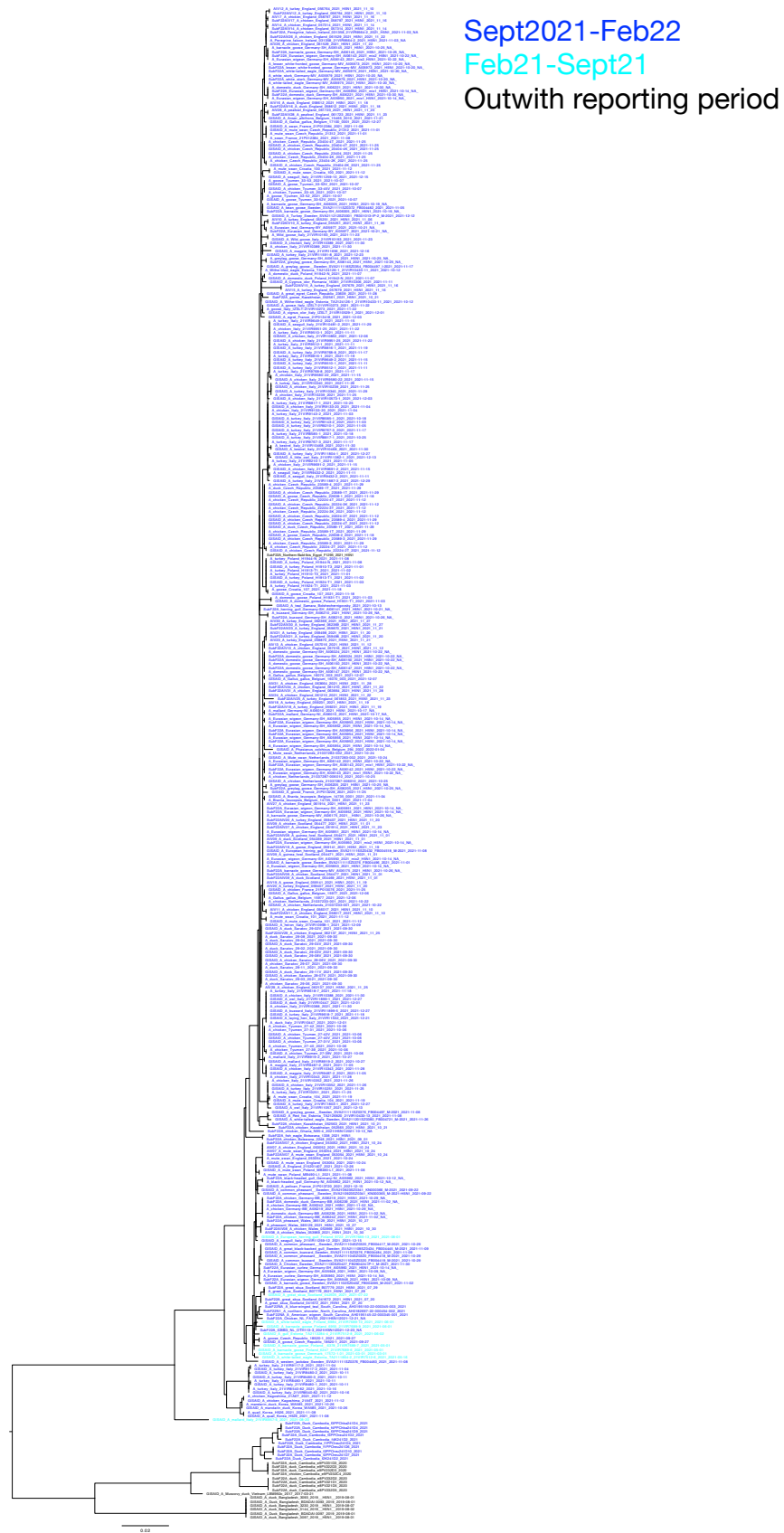
NA-N1 Influenza A viruses (Nucleotide)

Sept2021-Feb22
Feb21-Sept21
Outwith reporting period

Figure 7: Avian N1 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU.

H9 Influenza A viruses

H9 Phylogenetic tree (nucleotide)

Sept21-Feb22
Feb21-Sept21
2021 exact date unknown
Human
HI strain
CVV

Figure 8: Avian H9 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data from the reporting period downloaded from GISAID, FluDB (13th February 2022) and shared through OFFLU. The CVV for this clade is shown in red. Human strains are shown in pink. HI strains are shown in yellow and fold changes are annotated to the right of strains.

