# OFFLU AVIAN INFLUENZA REPORT

March 2021 to September 2021

## SCOPE

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

Contents

# 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 partner countries 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 (UK), ARRIAH (RUS), Canadian Food Inspection Agency (CAN), ANSES (FRA), FLI (GER), IZSVe (ITA), ICAR-NIHSAD (IND), Hokkaido University (JAP), NIAH (JAP), CAAS (CHN) and UP (SA) for sharing unpublished data and virus isolates via the OFFLU network.
We acknowledge and thank the OIE Reference laboratory and diagnostic laboratory teams at APHA, IZSVe, AAHL, FLI and NVSL for their expertise. Report analytical contributions were made from: APHA: Ian Brown, Nicola Lewis, Steve Essen; RVC: Nicola Lewis, Sara Lopes; OFFLU: Amelia Coggon. Phylogenetic and sequence analyses were performed at the Royal Veterinary College (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 and IZSVe EURL.

Analyses were conducted by subtype. Reference sequences and new data added from the beginning of this reporting period were downloaded from Genbank, NCBI and GISAID in addition to data provided through the OFFLU network. Sequences were aligned using MAFFT (Katoh and Standley, 2013) using default settings. Alignments were manually inspected and trimmed to the start and stop codon of HA1. Trees were run using IQ-TREE (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). Best matched strains were selected for testing against reference ferret antisera in haemagglutination inhibition (HI) assays.

## Avian influenza A virus vaccination

In many AI endemic countries, H5 and other vaccination is employed as part of overall control efforts. Recent H5 HPAI epizootic events have resulted in additional countries considering vaccination to control disease. China has had an extensive vaccination production system and current understanding is that in China a trivalent vaccine based on H5 and H7 (H5: Re-11 Re-12 and H7 Re-2) seed viruses are used. H5 HAs are derived from DK/GZ/S4184/17 - a clade 2.3.4.4d virus and CK/LN/SD007/17 a clade 2.3.2.1d virus. The H7 component is derived from CK/GX/SD098/17. Although the backbone HA sequence for the H7 component of the bivalent vaccine is based on A/pigeon/Shanghai/S1069/2013 (H7N9), the HA sequence was modified to reflect changes in more contemporary viruses in order to improve the antigenicity and titre of the vaccine stain. Institutions in China also produce various other H7 and H9 vaccines.

Currently, in China, vaccination of poultry is mandatory in all provinces (including chickens, ducks, geese, quails, pigeons and other rare birds in captivity).

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

# Global H5, H7 and H9 avian influenza events in animals

## Epidemiology

The majority of H5 events in this reporting period were due to the Goose/Guangdong (GsGD) H5 clades of HPAI. Relatively few countries reported H5 LPAI in domestic birds: H5N2 in South Africa and H5 in France. During this period there were reports of H7 HPAI viruses in Lithuania. While not officially reportable, H9N2 LPAI viruses were detected in Cambodia, China, India, Nigeria and Pakistan.

H5Nx clades have been steadily evolving since 2008 constituting an antigenically and genetically broad series of isolates. The vast majority of outbreaks this reporting period where sequence data were available were caused by H5 HPAI 2.3.4.4b.

Continued H5N8 circulation:
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. There have been numerous subsequent and continuing outbreaks throughout Europe, Asia and more recently Africa which appear genetically closely related.
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, and have continued in this reporting period.

Other H5 HPAI subtypes in this reporting period:
Throughout this reporting period H5N1 outbreaks occurred in Western Europe and Western Africa and subsequently in parts of Europe and additionally Viet Nam and India. Phylogenetic analysis of sequences available indicates the viruses circulating in India are of the 2.3.2.1a clade. Sporadic cases of H5N2 were seen in Taiwan in May, H5N3 in Denmark in April, H5N4 in Germany and Sweden between February and April. Cases of H5N5 have been seen throughout the year in Europe and Asia and since April H5N6 has been seen in China and Viet Nam.

