OFFLU AVIAN INFLUENZA VCM REPORT

February 2022 to September 2022

SCOPE

In this document we present a summary of H5, H7 and H9 avian influenza A virus events reported from 1st February 2022 to 1st September 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 We acknowledge ACDP, ANSES, APHA, ARRIAH, CAN, ICAR, IZSVe, NIAH, Institut Pasteur and WUR for sharing unpublished data and virus isolates via the OFFLU network.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: Sara Lopes, Amelia Coggon and Nicola Lewis. Phylogenetic and sequence analyses were performed by RVC and OFFLU. Avian influenza A virus haemagglutination inhibition (HI) assay antigenic data in this reporting period was generated using harmonised protocols by APHA, IZSVe and ACDP using ferret-origin reagents kindly provided by CDC, SJ-RCH, and WHOCC's. Additional ferret sera were generated at IZSVe from a selection of antigenically important viruses from contemporary avian Influenza strains to increase the resolution of antigenic characterisation of 2.3.4.4b viruses.

Analyses were conducted by subtype. Sequences collected between 1st January 2022 and 1st September 2022 were downloaded from GISAID, 2nd September 2022. These were added to data kindly provided through the OFFLU network and to a genetic reference dataset compiled by CDC. Sequences were analyses using the Augur pipeline by Nextstrain.

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 588 HPAI H5, 60 H7 LPAI and 89 H9 sequences were contributed to OFFLU by animal health laboratories in countries representing Europe, Asia, Africa, Oceania, and the Americas to which 459 H5, 0 H7 and 1 H9 sequences from Genbank and GISAID were added.

Avian influenza A virus vaccination

Usage of vaccination is expected to increase especially if wild bird cycles of infection and introduction continue in a manner similar to 2020/21 and 2021/22. Many countries where vaccination was previously banned are considering trialling the use of vaccines in poultry. Additionally some manufacturers have updated their vaccines to include 2.3.4.4b clade viruses as vaccine seed strains. Below are some examples of vaccines which are widely used in countries which regularly use vaccines to control AIV.

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: adjuvanted inactivated vaccines containing classic clade H5N2 seed strains A/chicken/Mexico/232/1994 or A/duck/Potsdam.1402-6/1986 (Nobilis influenza and CEVac Flukem). Inactivated recombinant vaccines containing clade 1 H5N3 virus seed strain A/chicken/Vietnam/C58/2004 (Zoetis). Vaccines containing RG clade 2.2.1.1 H5N1 A/chicken/Egypt/18-H/2009 (Egyflu), RG clade 2.2.1.2 H5N1 A/duck/Egypt/MD2583D/2010 (Me Flu VAC and SERA-VAC), 2.3.2 H5N1 A/duck/china/E319-2/2003 (Volvac B.E.S.T), OR RG 2.3.4 (A/duck/Anhui/1/2006) (RE-5 Merial) H5N1 seed strains.

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.

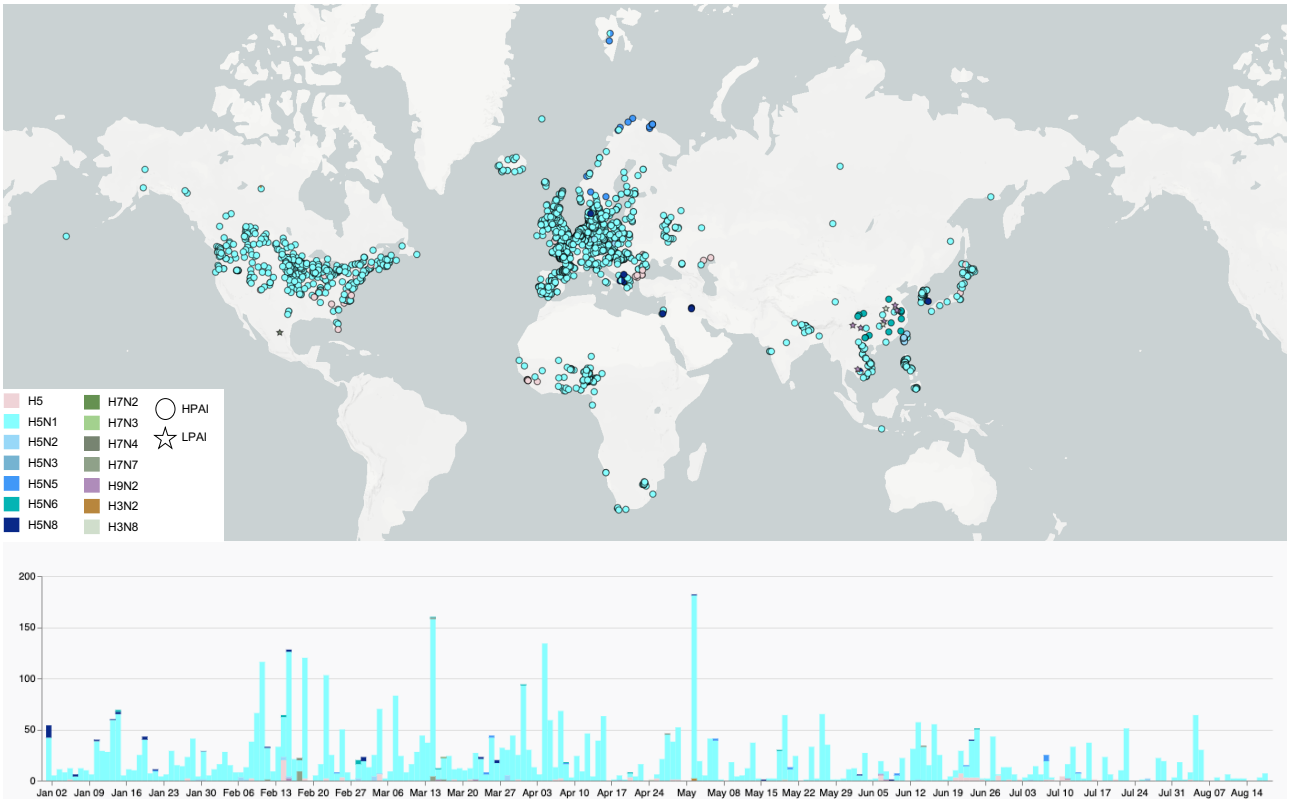
In Indonesia a vaccination program was implemented in 2004. Most recent information from 2020 indicates the currently used vaccine is based on an H5N1 clade 2.3.2.1 A/duck/Sukoharjo/BBVW-1428-9/2012.

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

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

Figure 1: Microreact map of H5, H7 and H9 events observed for the current reporting period 1st February 2022 to 1st September 2022. Points are coloured by subtype. Collection data is represented along a timeline: the x axis represents time and y axis number of reports. Data was harvested from Empres-Plus and complimented with information shared via the OFFLU network. If no observation date was available a random date within the reporting period was allocated.



Activity Table (HPAI H5) viruses

Table 1: 1st February 2022- 1st August 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
Albania	Poultry Wild	H5N8 (2.3.4.4b) H5N1 (2.3.4.4b)
Austria	Wild	H5N1 ()
Bangladesh	Poultry	H5N1 (2.3.4.4b, 2.3.2.1a)
Belgium	Poultry Wild	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b) H5 (2.3.4.4b)
Bulgaria	Poultry Wild	H () H ()
Cambodia	Poultry Mammal (Human)	H5N8 (2.3.4.4b) H5N1 (2.3.4.4b, 2.3.2.1c)
Canada	Poultry Wild Mammal (Skunk , Red Fox, Wild fox, Mink, Harbour Seal)	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b) H5N1 (2.3.4.4b)
China	Wild Poultry Mammal (Human)	H5N1 () H5N1 (2.3.4.4b) H5N6 (2.3.4.4b)
Croatia	Poultry Wild	H5N1 () H5N1 ()
Czech Republic	Poultry	H5N1 (2.3.4.4b)
Denmark	Wild Poultry	H5N1 () H5N1 ()
Egypt	Poultry	H5 (2.3.4.4b)
Estonia	Wild	H5N1 ()
Faroe Islands	Wild	H5N1 ()
Finland	Wild	H5N1 ()
France	Wild Poultry	H5N1 (2.3.4.4b) H5N1 ()
Gabon	Poultry	H5N1 ()
Germany	Wild Poultry	H5N1 () H5N2 () H5N1 ()
Ghana	Poultry	H5N1 ()
Greece	Wild	H5N1 (2.3.4.4b)
Guernsey	Wild	H5N1 ()
Guinea	Poultry	H5N1 (2.3.4.4b)
Hungary	Poultry Wild	H5N1 () H5N1 ()
Iceland	Poultry Wild	H5N1 () H5N1 ()
India	Poultry	H5N1 (2.3.2.1a)

Indonesia	Poultry	H5N1 (2.3.2.1e)
Iraq	Poultry	H5N8 ()
Ireland	Wild Mammal (Fox)	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b)
Israel	Wild	H5N8 ()
Italy	Wild Poultry	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b)
Japan	Poultry Wild Mammal (Fox, Racoon Dog)	H5N1 () H5N1 (2.3.4.4b) H5 () H5N1 (2.3.4.4b)
Jersey	Poultry	H5N1 (2.3.4.4b)
Kazakhstan	Wild	H5 ()
Kozovo	Poultry	H5N8 (2.3.4.4b)
Lithuania	Wild	H5N1 ()
Luxembourg	Wild	H5N1 ()
Mali	Poultry	H5N1 (2.3.4.4b)
Moldova, Republic of	Poultry	HPAI ()
Montenegro	Wild	H5N1 ()
Nepal	Wild Poultry	H5N1 () H5N1 (2.3.2.1a)
Netherlands	Wild Poultry Mammal (Fox, Polecat)	H5N1 (2.3.4.4b) H5N1 () H5N1 (2.3.4.4b)
Niger	Poultry	H5N1 ()
Nigeria	Poultry	H5N1 (2.3.4.4b)
North Macedonia	Wild	H5N1 ()
Norway	Wild	H5N1 () H5N5 (2.3.4.4b)
Philippines	Poultry	H5N1 (2.3.4.4b) H5N8 ()
Poland	Poultry Wild	H5N1(2.3.4.4b) H5N2 (2.3.4.4b) H5N1 (2.3.4.4b)
Portugal	Poultry Wild	H5N1 () H5N1 ()
Republic of Korea	Poultry Wild	H5N1 () H5N1 ()
Romania	Poultry Wild	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b)
Russian Federation	Poultry Wild	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b) H5 ()
Slovakia	Wild	H5N1 ()
South Africa	Poultry Wild Environment	H5N1 () H5N1 () H5N1 ()
Spain	Poultry Wild	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b)

Svalbard and Jan Mayen Islands	Wild	H5N1 () H5N5 ()
Sweden	Poultry Wild	H5N1 () H5N1 ()
Switzerland	Wild	H5N1 ()
Taiwan (Province of China)	Poultry Wild	H5N1 () H5N1 () H5N2 ()
U.K. of Great Britain and Northern Ireland	Poultry Wild	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b)
United States of America	Poultry Wild Mammal (Red Fox, Skunt, Fox, Coyote, Wild fox, Bobcat) Mammal (Human)	H5N1 (2.3.4.4b) H5N1 (2.3.4.4b) H5N1 (2.3.4.4b) H5N1 (2.3.4.4b)
Viet Nam	Poultry	H5N1 ()

Activity Table Non Gs/Gd viruses

Table 2: Non Gs/Gd viruses reported between 1st February 2022 and 1st September 2022. Data was collected through EMPRES-i, WOAH via the WAHIS system and through OFFLU collaborators, GISAID and WHO CCs.