H9 G1 comparative amino acid substitutions to clade CVV

Table 8: Avian H9 G1 clade sequence comparison to the CVV which is shown in red. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H9 numbering.

| site	CVV_A_Oman_2747_2019_H9N2_2019-03-21_HA	SubF22_A_Chicken_EGYPT_NLQP_FAO-S51-2021_08-09-202	GISAID_A_chicken_Egypt_FAO-S33_2021_2021-07-02	SubF22_Chicken_EGYPT_F423-1_2021_H9N2_2021-05-06	SubF22_A_Chicken_EGYPT_FAO-SI4_2021_H9N2_31-01-202	SubF22_Chicken_EGYPT_FAO-S22_2021_H9N2_11-05-2021	SubF22_A_chicken_Nigeria_VRD-21-239_21VIR7423-12_202	SubS2021_A_poultry_Niger_ET1_21VIR2131-37_2021_2021	subS2019_A_chicken_Niger_19VIR1322-37_2019_H9N2	SubF22_A_avian_Nigeria_VRD-21-222A_21VIR7423-36_202	Annotations
23	N	G	G	G	G	G					
28	Q	H	H	H	H	H					
29	A										
40	M	K	K	K	K	R					
45	N		S				K	K		K	
48	R	N	N	N	N	D					
54						A					
57	I	V	V	V	V	V					
60						I					
69	M	L	L	L	L						
70						F					
75						K					
77	S						A	A	A	A	
86	V						A	A		A	
94	N						S				
95									I		
103	T	I	I	I	I	V				A	
104	L						F	F	F	F	
114	V	I	I	I	I	I					
116	L	M	M	M	M	M					
120	S	T	T	T	T	T					Published escape mutation
121	I					T					
127	T	S	S	S	S	S					Published escape mutation
135								G			Published escape mutation
148								D			Published escape mutation
150	G	N	N	N	N	N	N	N	S	N	Published escape mutation
153	I	V	V	V		V					
156						T					
161	N	T	T	T	T						
162						Q					Published escape mutation
165	D	N	N	N							
180	A				T						
187	X	R	R	R	R	R	R	R		R	
194	V	I	I	I	I	I					
198	T	S	S	S	S	S				S	
226	V	I									
260	R	K	K	K	K	K					
262	D						N	N		N	
264	S	N	N	N	N	N					Published escape mutation
265	S					I					
269						L					
283	M	L	L	L	L	L					
295	T	N	N	N	D	D					
302	V				A	E					
315	H	P	P	P	P	P					
317	S								K		
Total		24	23	24	23	29	9	7	7	9	
Antigenic		4	4	4	4	5	1	1	3	1	

H9 Y280 comparative amino acid substitutions to clade CVV

Table 9: Avian H9 Y280 clade sequence comparison to the CVV which is shown in red. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H9 numbering. Sequences within the reporting period were coloured dark blue.

Site	CVV_A_Hong_Kong_308_2014_Y280	SubF22_A_Chicken_Cambodia_f10PPChba241C5_2021_H9N2_2022-01-;	SubF22_A_Chicken_Cambodia_f10T241C6_2021_H9N2_2022-01-29	SubF22_A_Chicken_Cambodia_f3K222C2_2021_H9N2_2021-09-10	SubF22_A_Chicken_Cambodia_f5K222C1_2021_H9N2_2021-11-11	SubF22_A_Chicken_Cambodia_f5PV222C1_2021_H9N2_2021-11-12	SubF22_A_Chicken_Cambodia_f10T241C2_2021_H9N2_2022-01-29	SubF22_A_Chicken_Cambodia_f10PV231C4_2021_H9N2_2022-01-25	Annotations
69	L	P	P	P	P	P	P	P	
71	L	P	P	P	P	P	P	P	
72	E	D	D	D	D	D	D	D	Published Escape Mutation
73	G	E	E	E	E	E	E		
109	R							K	Published Escape Mutation
131	K	N	N	K	N	N	N	N	Published Escape Mutation
132	A	T	T	T	T	T	T	T	
140	S	N	N	N	N	N	N	N	
158	Y	F	F	F	F	F			
165	N						D		
174	H	N	N	N	N	N	N	N	
188	T		I	I					Published Escape Mutation
199	I	M	M	M	M	M	M	M	
238	D							N	
242	I	V	V	V	V	V	V	V	
248	H	Y	Y	Y	Y	Y	Y	Y	
251	S	L	L	L	L		L	L	
260	K	R	R	R	R	R	R	R	
267	S						I		
315	P				H				
319	S							N	
Total		14	15	14	15	13	15	15	
Antigenic		2	3	2	2	2	2	3	