# Avian influenza regional geographic summary and context

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

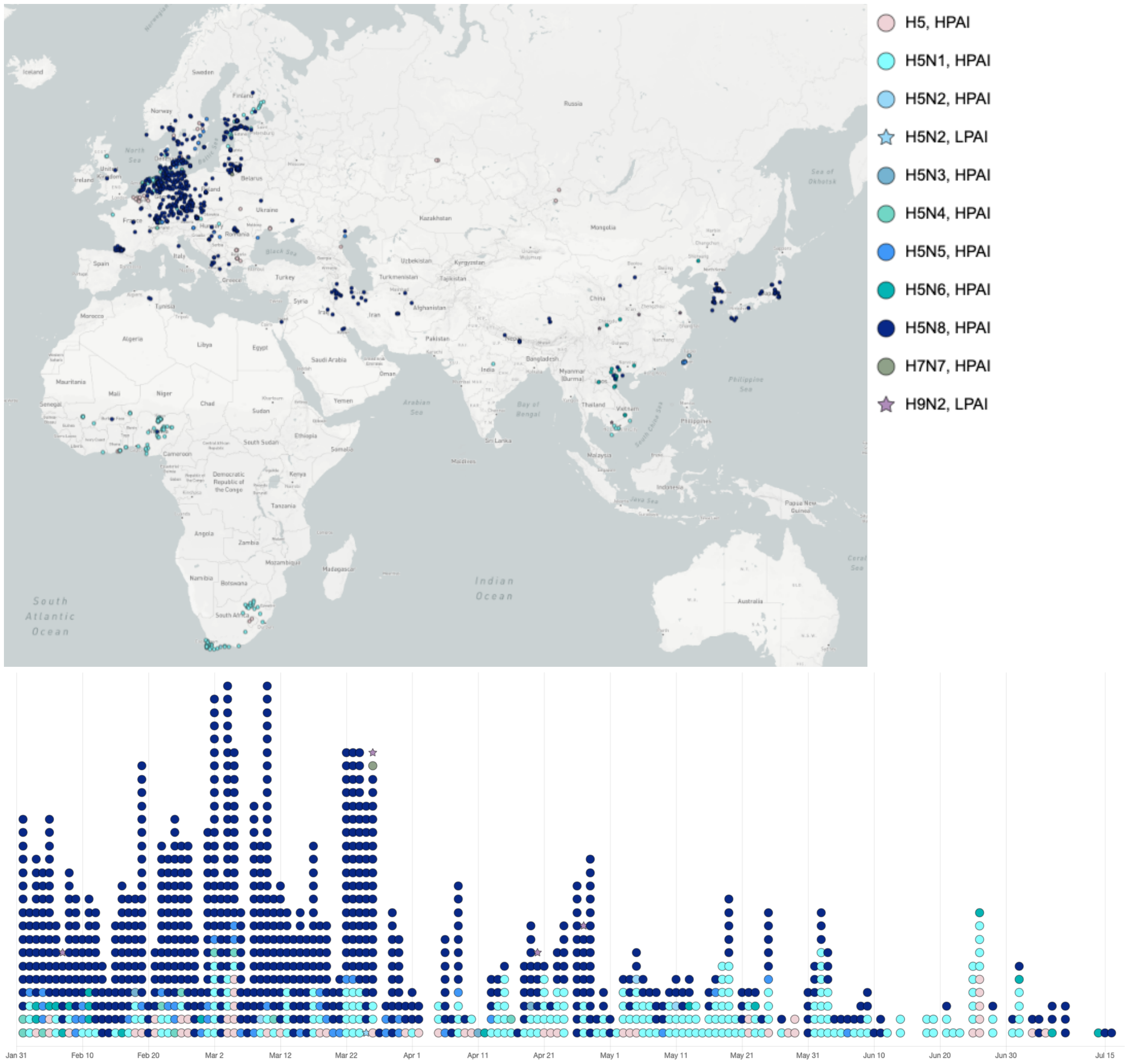


*Figure 1: MicroReact map of H5, H7 and H9 events for the current reporting period 1st February 2021 to 23rd September 2021 (N=1083). Points are coloured by subtype. HPAI events are denoted by a circle and LPAI events a star. Collection data is represented along the timeline. Data was harvested from EMPRES-Plus and complimented with information shared via the OFFLU network. If no observation date was available a random date within the reporting*

## HPAI activity within reporting period

**EUROPE**:

H5:

Bulgaria, Sweden and Austria from February onwards. Ukraine, Belgium, Sweden from March onwards and the Russian Federation from May onwards.

H5N1:

U.K. from February onwards. Denmark, Netherlands, Germany, France from March onwards. Hungary, Slovakia from April onwards. Estonia, Germany and Finland from May onwards and Latvia from June onwards.

H5N3:

Germany from February onwards and Denmark from April onwards.

H5N4:

Germany, Netherlands and Switzerland from February onwards and Sweden from March onwards.

H5N5:

Austria, Czech republic, Denmark, Germany, Hungary, Romain, Sweden and Switzerland from February onwards.

H5N8:

Austria, Czech republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy Latvia, Lithuania, Russian Federation, Poland, Sweden, UK and Ukraine from February onwards. Croatia, Netherlands, Norway and Serbia from March onwards. Albania from April onwards and Lithuania, Kosovo and Romania from May onwards. Luxembourg in September.

**ASIA:**

H5N1:

India from March onwards and Viet Nam from July onwards.

H5N2:

Taiwan from May onwards.

H5N5:

Taiwan from February onwards.

H5N6:

Viet Nam, China and Lao people's republic from February onwards

H5N8:

Afghanistan, Korea, Japan, Iran, from February onwards. Kuwait, China, Nepal from March onwards. Israel and Iraq from April onwards. Iraq, Viet Nam from June onwards.

**AFRICA:**

H5:

South Africa from April onwards. Lethoso from May onwards. Ghana from June onwards and Benin from July onwards.

H5N1:

Niger, Nigeria from February onwards. Mali, South Africa from March onwards. Ghana, Togo from June onwards. Cote d'Ivoire and Botswana from July onwards.

H5N8:

Algeria, Burkina Faso, Nigeria, from February onwards.

Data for 252 H5 and 4 H9 sequences were contributed to OFFLU by animal health laboratories in countries representing Europe, Asia, Africa, Oceania, and the Americas to which 637 H5 and 261 H9 sequences from Genbank and GISAID were added. 45 of the OFFLU contributions were partial H5 sequences and thus were not included in final phylogenetic analyses.