Bangladesh	Poultry	H5N3 LPAI, H9N2
Canada	Poultry	H7N4 LPAI
Cambodia	Human	H9N2, H7N7 LPAI
China	Human	H3N8, H9N2
Netherlands	Wild Bird	H7Nx LPAI
Korea	Poultry	H5N3 LPAI
Mexico	Poultry	H7N3

Global H5, H7 and H9 avian influenza events in humans and mammals

Table 3: Data taken from Influenza at the human animal interface summary and assessments (April, May, June) Table contains data reported to the WHO within the reporting period of 1st February 2022- 1st September 2022.

Onset date	Subtype	Location	Gender (Age)	State	Exposure
20/02/2022	A(H5N6)	Jiangxi, China	F (51)	Critical	Poultry
18/03/2022	A(H5N6)	Henan, China	M (28)	Critical	Poultry
24/03/2022	A(H5N6)	Jiangsu, China	F (53)	Critical	Poultry
29/01/2022	A(H9N2)	Anhui, China	F (2)	Mild	Poultry
27/02/2022	A(H9N2)	Siem Reap, Cambodia	F (1)	Mild	Poultry
31/03/2022	A(H5N6)	Sichuan, China	M (56)	Severe	Poultry
20/04/2022	A(H5N1)	Colorado, USA	M (?)	Mild	Poultry
05/04/2022	A(H3N8)	Henan, China	M (4)	Critical	Poultry
09/05/2022	A(H3N8)	Hunan, China	M (5)	Mild	Poultry market
16/04/2022	A (H5N6)	Guangxi, China	M (49)	Fatal	Poultry
02/06/2022	A (H5N6)	Jiangxi, China	M (58)	Critical	Poultry
08/05/2022	A (H9N2)	Guizhou China	M (2)	Mild	Poultry market
26/04/2022	A (H9N2)	Hunan China	M (5)	Mild	Unknown
28/04/2022	A (H9N2)	Sichuan, China	M (1)	Mild	Unknown
1/08/2022	A (H9N2)	China, Guangdong	Child	Mild	Unknown

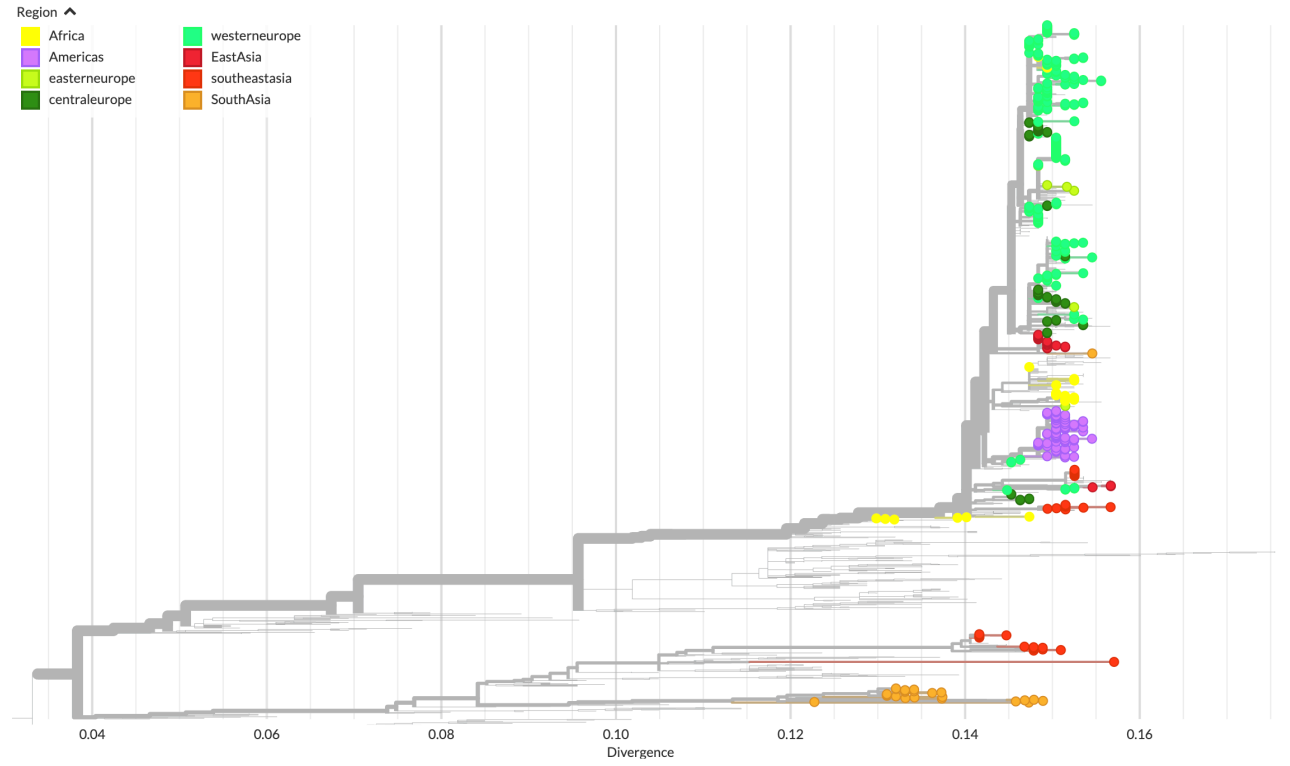
Table 4: Infections in Mammals within this reporting period taken from Global Influenza situation update and EFSA's Avian influenza report

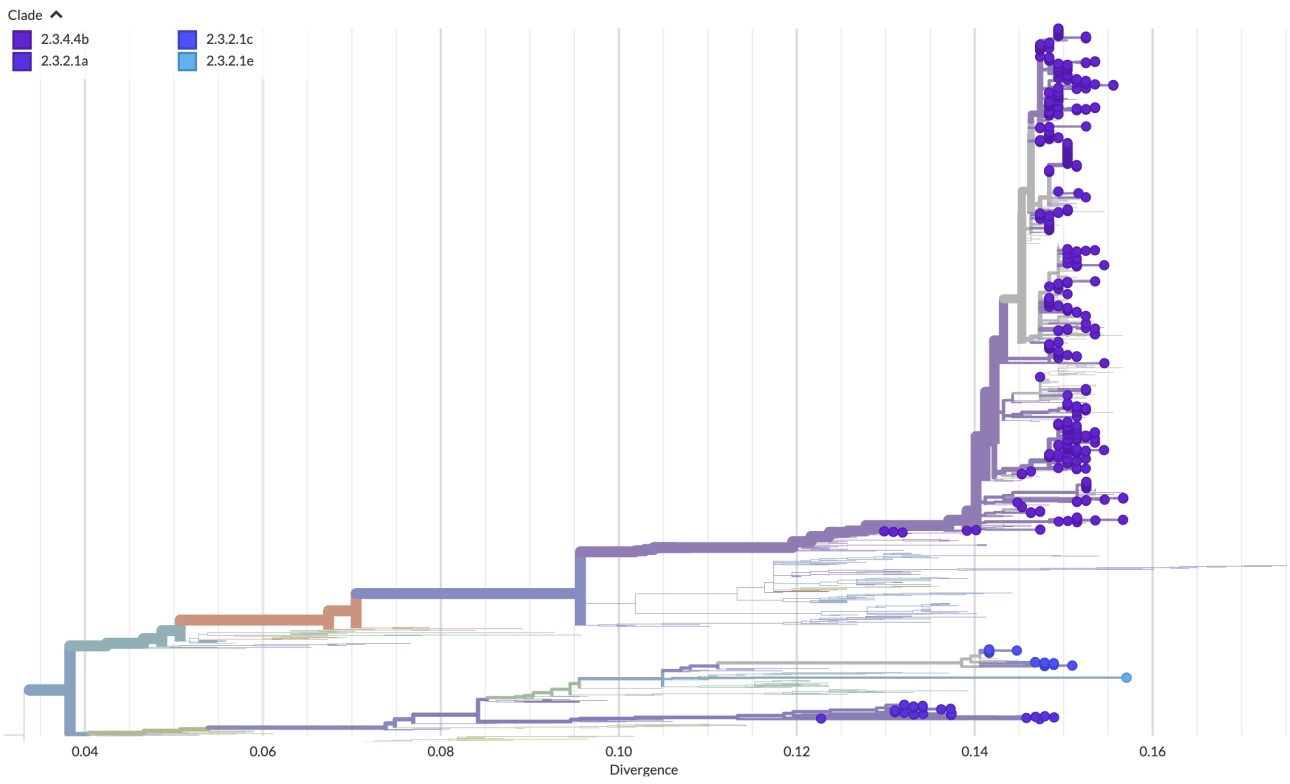
Canada	Skunk , Red Fox, Wild fox, Mink, Harbor Seal, Bear	H5N1 (2.3.4.4b)
Ireland	Fox	H5N1 (2.3.4.4b)
Japan	Fox, Racoon Dog	H5N1 (2.3.4.4b)
Netherlands	Polecat, Fox	H5N1 (2.3.4.4b)
United States of America	Red Fox, Skunk, Fox, Coyote, Wild fox, Bobcat, Seal, Racoon, Opossumn, Dolphin	H5N1 (2.3.4.4b)
Sweden	Porpoise	H5N1 (2.3.4.4b)
Thailand	Cats	H5N1 (unknown)

H5 Influenza A viruses

H5 Phylogenetic tree coloured by geographic location (nucleotide)