H9 Antigenic Analysis

Table 10: H9 Antigenic Analysis. Fold changes are indicated by colour. Analysis was performed by IZSVe

	Homologous
	2 fold
	4 fold
	8 fold
	16 fold
	< 32 fold
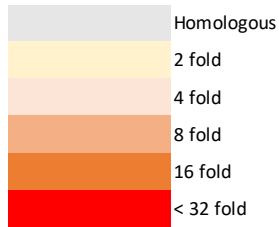
REFERENCE FERRET ANTISERA

					Clade	G1
					Subtype	H9N2
					Ferret ID	OMAN/2747
Semester			**Clade**	**Subtype**		
		Reference Antigen				
		A/Oman/2747/2019	G1	H9N2		640
		Test antigen				
Sept21-Feb22	1	A/poultry/Niger/Et1_21VIR2131-37/2021	G1	H9N2		640
	2	A/avian/Niger/19VIR1322-37/2019	G1	H9N2		320
	3	A/chicken/Nigeria/VRD_21_239/21VIR7423-12/2021	G1	H9N2		640
	4	A/chicken/Nigeria/VRD_21_222A/21VIR7423-36/2021	G1	H9N2		1280

Annex:
Humanising substitutions A/England/215201407/2021

Routine nasophyarngeal samples were taken from an asymptomatic 79 year old male in England who had experienced exceptionally close contact with infected birds from the 22nd December 2021 . Swabs testing positive 7 days post exposure were whole genome sequenced. No virus was able to be isolated and serology results are pending.

- This is an H5N1 clade 2.3.4.4b virus with all internal genome segments related to currently circulating avian european H5 2.3.4.4b viruses.
- This virus has one amino acid difference within the HA1 protein compared to A/Astrakhan/3212/2020 present in a putative antigenic site.
- All changes linked to increased alpha 2-6 linked receptor binding are present in the HA of the sequence of many to most currently circulating viruses.
- The amino acid change E627K is not present in this virus.
- There are no substitutions indicating antiviral drug resistance.

Humanising substitutions analysis A/Hangzhou/01/2021

Broncheoavleolar lavage samples were taken from 59 year old year old female in the province of Hangzhou in China. The whole genome sequence was released onto GISAID on the 22nd December 2021.

- This is an H5N6 clade 2.3.4.4b virus
- This virus has 2 changes in the HA1 protein compared to current A/Astrakhan/3212/2020 of which two are in putative antigenic sites and one change is linked to linked to increased alpha 2-6 linked receptor binding.
- The amino acid change E627K is not present in this virus.
- There are no substitutions indicating antiviral drug resistance.

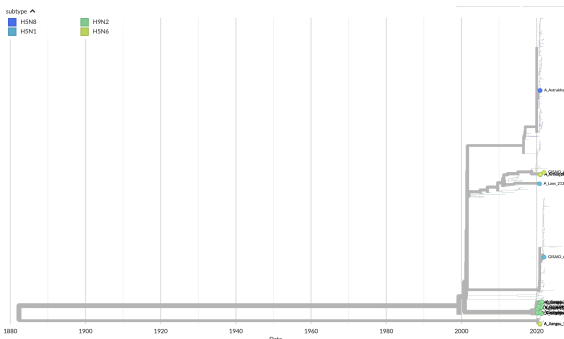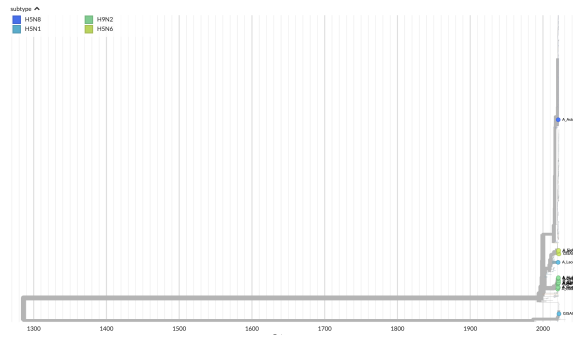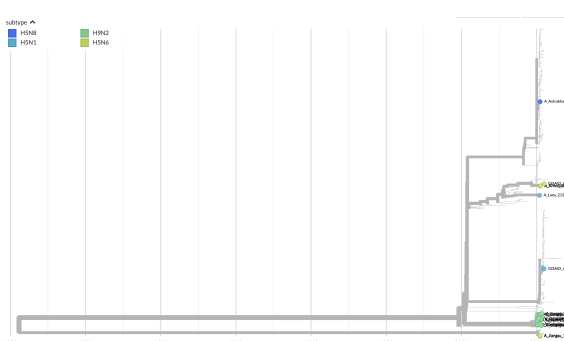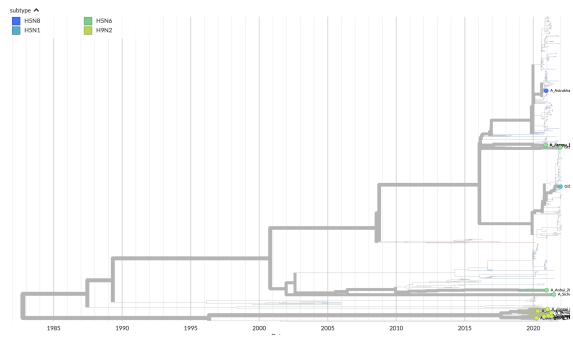# Whole genome analysis of currently circulating strains.