# Activity Table (H5) viruses

*Table 1: 01/02/2021 to 23/09/2021 A(H5) viruses of the A/goose/Guangdong/1/96 lineage detected in domestic and wild birds as summarised below. Where sequences have been available for analysis, clades have been included. Data was collected through EMPRES-i, OIE via the WAHIS system or through OFFLU collaborators.*

| **Country, area or territory** | **Host** | **Genetic clade** |
|---|---|---|
| Afghanistan | Poultry | H5N8 2.3.4.4b |
| Albania | Poultry | H5N8 |
| Algeria | Poultry<br>Wild Birds | H5N8<br>H5N8 |
| Austria | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N5/8 2.3.4.4b |
| Belgium | Poultry<br>Wild Birds | H5<br>H5/N8 |
| Bulgaria | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N5 2.3.4.4b |
| Burkina Faso | Poultry | H5N8 2.3.4.4b |
| Botswana | Poultry | H5N1 2.3.4.4b |
| China | Poultry<br>Wild Birds<br>Human | H5N6/8<br>H5N6/8 2.3.4.4b<br>H5N6 2.3.4.4b |
| Cote d'Ivoire | Poultry | H5N1 |
| Croatia | Wild Birds | H5N8 2.3.4.4b |
| Czechia | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N8 2.3.4.4b |
| Denmark | Wild Birds | H5N1/5/8 |
| Estonia | Poultry<br>Wild Birds | H5N8<br>H5N8 |
| Finland | Poultry<br>Wild Birds | H5N8<br>H5N1/8 |
| France | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N1/8 2.3.4.4b |
| Germany | Poultry<br>Wild Birds | H5N1/5/8 2.3.4.4b<br>H5N1/3/4/8 2.3.4.4b |
| Ghana | Poultry | H5 |
| Greece | Wild Birds | H5N8 2.3.4.4b |
| Hungary | Wild Birds | H5N1/8 |
| India | Wild Birds<br>Human | H5N1 2.3.2.1a<br>H5N1 |
| Iran (The Islamic Republic of) | Poultry | H5N8 |
| Iraq | Poultry | H5N8 |
| Ireland | Wild Birds | H5N3 2.3.4.4b |
| Israel | Wild Birds | H5N8 |
| Italy | Poultry<br>Wild Birds | H5N8<br>H5N8 2.3.4.4b |
| Japan | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N8 2.3.4.4b |
| Kosovo | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N8 2.3.4.4b |
| Kuwait | Wild Birds | H5N8 |
| Lao People's Democratic republic | Human | H5N6 |
| Latvia | Wild Birds | H5N1 |
| Lesotho | Poultry | H5 |
| Lithuania | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N8 2.3.4.4b |
| Luxembourg | Wild Birds | H5N8 |
| Mali | Avian | H5N1 2.3.4.4b |
| Nepal | Poultry | H5N8 |
| Netherlands | Wild Birds | H5N1/8 |
| Niger | Poultry | H5N1 2.3.4.4b |
| Nigeria | Poultry<br>Human | H5N1/8 2.3.4.4b<br>H5 2.3.4.4b |
| Norway | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N8 2.3.4.4b |
| Pakistan | Poultry | H5N8 |
| Poland | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N1/8 2.3.4.4b |
| Republic Of Korea | Poultry | H5N8 |
| Romania | Poultry<br>Wild Birds | H5N5/8 2.3.4.4b<br>H5N5/8 2.3.4.4b |
| Russian Federation | Wild Birds<br>Poultry | H5N1/5/8 2.3.4.4b<br>H5N1 |
| Senegal | Poultry<br>Wild Birds | H5N1 2.3.4.4b<br>H5N1 2.3.4.4b |
| Serbia | Wild Birds | H5N8 |
| South Africa | Poultry<br>Wild Birds | H5N1 2.3.4.4b<br>H5N1 2.3.4.4b |
| Spain | Wild Birds | H5N8 2.3.4.4b |
| Sweden | Wild Birds<br>Poultry | H5N4/5/8<br>H5N8 |
| Switzerland | Wild Birds | H5N4 2.3.4.4b |
| Taiwan (Provence of China) | Poultry<br>Wild Birds | H5N2/5<br>H5N2 |
| Togo | Avian | H5N1 2.3.4.4b |
| Ukraine | Poultry | H5N8 2.3.4.4b |
| United Kingdom of Great Britain and Northern Ireland | Poultry<br>Wild Birds | H5N8 2.3.4.4b<br>H5N3 2.3.4.4b |
| Viet Nam | Poultry | H5N1/6/8 2.3.4.4b |