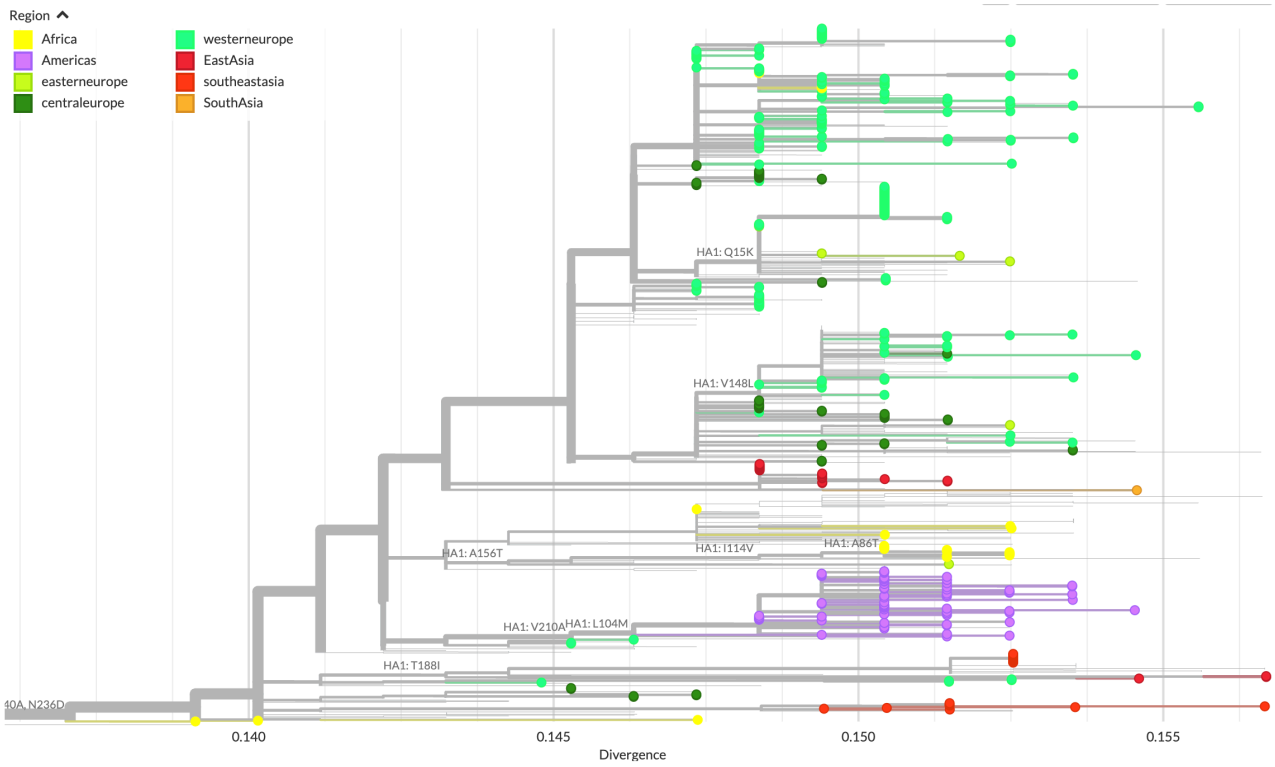
Figure 2: Avian H5 global summary maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences, data downloaded from GISAID (1st January 2022 to 1st September 2022) and shared by the OFFLU network. Sequences were coloured by geographic region. Only tips with sampling dates confirmed to be within the reporting period 1st February 2022 to 1st September 2022 were coloured by geographical region and clade.





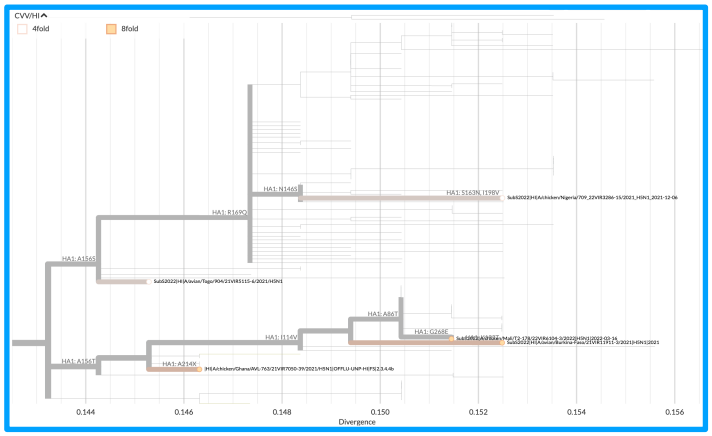
2.3.4.4b Tree - overview

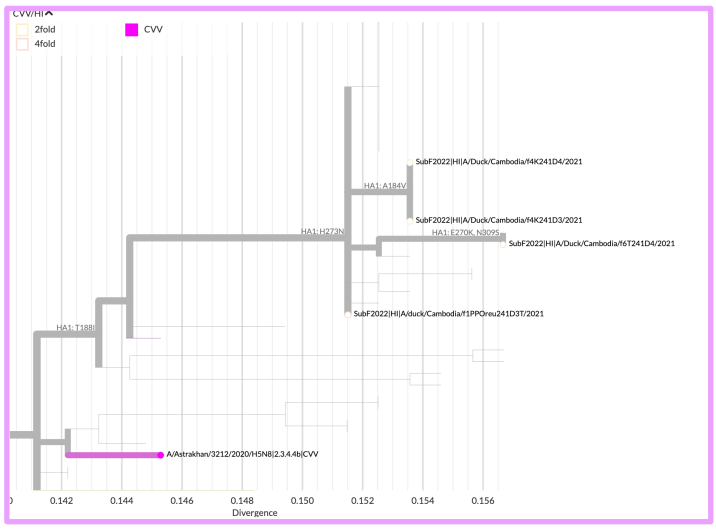
Figure 3: Avian H5 2.3.4.4b global summary maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences, data downloaded from GISAID (1st January 2022 to 1st September 2022) and shared by the OFFLU network. Sequences were coloured by geographic region. Only tips with sampling dates confirmed to be within the reporting period 1st February 2022 to 1st September 2022 were coloured. Amino acid changes were annotated on the nodes



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

Figure 4: Avian H5 2.3.4.4b global summary maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences, data downloaded from GISAID (1st January 2022 to 1st September 2022) and shared by the OFFLU network. Sequences HI'd by OFFLU collaborating laboratories are highlighted. Amino acid changes in the HA1 are annotated on the tree.





H5 2.3.4.4b HI Strains comparative amino acid substitutions to clade CVV

Table 5: 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. Antigenic sites have been coloured in grey. HI strains are coloured yellow and CVVs coloured red.

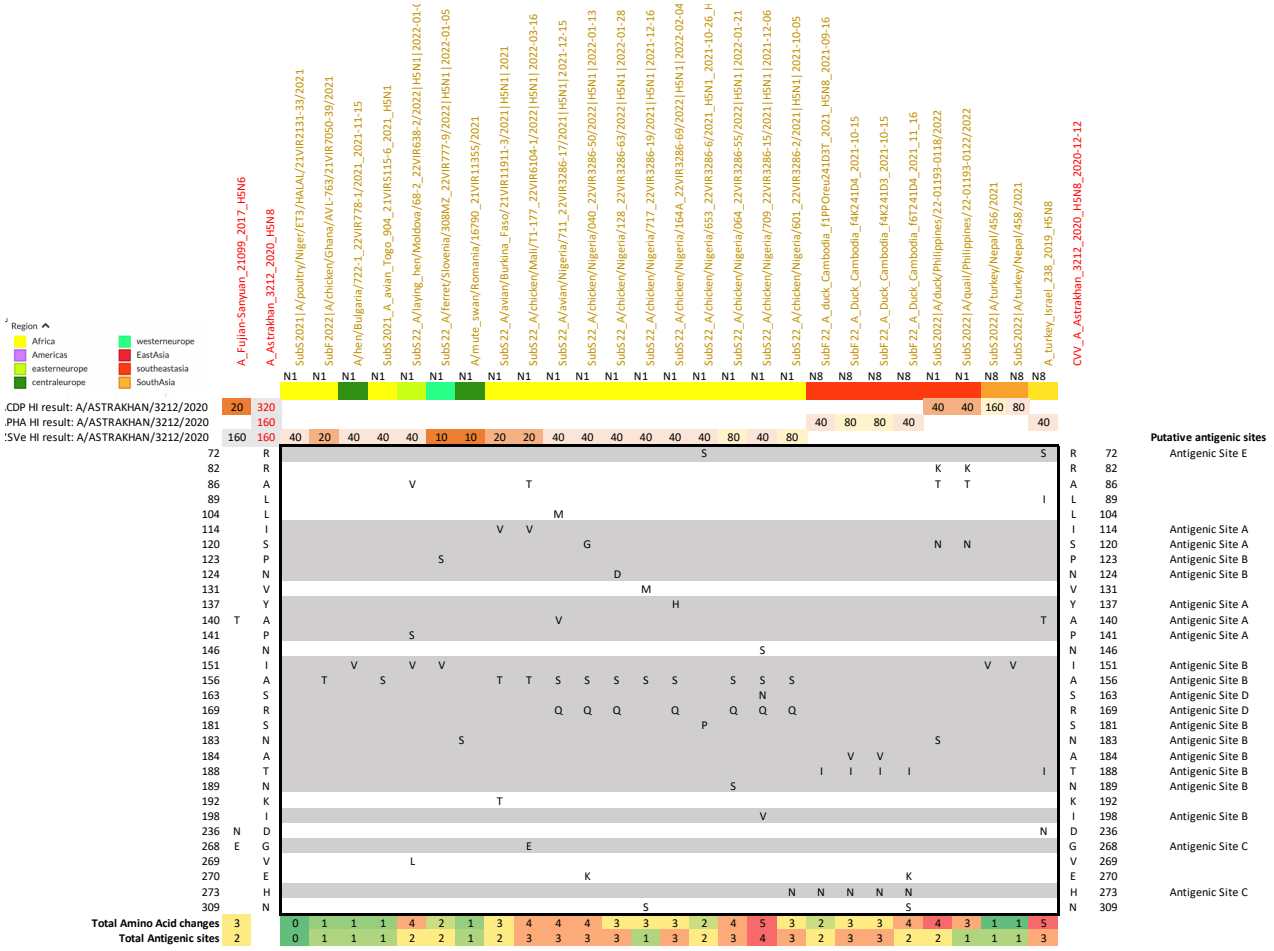
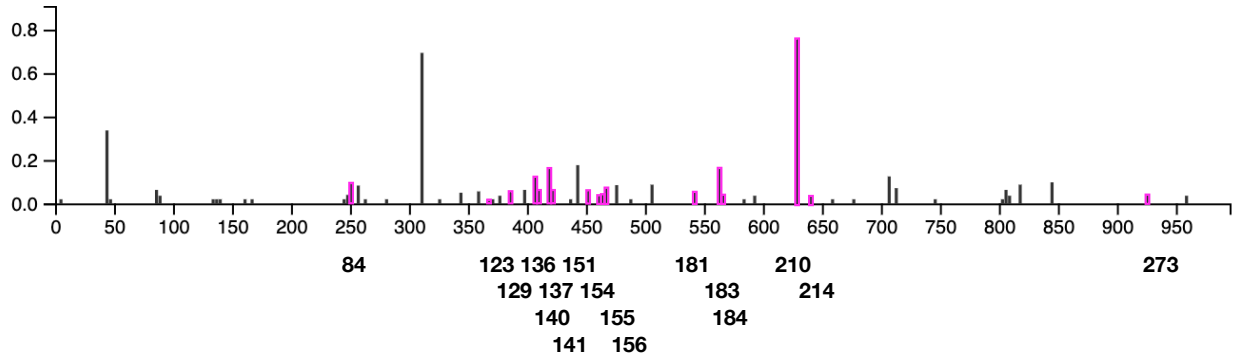


Table 6: Normalised shannon entropy of all 2.3.4.4b clade viruses within reporting period at each given codon position. Putative antigenic sites highlighted in pink with antigenic amino acid site position annotated underneath



H5 2.3.4.4b Antigenic Analysis

Table 7: 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 ▲. Strains are annotated by region

Homologous
2 fold
4 fold
8 fold
16 fold
< 32 fold

REFERENCE FERRET ANTISERA

Region: Africa, Americas, easterneurope, centraleurope, westerneurope, EastAsia, southeastasia, SouthAsia