HA
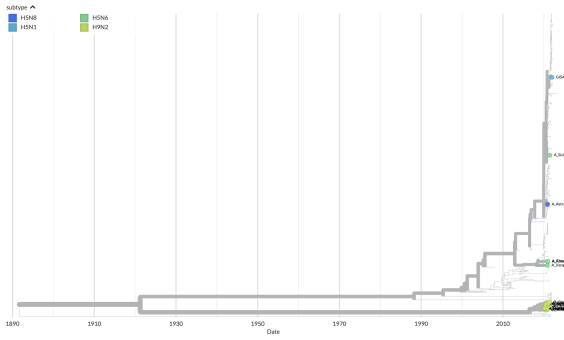
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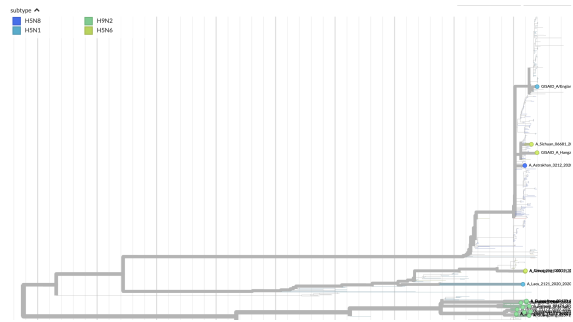
NA

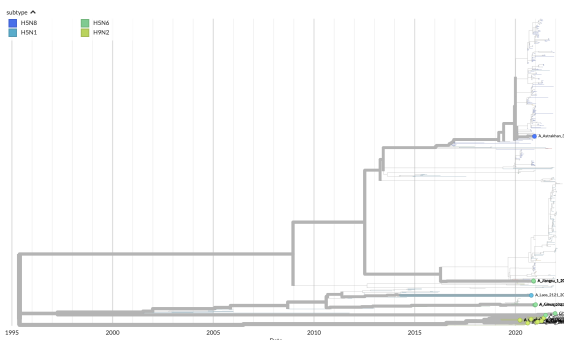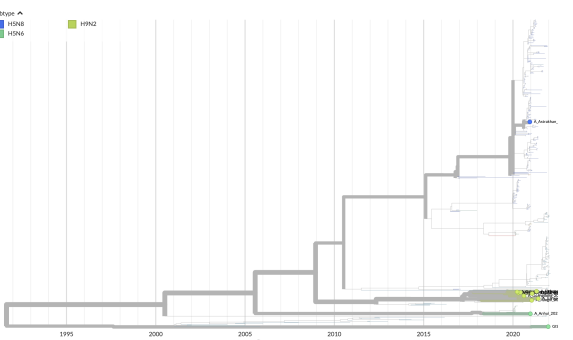

NP

NS

PA

PB1

PB2

H7 Tree

Figure 9: Avian H7 maximum likelihood phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. Data within this reporting period is coloured dark blue.