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

*Table 2: Data taken from Influenza at the human-animal interface summary and assessment (April 2021, May 2021, June 2021 and August 2021). Table contains data reported to the WHO within the reporting period of 1st March 2021 to 1st September 2021. Where sequence data were available from GISAID, cases are marked by an asterisk* and annotated with clade.*

| Reporting Date | State (Country) | Subtype | Age (Gender) | Further Information |
|---|---|---|---|---|
| 2-3-21 | Guangxi (China) | H5N6 | 50 (Male) | Deceased |
| 4-3-21 | Kano & Plateau (Nigeria) | PCR+ H5N? 2.3.4.4b | 7 bird handlers | Asymptomatic. No further data confirming infection. |
| 15-3-21 | Lunag Prabang (Lao people's democratic republic) | H5N6 | 5 (Male) | Recovered |
| 17-3-21 | Siem Reap (Cambodia) | H9N2* | 3 (Male) | Mild |
| 9-4-21 | Fujan (China) | H9N2 | 10 (Male) | Mild |
| 9-4-21 | Hubei (China) | H9N2 | 2 (Female) | Mild |
| 30-5-21 | Sichuan (China) | H5N6 | 49 (Female) | Deceased |
| 9-6-21 | Sichuan (China) | H9N2 | 2 (Male) | Outpatient care |
| 9-6-21 | Jiangsu (China) | H9N2* | 78 (Female) | Hospitalised |
| 7-7-21 | Sichuan (China) | H5N6 | 55 (Male) | Critical |
| 12-7-21 | Chongqing (China) | H5N6 | 66 (Male) | Critical |
| 12-7-21 | Sichuan (China) | H5N6 | 57 (Male) | Critical |
| 12-7-21 | Sichuan (China) | H5N6* 2.3.4.4b | 51 (Female) | Deceased |
| 21-7-21 | Haryana (India) | H5N1 | 18 (Male) | Deceased. Previous underlying illness |
| 22-7-21 | Sichuan (China) | H5N6 | 65 (Female) | Critical |
| 22-7-21 | Guangxi (China) | H5N6 | 61 (Female) | Mild |

# H5 Influenza A viruses

## H5 phylogenetic tree: overview

**2021 SEPTEMBER REPORTING PERIOD OFFLU SUBMISSIONS**
**2021 OTHER SUBMISSIONS**
**2020 SUBMISSIONS**
**CVV**
**HUMAN VARIANTS**
**LOW PATHOGENIC STRAINS**

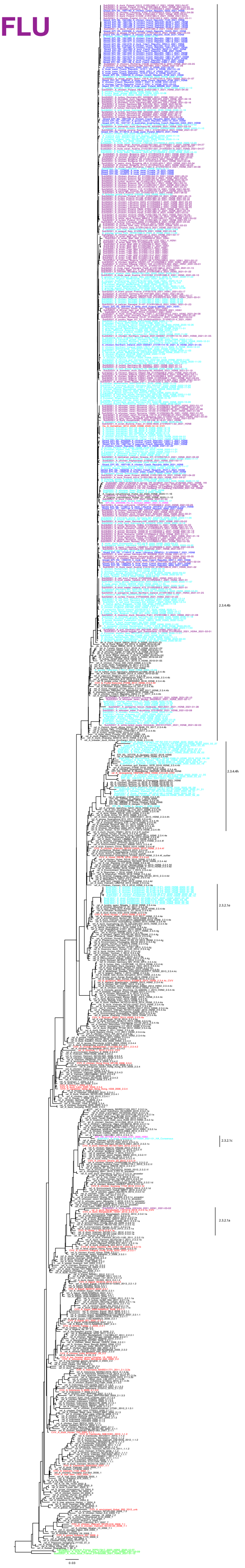


*Figure 2: Avian H5 global summary maximum likelihood phylogenetic tree annotated by clades. Analyses were conducted with reference sequences and data downloaded from GISAID or shared by the OFFLU network.*