Semester			Clade	Subtype / Ferret ID Subtype	A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	A/ASTRAKHAN/3212/2020	A/TURKEY/ITALY/17VIR576-11/2017	A/poultry/Niger/ET3/HALAL/21VIR2131-33/2021	A/chicken/Ghana/AVL-763/21VIR7050-39/2021	A/hen/Bulgaria/722-1/22VIR778-1/2021	A/avian/Togo/904/21VIR5115-6/2021	Aadiffs
			2.3.4.4b	H5N6	2.3.4.4b	H5N8	H5N8	H5N1	H5N1	H5N1	H5N1	
								Niger 2131	Ghana 7050	Bulgaria 778	Togo 5115	
				*								
		Reference Antigen										
		A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	2.3.4.4b	H5N6	40	160	640	1280	1280	640	1280	▲T140A, N236E, ▲G268E
		A/ASTRAKHAN/3212/2020	2.3.4.4b	H5N8	80	160	1280	1280	640	640	1280	*
		A/TURKEY/ITALY/17VIR576-11/2017	2.3.4.4b	H5N8	10	80	160	640	320	320	640	▲R72S, L89I, ▲T188I
		A/poultry/Niger/ET3/HALAL/21VIR2131-33/2021	2.3.4.4b	H5N1	10	40	160	640	320	160	640	*
		A/chicken/Ghana/AVL-763/21VIR7050-39/2021	2.3.4.4b	H5N1	10	20	160	640	320	80	1280	▲A156T
		A/hen/Bulgaria/722-1/22VIR778-1/2021	2.3.4.4b	H5N1	10	40	40	2560	640	640	1280	▲I151V
		A/avian/Togo/904/21VIR5115-6/2021	2.3.4.4b	H5N1	10	40	160	320	160	40	320	▲A156S
		Test antigen										
ISZVe Feb22-Sep22	1	A/laying-hen/Moldova/68-1_22VIR638-1/2022	2.3.4.4b	H5N1	10	40	80	640	320	320	160	A86V, ▲P141S, I151V, V269L
	2	A/ferret/Slovenia/308/22VIR777-9/2022	2.3.4.4b	H5N1	10	10	20	1280	640	640	1280	P123S, ▲I151V
	3	A/Swan/Romania/16381/21VIR11355-1/2021	2.3.4.4b	H5N1	10	10	10	1280	320	320	640	▲ N183S
	4	A/avian/Burkina Faso/21VIR11911-3/2021	2.3.4.4b	H5N1	10	20	320	320	640	80	1280	I114V, ▲A156T, ▲K192T
	5	A/chicken/Mali/T1-177/22VIR6104-1/2021	2.3.4.4b	H5N1	10	20	160	320	640	80	1280	A86T, I114V, ▲A156T, G268E
	6	A/avian/Nigeria/711/22VIR3286-17/2021	2.3.4.4b	H5N1	10	40	160	320	320	40	320	L104M, ▲A140V, ▲A156S, ▲R169Q
	7	A/chicken/Nigeria/040/22VIR3286-50/2022	2.3.4.4b	H5N1	10	40	160	320	320	40	640	S120G, ▲A156S, ▲R169Q, E270K
	8	A/chicken/Nigeria/128/22VIR3286-63/2022	2.3.4.4b	H5N1	10	40	320	320	640	80	1280	N124D, ▲A156S, ▲R169Q
	9	A/chicken/Nigeria/717/22VIR3286-19/2021	2.3.4.4b	H5N1	10	40	160	320	320	40	640	V131M,▲A156S, N309S
	10	A/chicken/Nigeria/164A/22VIR3286-69/2022	2.3.4.4b	H5N1	10	40	160	320	320	40	640	Y137H, ▲A156S, ▲R169Q
	11	A/chicken/Nigeria/653/22VIR3286-6/2021	2.3.4.4b	H5N1	20	40	320	1280	320	320	640	▲R72S, ▲S181P
	12	A/chicken/Nigeria/064/22VIR3286-55/2022	2.3.4.4b	H5N1	10	80	320	640	320	40	320	▲A156S, ▲R169Q, ▲N189S, V200I
	13	A/chicken/Nigeria/709/22VIR3286-15/2021	2.3.4.4b	H5N1	10	40	80	320	320	40	640	N146S, ▲A156S, ▲S163N, ▲R169Q, I198V
	14	A/chicken/Nigeria/601/22VIR3286-2/2021	2.3.4.4b	H5N1	10	80	320	640	320	80	640	▲A156S, ▲R169Q, H273N

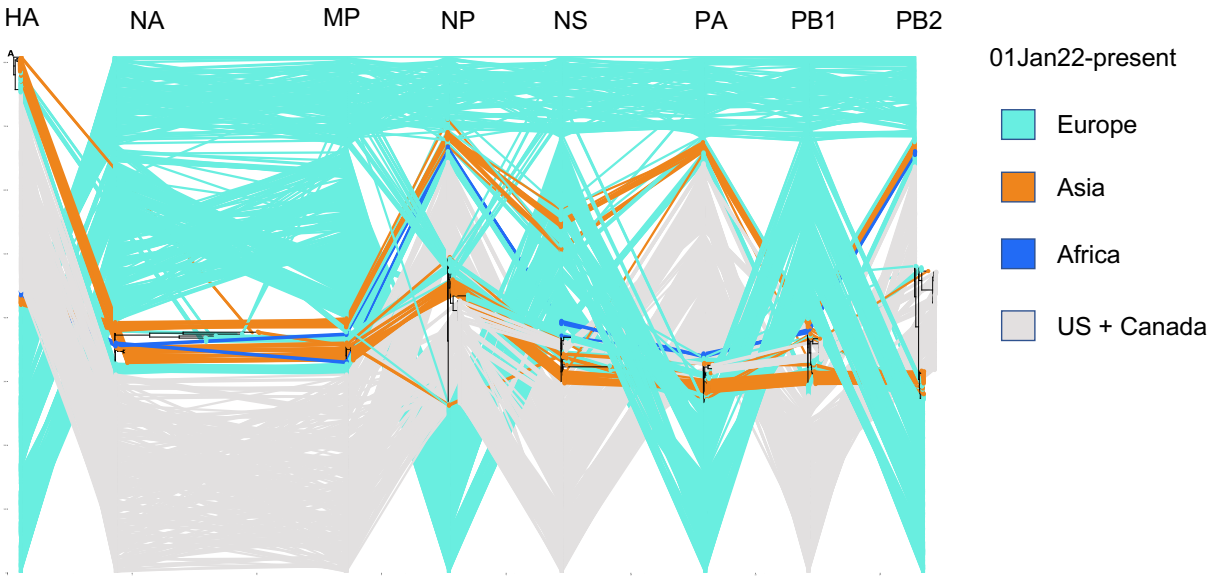
		Reference Antigen										
		A/ASTRAKHAN/3212/2020	2.3.4.4b	H5N8		160						
		Test antigen										
APHA Feb22-Sep22	1	A/Duck/Cambodia/f-1PPOreu-24-1-D3/2021	2.3.4.4b	H5N8		40						▲T188I, H273N
	2	A/Duck/Cambodia/f-4K-24-1-D4/2021	2.3.4.4b	H5N8		80						A183V, ▲T188I, H273N
	3	A/Duck/Cambodia/f-4K-24-1-D3/2021	2.3.4.4b	H5N8		80						A183V, ▲T188I, H273N
	4	A/Duck/Cambodia/f-6T-24-1-D4/2021	2.3.4.4b	H5N8		40						▲T188I, E270K, H273N, N309S
	5	A/turkey/Israel/238/2019	2.3.4.4b	H5N8		40						▲R72S, L89I, ▲A140T, ▲T188I, D236N,

		Reference Antigen										
		A/ASTRAKHAN/3212/2020	2.3.4.4b	H5N8		320		320	160	160	160	*
		A/poultry/Niger/ET3/HALAL/21VIR2131-33/2021	2.3.4.4b	H5N1		40		1280	320	320	640	*
		A/chicken/Ghana/AVL-763/21VIR7050-39/2021	2.3.4.4b	H5N1		40		640	640	160	640	▲A156T
APHA-bis Feb22-Sep22		A/hen/Bulgaria/722-1/22VIR778-1/2021	2.3.4.4b	H5N1		20		1280	640	1280	1280	▲I151V
		A/avian/Togo/904/21VIR5115-6/2021	2.3.4.4b	H5N1		80		640	320	80	2560	▲A156S
		Test antigen										
	1	A/Duck/Cambodia/f-4K-24-1-D3/2021	2.3.4.4b	H5N8		40		320	320	160	320	A183V, ▲T188I, H273N
	2	A/turkey/Israel/238/2019	2.3.4.4b	H5N8		40		320	160	320	320	▲R72S, L89I, ▲A140T, ▲T188I, D236N,

		Reference Antigen										
		A/FUJIAN-SANYUAN/21099/2017 xA/PR/8/1934	2.3.4.4b	H5N6	80	20						
		A/ASTRAKHAN/3212/2020	2.3.4.4b	H5N8	<20	320						
		Test antigen										
ACDP Feb22-Sep22	1	A/duck/Philippines/22-01193-0118/2022	2.3.4.4b	H5N1	<20	40						R82K, A86T, ▲S120N, N183S
	2	A/quail/Philippines/22-01193-0122/2022	2.3.4.4b	H5N1	<20	40						R82K, A86T, ▲S120N
	3	A/turkey/Nepal/456/2021	2.3.4.4b	H5N8	<20	160						▲I151V
	4	A/turkey/Nepal/458/2021	2.3.4.4b	H5N8	<20	80						▲I151V

H5 2.3.4.4b Whole Genome Incongruence Analysis

Figure 5: Linked maximum likelihood phylogenies for each gene segment, coloured by region. See annex 2 for HA tree with taxon names included. Data included was downloaded from GISAID.



Viruses found in poultry in Burkina Faso in December 2021 had PA segments which cluster with H9N2 viruses of the G1 lineage which have been detected in West Africa between 2017 and 2020 A screenshot of a table containing whole genome mutations analysis is included below. Taken from (Ouoba et al., 2022).