American lineage sequences from reporting period

Eurasian lineage sequences from reporting period

Feb21-Sept21

CVV

Outwith reporting period
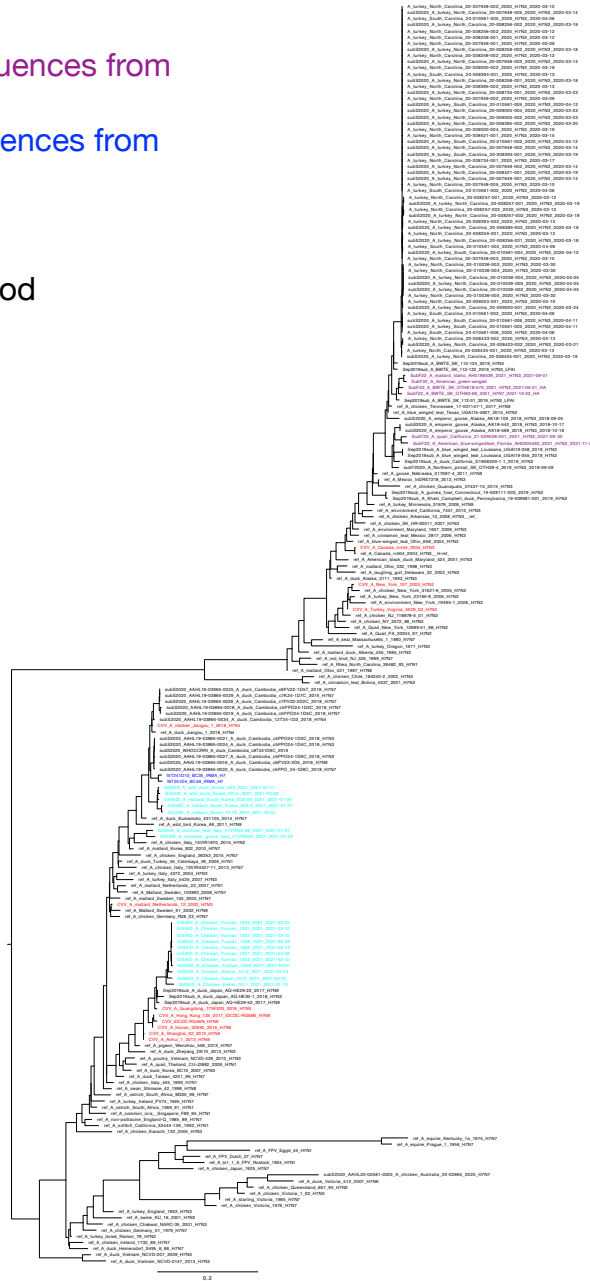
H7 amino acid substitutions

Table 11: H7 Amino acid differences tables from eurasian lineage viruses currently circulating strains in Cambodia.

site	CVV_A_chicken_Jiangsu_1_2018	f5T241D10_BC35_IRMA_H7	f6T241D4_BC49_IRMA_H7
49	V		I
84	S		N
95	D	E	E
121	R	K	
155	M	L	L
164	R	K	K
199	K	N	N
205	V	I	I
224	Q	H	H
237	T	S	S
256	L	P	P
262	G	E	
Total		10	10

Summary of Mammalian H5 cases

Table 12: Taken from EFSA Avian influenza overview [report](#) 2021.

Table 2: Avian influenza virus A(H5Nx) detections in other mammal species related to circulating viruses in Europe, 2016-2021

Date	Country	Animal (species)	Virus	Reference
November 2016, April 2017	Poland	2 grey seals (*Halichoerus grypus*)	A(H5N8) clade 2.3.4.4b	(Shin et al., 2020)
December 2016 to May 2017	France	Domestic pigs (*Sus scrofa*), serological detection	A(H5N8) clade 2.3.4.4b	(Herve et al., 2021)
2017	Germany	Wild boar serological detection	A(H5N8)	(Schülein et al., 2021)
Late 2020	The United Kingdom	4 juvenile common seals (*Phoca vitulina*), 1 juvenile grey seal (*Halichoerus grypus*), 1 juvenile red fox (*Vulpes vulpes*)	A(H5N8) clade 2.3.4.4b	(Floyd et al., 2021)
April, February, and September 2021	Sweden	1 gray seal (*Halichoerus grypus*), 2 red foxes (*Vulpes vulpes*)	A(H5N8) clade 2.3.4.4b, A(H5N1) clade 2.3.4.4b, A(H5N1)[*]	(SVA, online: Personal communication by Siamak Zohari, SVA)
May 2021	Netherlands	2 red fox cubs (*Vulpes vulpes*)	A(H5N1) clade 2.3.4.4b	(Rijks et al., 2021)
August 2021	Germany	3 harbour seals (*Phoca vitulina*)	A(H5N8) clade 2.3.4.4b	(Ärzteblatt, online)
September and November 2021	Finland	2 fox (*Vulpes vulpes*), 1 otter (*Lutra lutra*)	A(H5N1) clade 2.3.4.4b	(FFA, online)
November 2021	Estonia	Fox (*Vulpes vulpes*)	A(H5N1) clade 2.3.4.4b	(OIE, online-b)
November 2021	Italy	Domestic pigs (*Sus scrofa*) (serological detection in HPAI poultry outbreak)	A(H5N1) clade 2.3.4.4b	EURL

[*] No information on the clade of HPAI A(H5N1) detected in the second red fox is currently available