## H5 phylogenetic tree coloured by geographic location (nucleotide)

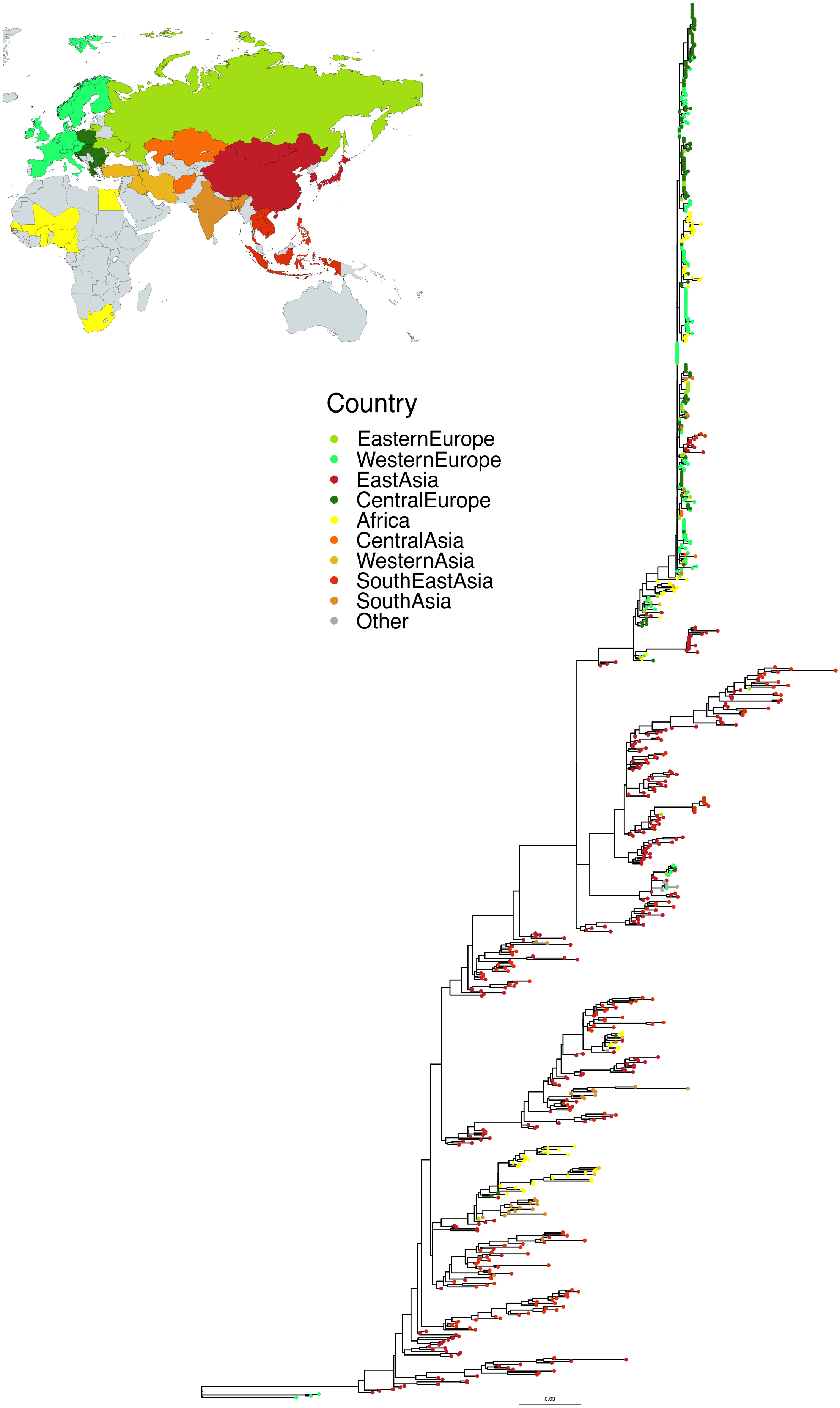


*Figure 3: Avian H5 global summary maximum likelihood phylogenetic tree annotated by geographic location using ggtree.*

# H5 2.3.4.4b phylogenetic tree (nucleotide)

**2021 SEPTEMBER REPORTING PERIOD**
**OFFLU SUBMISSIONS**
**2021 OTHER SUBMISSIONS**
**2020 SUBMISSIONS**
**CVV**
**HUMAN VARIANTS**
**LOW PATHOGENIC STRAINS**

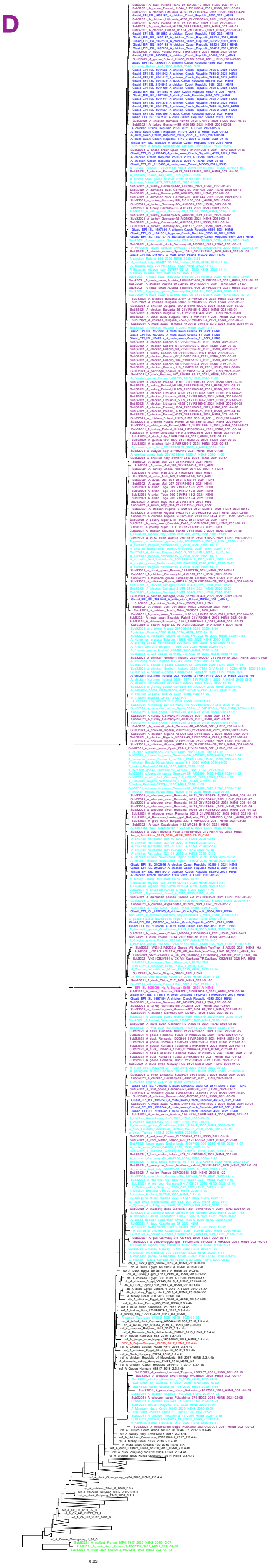


*Figure 4: Avian H5 clade 2.3.4.4b phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. Black arrows mark strains linked to human cases in Nigeria and China.*