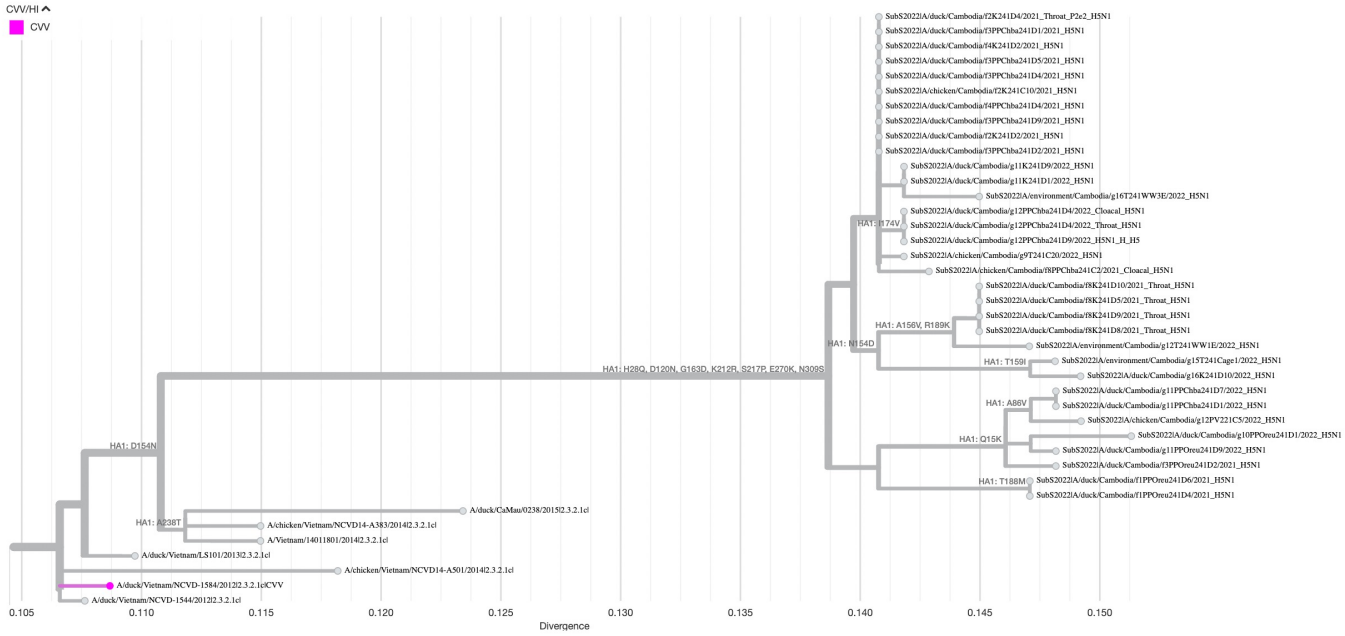
Table 2. Mutations identified in the HPAI H5N1 viruses from Burkina Faso.

Protein	Mutation	HPAI H5N1 Viruses Burkina Faso	Effect	Reference
HA	S137A, H3 numbering (S149A from the initial Met)	All 3 viruses	Increased pseudovirus binding to α2–6.	[28]
HA	HA-A158T, H3 numbering (A172T from the initial Met)	All 3 viruses	Creates a new potential N-glycosylation site. The motif at positions 156–158 changed from NDA to NDT.	n.a.
PB2	T105V and A661T	All 3 viruses	Host specificity markers identified through statistical methods (T in avian, V in human for T105V and A in avian, T in human for A661T). The mutations lie in regions of PB2 both known for binding to PB1 and NP.	[29]
PA	L268I and S409N	All 3 viruses	Host specificity markers identified through statistical methods (L in avian, I in human for L268I and S in avian, N in human for S409N).	[30]
NA	NA stalk deletion	All 3 viruses, similarly to the H5N1 viruses identified in Nigeria in 2021	A deletion in the stalk region of the NA decreases the ability of NA to release the virus from cells and increases the virulence of the virus in mice and chickens. In addition, it is a marker of virus adaptation from wild aquatic birds to poultry.	[31–38]
NS1	Deletion at positions 78–80	A/chicken/ Burkina_Faso/ 21VIR11911-5/2021	This 3-amino acid deletion overlaps with a deletion of five amino acids previously described [39]. Viruses with both a deletion of 20 amino acids in the stalk of the NA glycoprotein and a deletion of 5 amino acids at positions 80 to 84 in the NS1 protein contribute to the high pathogenicity of H5N1 AIVs in ducks.	[39]
NS1	P42S	All 3 viruses	Increased virulence in mice.	[40]
NS1	P87S	All 3 viruses	P87S is a host specificity marker identified through statistical means (S in human, P in avian).	[41]
NS1	103F	All 3 viruses	Increased virulence in mice.	[42,43]
NS2	T47A (with NS1-205S)	A/chicken/Burkina_Faso/ 21VIR11911-5/2021	Decreased antiviral response in ferrets.	[44]

HA: hemagglutinin; PB2: polymerase basic 2; PA: polymerase acidic; NA: neuraminidase; NS1: non-structural 1; NS2: non-structural 2; n.a.: not available; AIVs: avian influenza viruses.

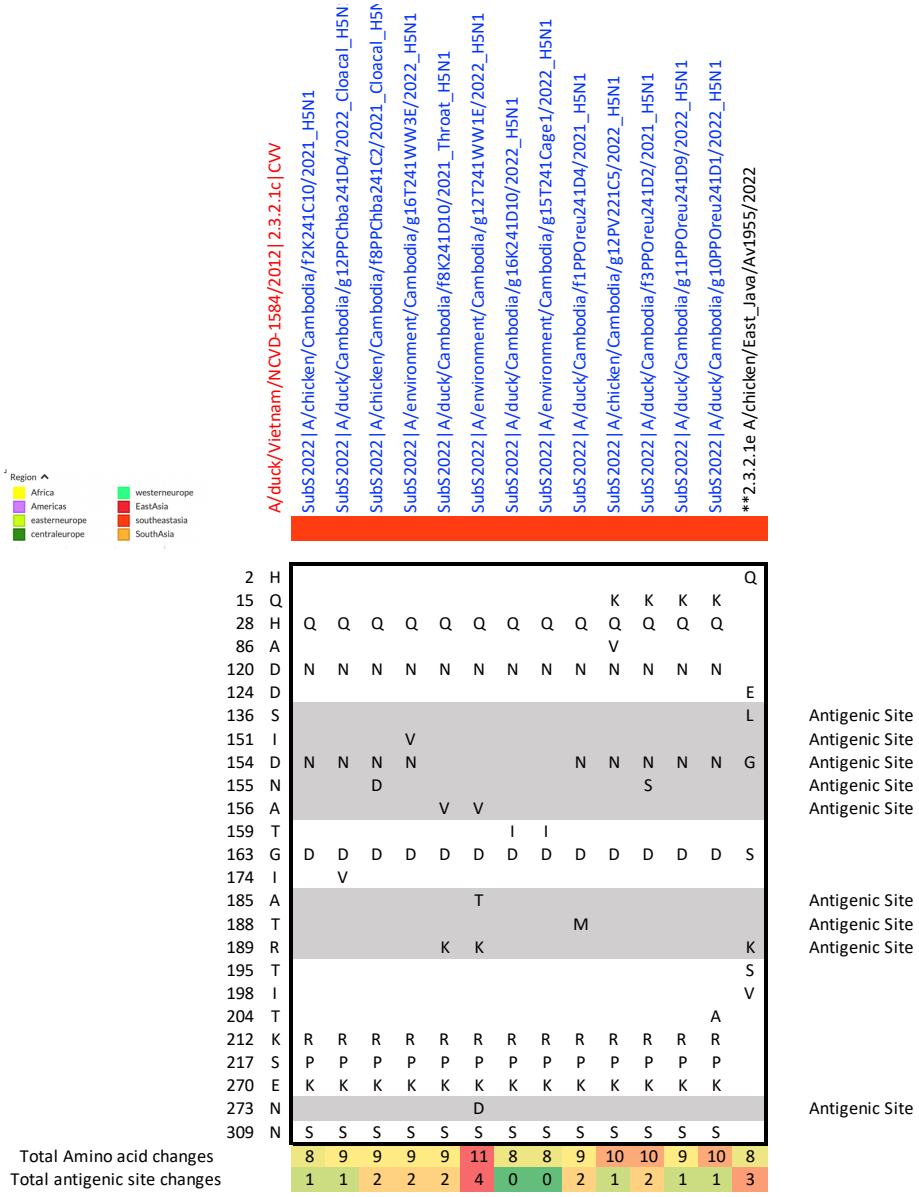
H5 2.3.2.1c phylogenetic tree (Nucleotide) annotated with amino acid changes

Figure 6: An ML nucleotide phylogenetic tree inferred from HA1 amino acid sequences have been included to complement the HI table and amino acid difference table. Amino acid differences were annotated at the nodes.



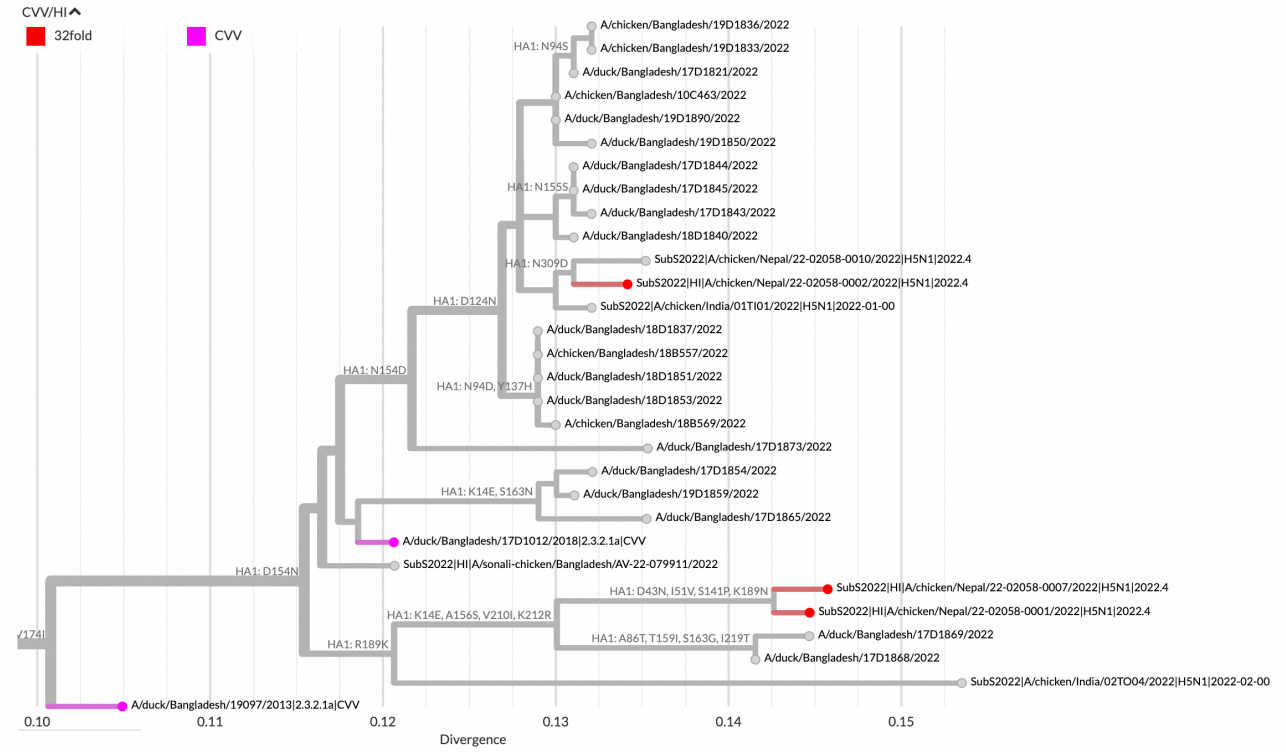
H5 2.3.2.1c strains comparative amino acid substitutions to clade CVV

Table 8: Avian H5 2.3.2.1c clade sequence comparison to the closest within clade CVV. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering. Antigenic sites have been coloured in grey. CVVs are coloured red.



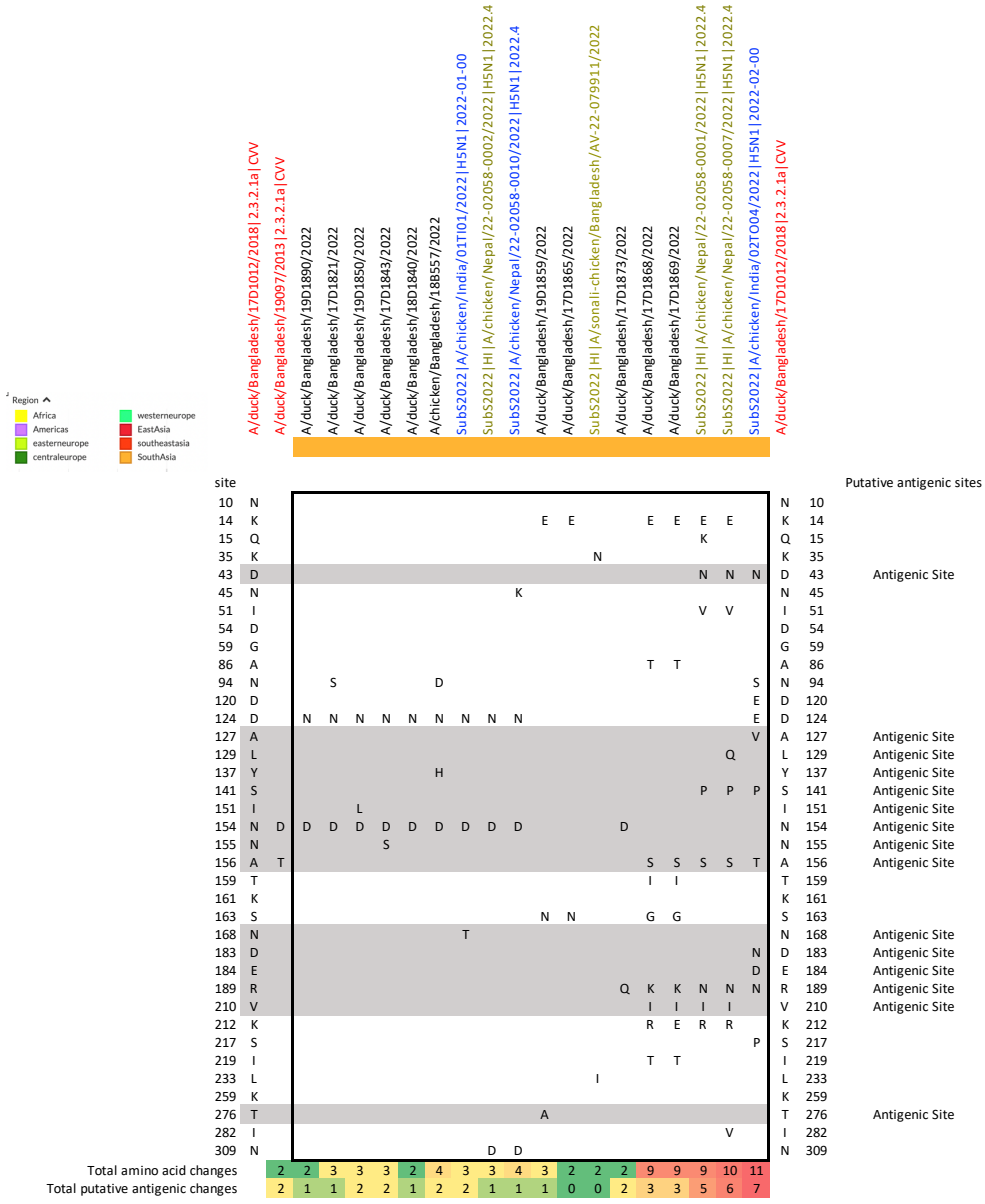
H5 2.3.2.1a phylogenetic tree (Nucleotide) annotated with amino acid changes

Figure 7: An ML nucleotide phylogenetic tree inferred from HA1 amino acid sequences have been included to complement the HI table and amino acid difference table. Amino acid differences were annotated at the nodes.



H5 2.3.2.1a HI Strains comparative amino acid substitutions to clade CVV

Table 9: Avian H5 2.3.2.1a clade sequence comparison to the CVV A/duck/Bangladesh/17D1012/2018. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering. Antigenic sites have been coloured in grey. HI strains are coloured yellow and CVVs coloured red.



H5 2.3.2.1a Antigenic Analysis

Table 10: H5 2.3.2.1a Antigenic Analysis. Fold changes are indicated by colour. Amino acid substitutions are annotated to the right of each strain, relative to closest CVV A/duck/Bangladesh/17D1012/2018. Antigenic sites are marked by ▲.

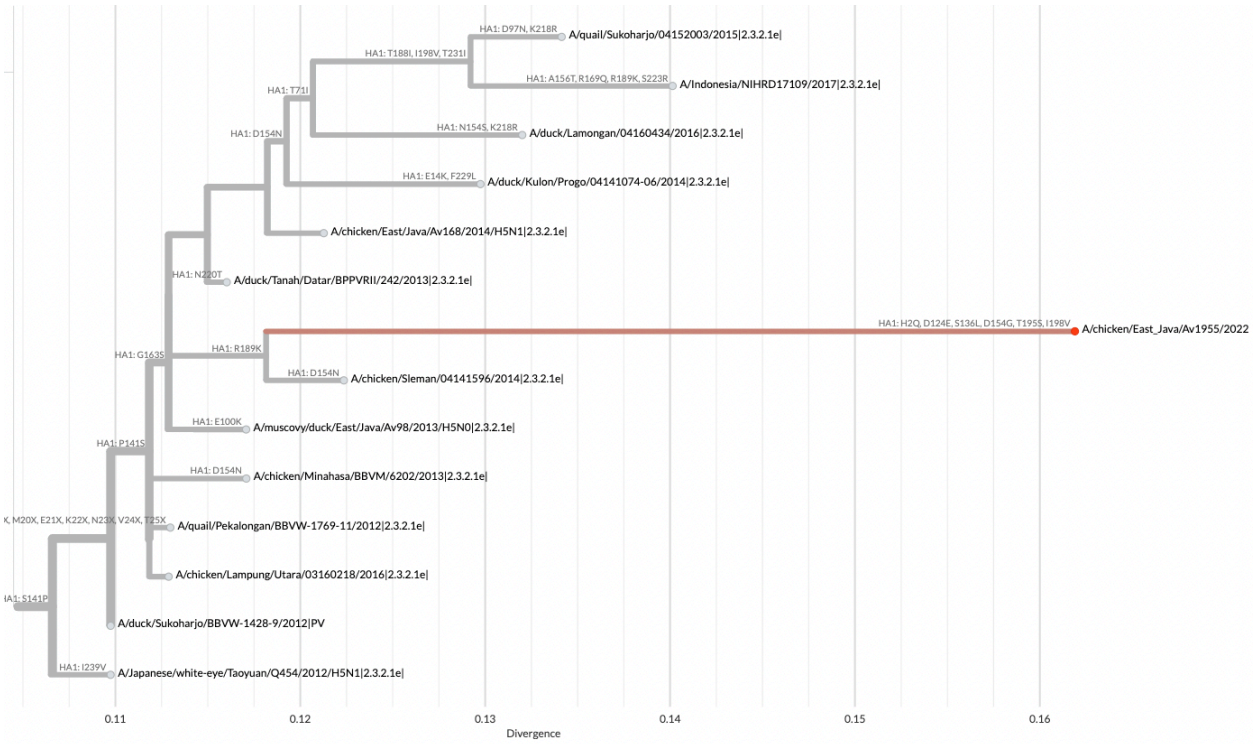
Homologous
2 fold
4 fold
8 fold
16 fold
< 32 fold

Semester		REFERENCE FERRET ANTISERA	Clade	Ferret ID Subtype	A/HUBEI/1/2010	A/DUCK/BANGLADESH/19097/2013	A/DUCK/BANGLADESH/17D1012/2018	
			Clade	Subtype	2.3.2.1a H5N1	2.3.2.1a H5N1	2.3.2.1a H5N1	
							*	
		Reference Antigen						
		A/HUBEI/1/2010	2.3.2.1a	H5N1	640	640	<20	
		A/DUCK/BANGLADESH/19097/2013	2.3.2.1a	H5N1	320	320	<20	
ACDP Feb22-Sep22		A/DUCK/BANGLADESH/17D1012/2018	2.3.2.1a	H5N1	160	160	1280	
		Test antigen						
	1	A/chicken/Nepal/22-02058-0001/2022	2.3.2.1a	H5N1	<20	<20	<20	K14E, Q15K, ▲D43N, I51V, ▲S141P, ▲A156S, ▲R189N, ▲V210I, K212R
	2	A/chicken/Nepal/22-02058-0002/2022	2.3.2.1a	H5N1	20	40	<20	D129N, ▲N154D, D309N
	3	A/chicken/Nepal/22-02058-0007/2022	2.3.2.1a	H5N1	20	<20	<20	K14E, ▲D43N, ▲L129Q, I51V, ▲S141P, ▲A156S, ▲R189N, ▲V210I, K212R

		Reference Antigen				
APHA Feb22-Sep22		A/DUCK/BANGLADESH/17D1012/2018	2.3.2.1a	H5N1	1280	
		Test antigen				
	1	A/Sonali-chicken/Bangladesh/AV-22-079911/2022	2.3.2.1a	H5N1	2560	K35N, L233I

H5 2.3.2.1e phylogenetic tree (Nucleotide) annotated with amino acid changes

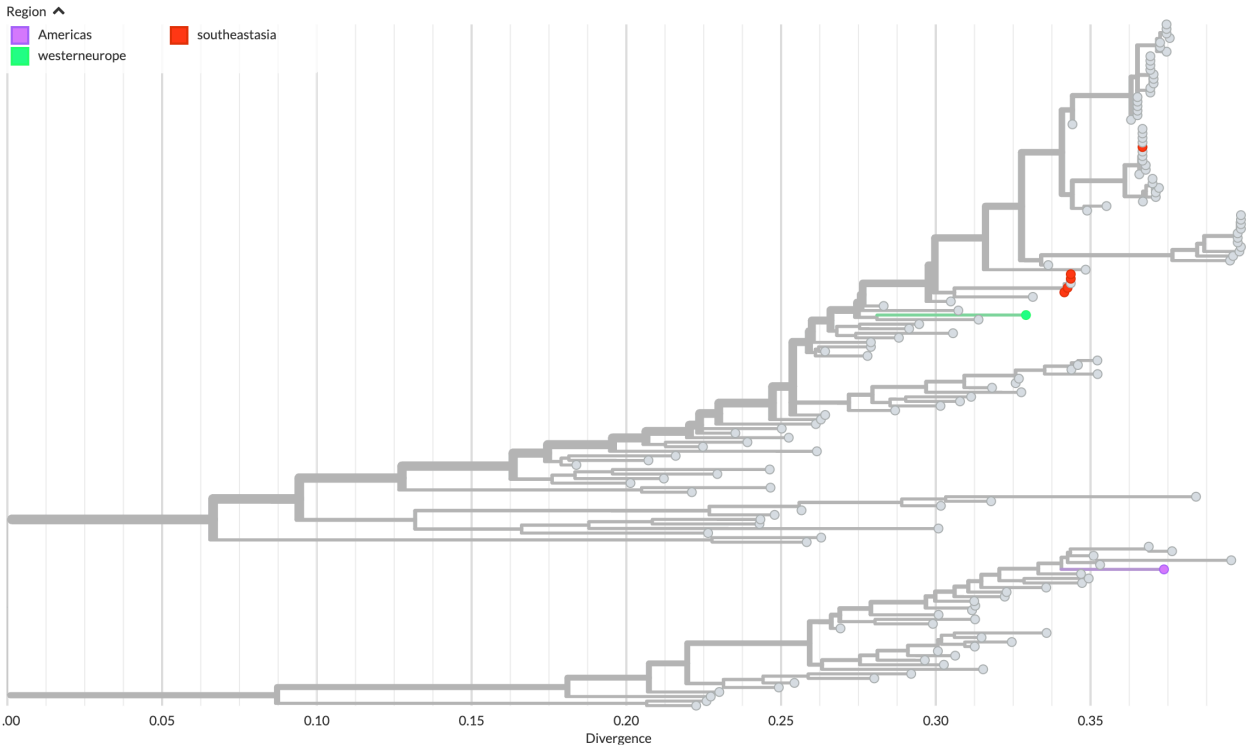
Figure 8: Zoom in on Avian H5 2.3.2.1e clade with amino acid differences annotated at the nodes.