# H5 annotated phylogenetic tree by geographic region (amino acid)

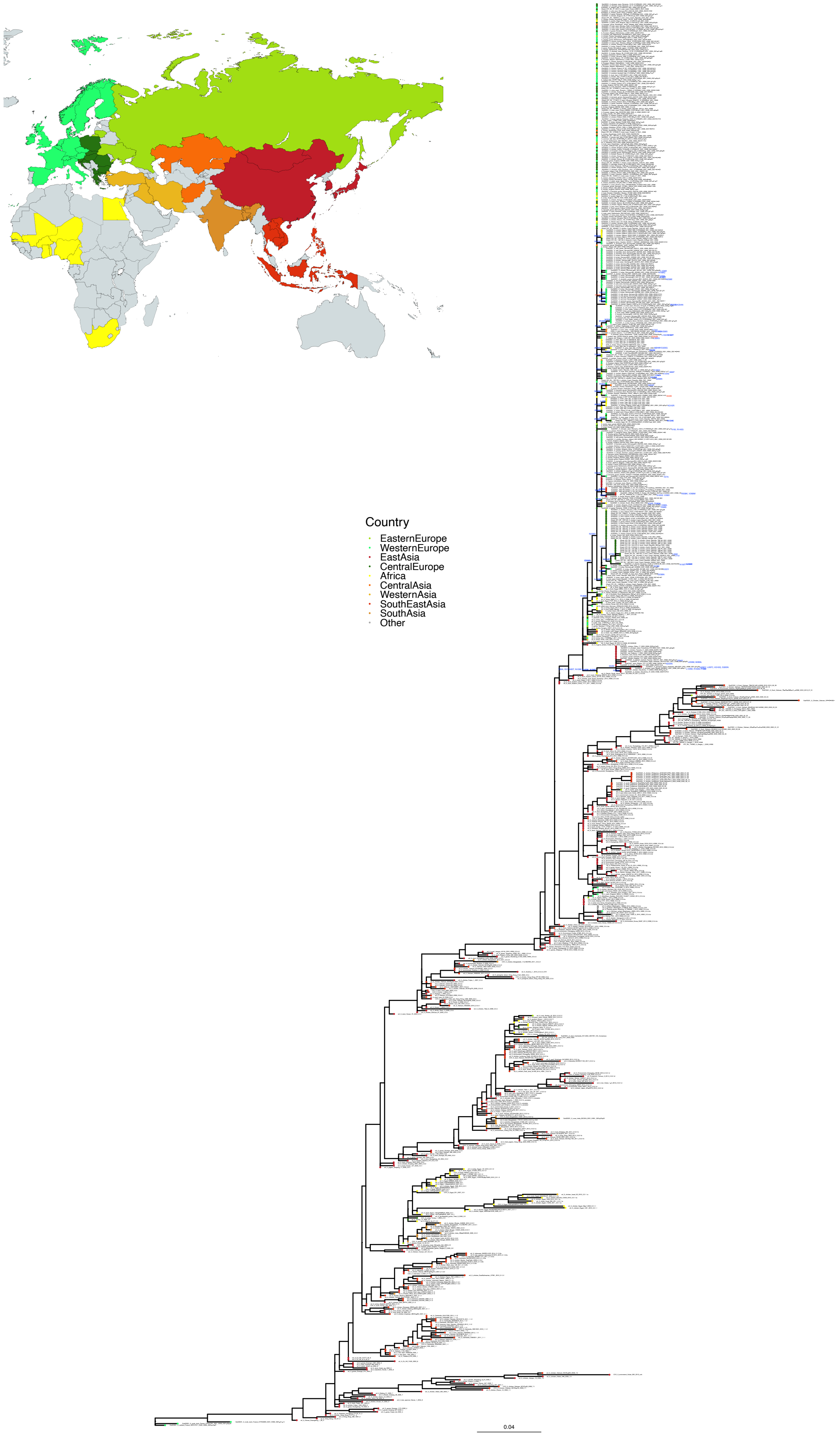


*Figure 5: Avian H5 phylogenetic tree coloured by geographical location and with clade 2.3.4.4b strains annotated compared to the CVV A/Astrakhan/3212/2020/H5N8/2020-12-12. Substitutions in putative antigenic sites are in bold and substitutions in cleavage site in red.*

# H5 2.3.4.4b phylogenetic tree (amino acid)

**2021 SEPTEMBER REPORTING PERIOD**
**OFFLU SUBMISSIONS**
**2021 OTHER SUBMISSIONS**
**2020 SUBMISSIONS**
**CVV**
**HUMAN VARIANTS**
**LOW PATHOGENIC STRAINS**

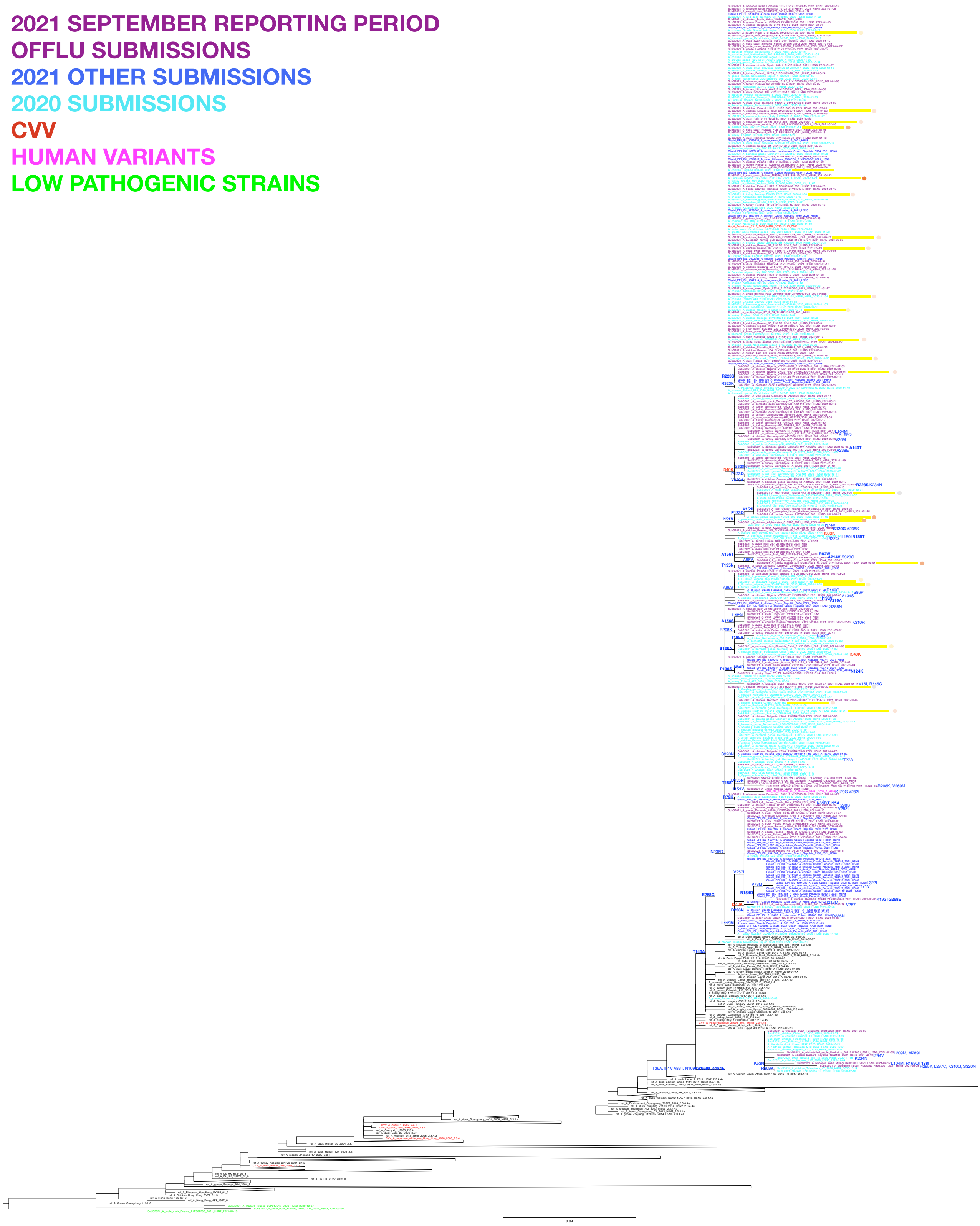
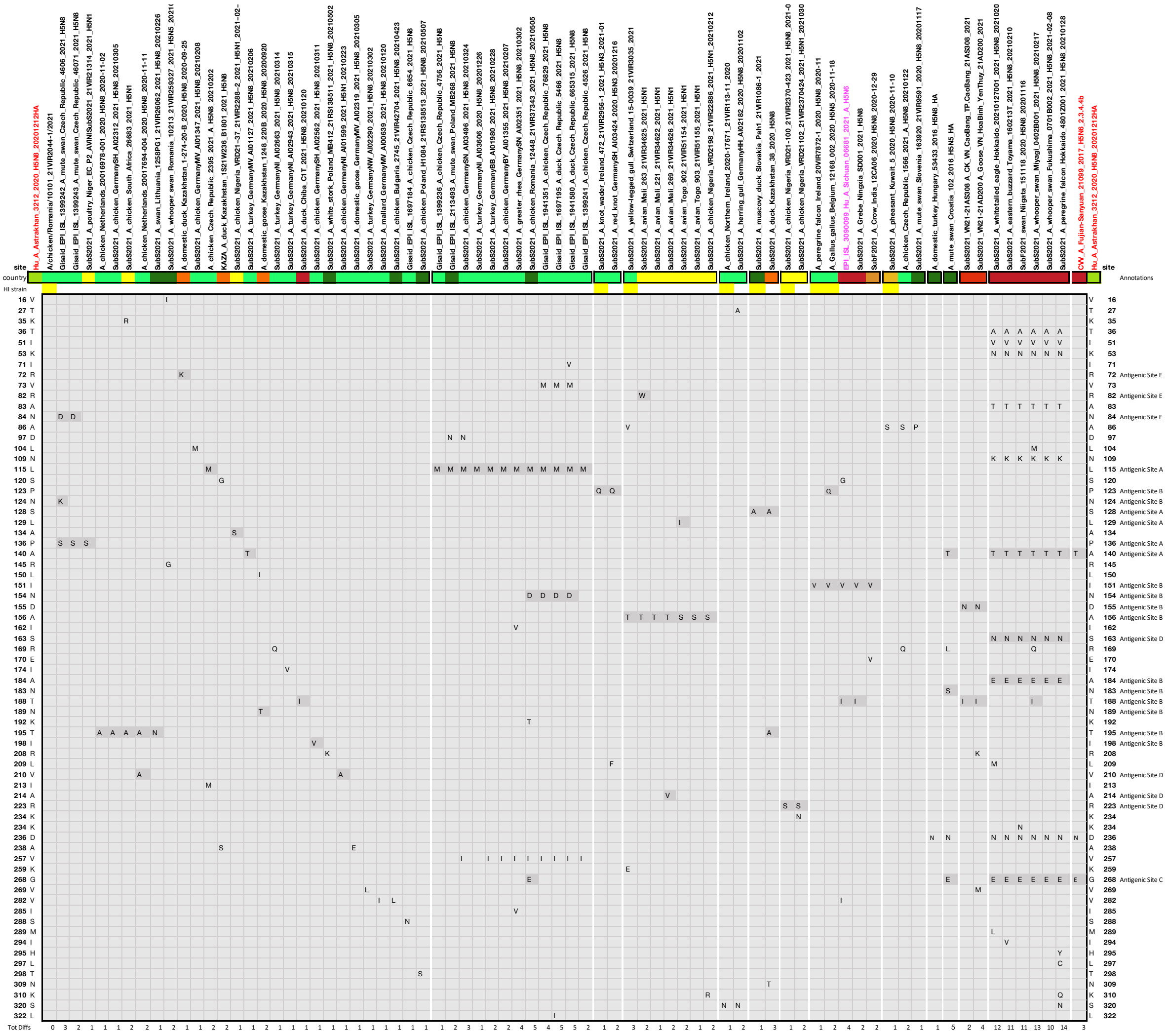