H7 Influenza A viruses

H7 Phylogenetic tree (nucleotide)

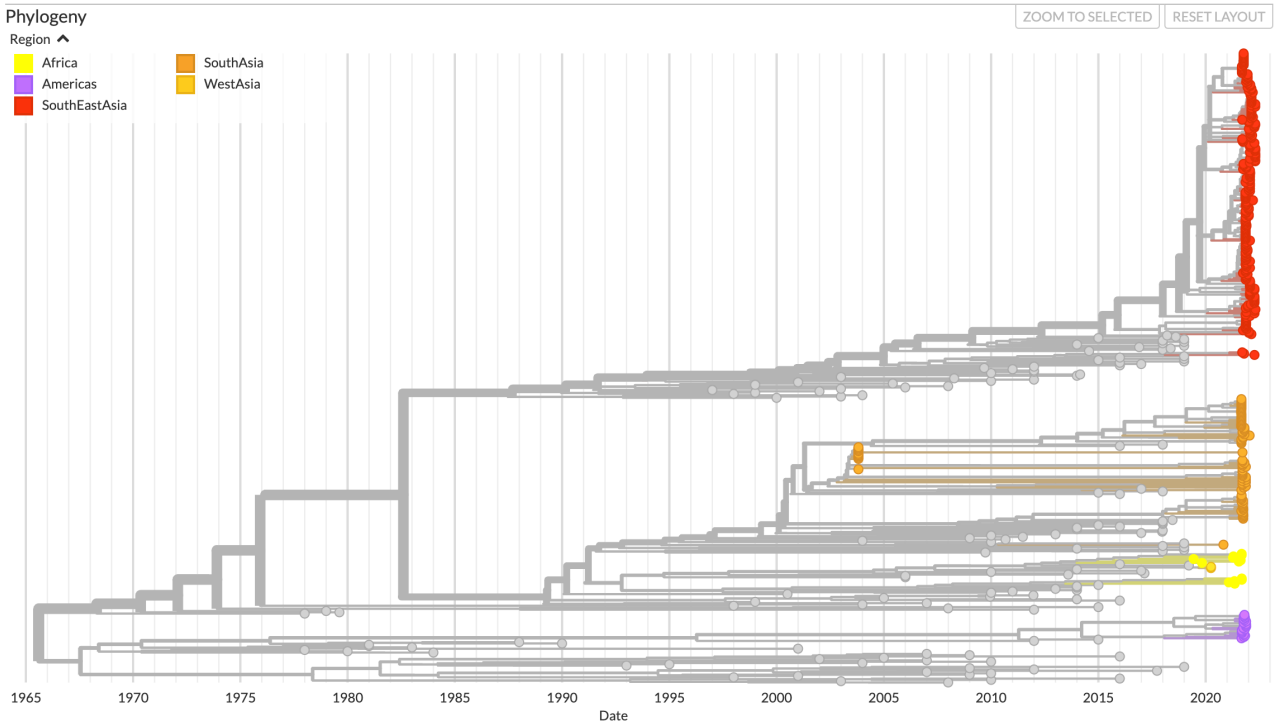
Figure 9: Avian H7 global summary maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data shared by the OFFLU network (all LPAI). Sequences were coloured by geographic region. Only tips with sampling dates confirmed to be within the reporting period 1st February 2022 to 1st September 2022 were coloured.



H9 Influenza A viruses

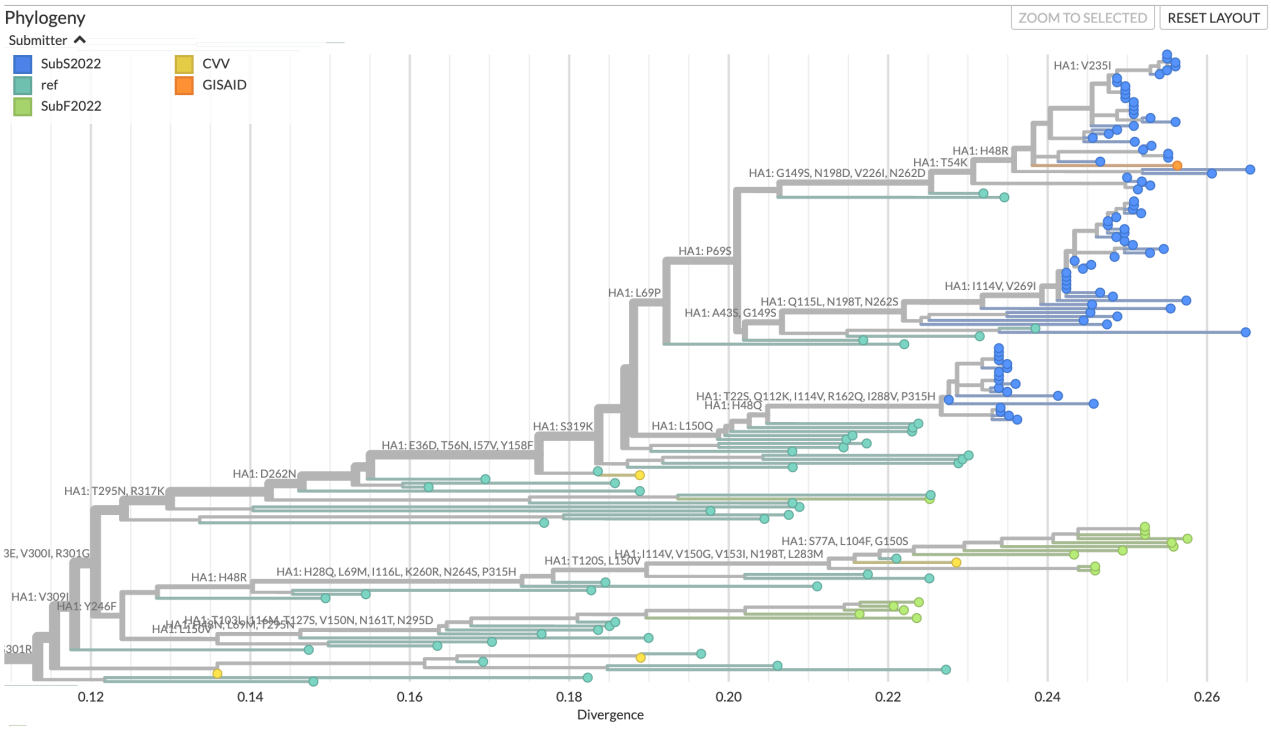
H9 Phylogenetic tree (nucleotide)

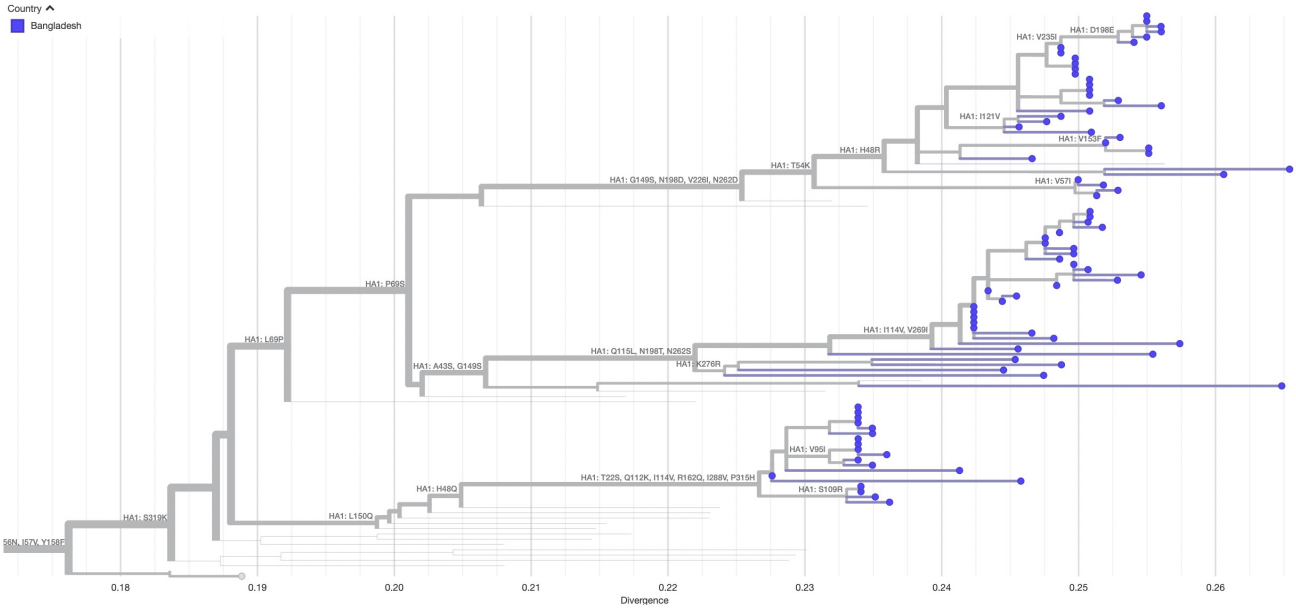
Figure 10: Avian H9 maximum likelihood time resolved phylogenetic tree midpoint rooted. Analyses were conducted with reference sequences and data from the reporting period downloaded from GISAID and shared through the OFFLU network.



H9 G1 phylogenetic tree (nucleotide) annotated with amino acid changes

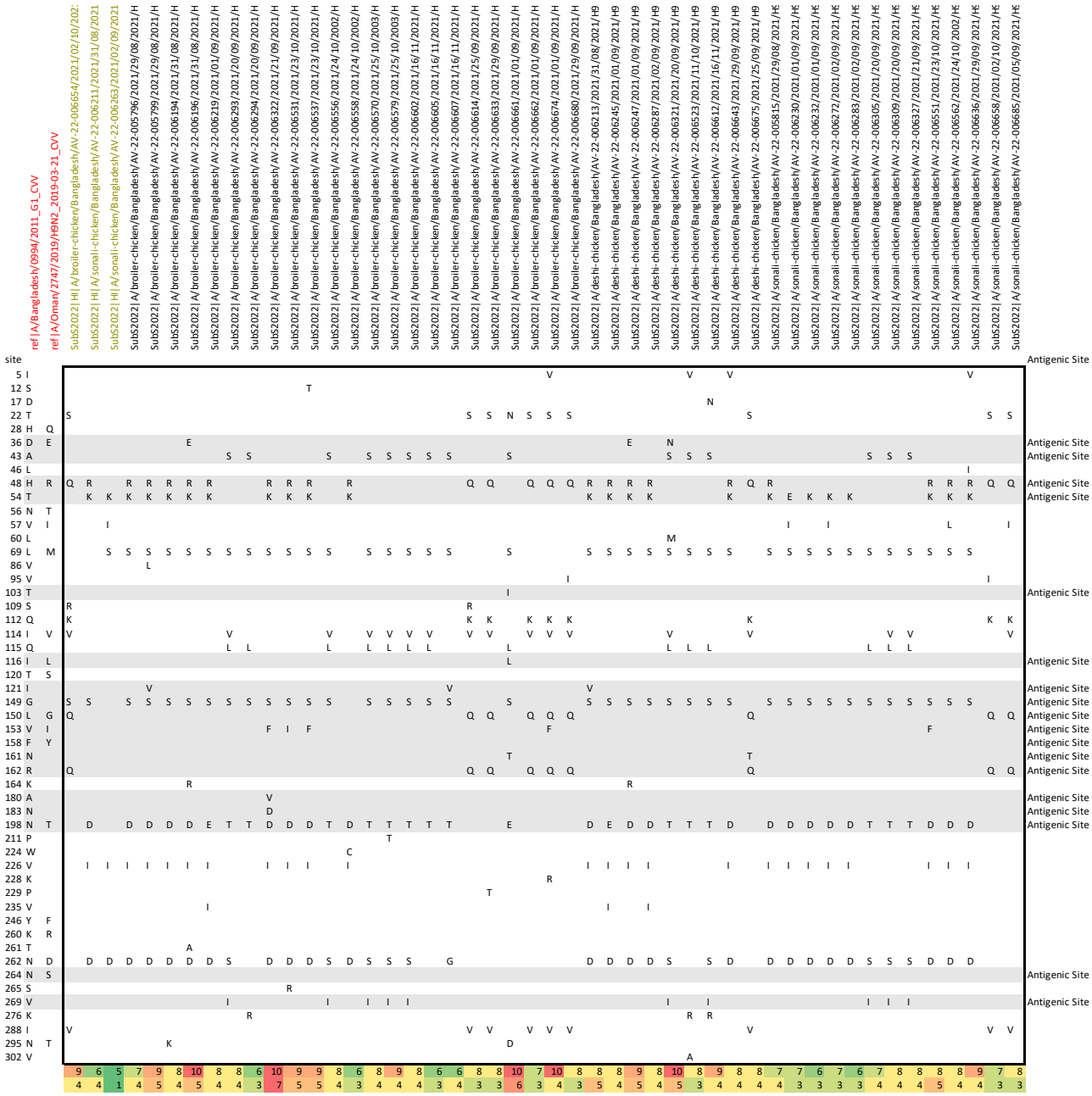
Figure 11: An ML nucleotide phylogenetic tree inferred from HA1 amino acid sequences have been included to complement the HI table and amino acid difference table. Amino acid differences were annotated at the nodes.





H9 G1 Amino acid difference table

Table 11: Amino acid substitutions relative to closest CVVs Antigenic sites are shaded in grey.



H9 G1 Antigenic Data

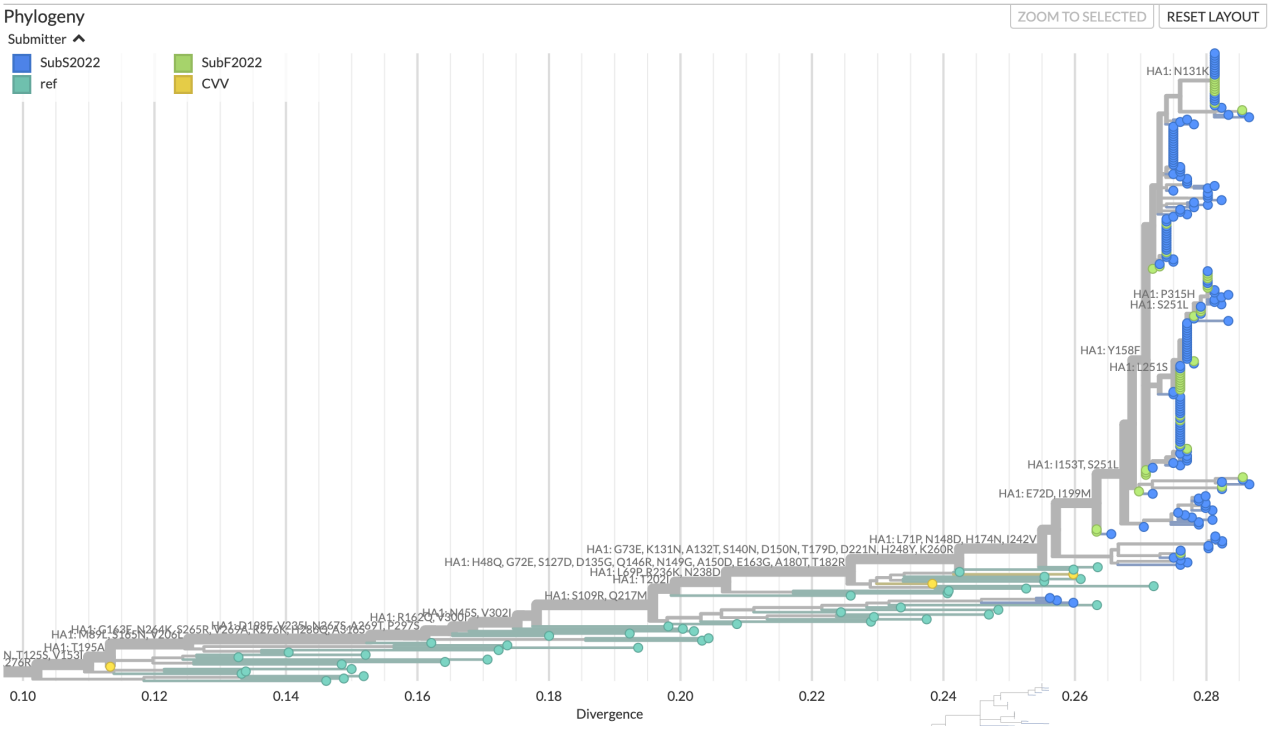
Table 12: Fold changes are indicated by colour. Amino acid substitutions are annotated to the right of each strain, relative to closest CVV. Antigenic sites are marked by *.

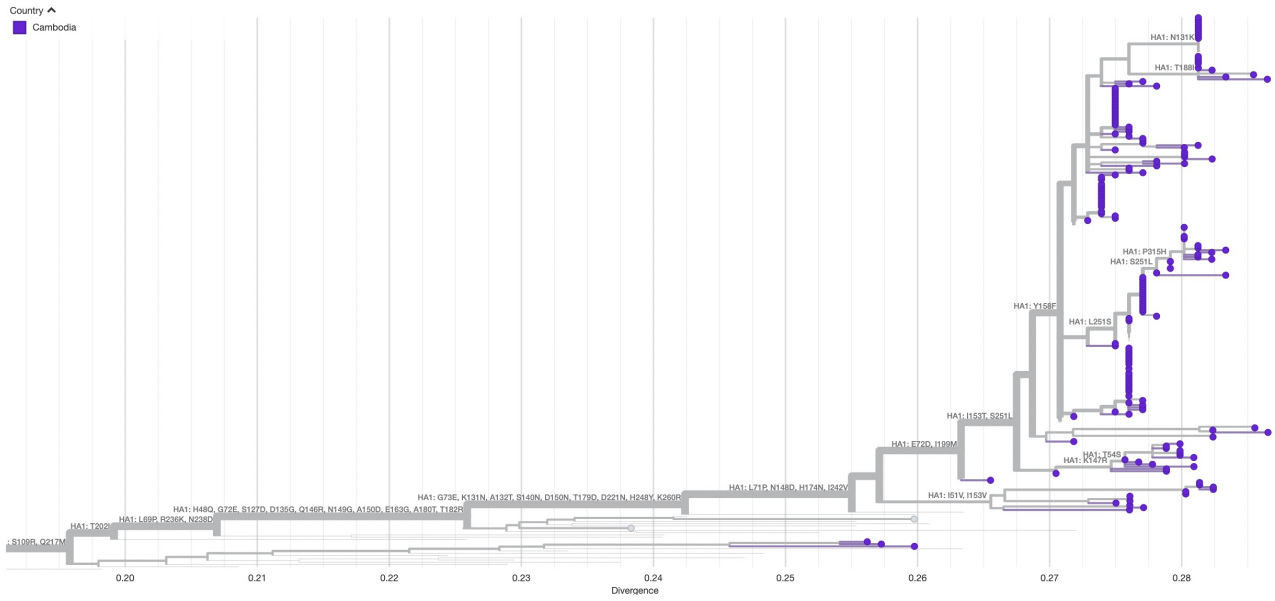
Homologous
2 fold
4 fold
8 fold
16 fold
< 32 fold

REFERENCE FERRET ANTISERA					A/Bangladesh/0994/2011_G1_CVV	A/Oman/2747/2019/H9N2/2019-03-21_G1_CVV	
			Clade		G1	G1	
			Subtype		H9N2	H9N2	
Semester		Clade	Ferret ID Subtype		*		AA diff from *
	Reference Antigen						
	A/Oman/2747/2019/H9N2/2019-03-21_G1_CVV	G1	H9N2		320	2560	
	A/Bangladesh/0994/2011_G1_CVV	G1	H9N2		2560	640	
	Test antigen						
1	A/sonali-chicken/Bangladesh/AV-22-006211/2021_31/08/2021	G1	H9N2		320	1280	*H48R, *T54K, *G149S,* N198D, V226I, N262D
2	A/sonali-chicken/Bangladesh/AV-22-006263/2021_02/09/2021	G1	H9N2		320	1280	*T54K, V57I, L69S, V226I, N262D
3	A/broiler-chicken/Bangladesh/AV-22-006654/2021_02/10/2021	G1	H9N2		60	640	T22S, *H48Q, S109R, Q112K, I114V, *G149S, *L150Q, *R162Q, I288V

H9 Y280 phylogenetic tree (nucleotide) annotated with amino acid changes

Figure 12: An ML nucleotide phylogenetic tree inferred from HA1 amino acid sequences have been included to complement the HI table and amino acid difference table. Amino acid differences were annotated at the nodes.





Annex 1: H5 Maximum Likelihood tree (all data)

Annex 2: HA tree for incongruence analyses (H5 2.3.4.4b)