*Figure 6: Phylogenetic tree inferred from HA1 amino acid sequences has been included to complement the HI table and amino acid difference table. Amino acid differences relative to proposed CVV A/Astrakhan/3212/2020/H5N8/2020-12-12 were included at the nodes. Changes in putative antigenic sites are marked in bold. Strains used for HI testing are marked by yellow bars with fold changes indicated by coloured balls. Cleavage site changes are noted in red.*

# 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/H5N8/2020-12-12 which is shown in red. Geographic location is indicated by colour as in figure 3. HI strains are annotated by a yellow box. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering. Note that cleavage site was not included in this table.*



| site | Hu_A_Astrakhan_3212_2020_H5N8_20201212HA | A/chicken/Romania/10101_21VIR2044-1/2021 | Gisaid_EPI_ISL_1399242_A_mute_swan_Czech_Republic_4606_2021_H5N8 | Gisaid_EPI_ISL_1399243_A_mute_swan_Czech_Republic_46071_2021_H5N8 | SubS2021_A_poultry_Niger_EC_P2_AVINISubS2021_21VIR21314_2021_H5N1 | A_chicken_Netherlands_20016978-001_2020_H5N8_2020-11-02 | SubS2021_A_chicken_GermanySH_AI02312_2021_H5N8_20210305 | SubS2021_A_chicken_South_Africa_26683_2021_H5N1 | A_chicken_Netherlands_20017694-004_2020_H5N8_2020-11-11 | SubS2021_A_swan_Lithuania_1258PG1_21VIR26062_2021_H5N8_20210226 | SubS2021_A_whooper_swan_Romania_10213_21VIR259327_2021_H5N5_2021 | A_domestic_duck_Kazakhstan_1-274-20-B_2020_H5N8_2020-09-25 | SubS2021_A_chicken_GermanyMV_AI01347_2021_H5N8_20210208 | A_chicken_Czech_Republic_2395_2021_A_H5N8_20210202 | KAZA_A_duck_Kazakhstan_1S21W236_B1801_2021_H5N8 | SubS2021_A_chicken_Nigeria_VRD21–37_21VIR2288–2_2021_H5N1_2021–02– | SubS2021_A_turkey_GermanyMV_AI01127_2021_H5N8_20210206 | A_domestic_goose_Kazakhstan_1248_220B_2020_H5N8_20200920 | SubS2021_A_turkey_GermanyNI_AI02663_2021_H5N8_20210314 | SubS2021_A_turkey_GermanyNI_AI02943_2021_H5N8_20210315 | SubS2021_A_duck_Chiba_C1T_2021_H5N8_20210120 | SubS2021_A_chicken_GermanySH_AI02562_2021_H5N8_20210311 | SubS2021_A_white_stork_Poland_MB412_21RS138511_2021_H5N8_20210502 | SubS2021_A_chicken_GermanyNI_AI01599_2021_H5N1_20210223 | SubS2021_A_domestic_goose_GermanyMV_AI02319_2021_H5N8_20210305 | SubS2021_A_turkey_GermanyNW_AI02290_2021_H5N8_20210302 | SubS2021_A_mallard_GermanyMV_AI00639_2021_H5N8_20210120 | SubS2021_A_chicken_Bulgaria_2745_21VIR42704_2021_H5N8_20210423 | Gisaid_EPI_ISL_1697184_A_chicken_Czech_Republic_6654_2021_H5N8 | SubS2021_A_chicken_Poland_H1084_21RS138513_2021_H5N8_20210507 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| country | 🟩 | 🟩 | 🟩 | 🟩 | 🟨 | 🟩 | 🟩 | 🟨 | 🟩 | 🟩 | 🟩 | 🟧 | 🟩 | 🟩 | 🟧 | 🟨 | 🟩 | 🟧 | 🟩 | 🟩 | 🟥 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| HI strain | | 🟨 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 16 | V | | | | | | | | | | I | | | | | | | | | | | | | | | | | | | |
| 27 | T | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 35 | K | | | | | | | R | | | | | | | | | | | | | | | | | | | | | | |
| 36 | T | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 51 | I | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 53 | K | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 71 | I | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 72 | R | | | | | | | | | | | K | | | | | | | | | | | | | | | | | | |
| 73 | V | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 82 | R | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 83 | A | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 84 | N | | D | D | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 86 | A | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 97 | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 104 | L | | | | | | | | | | | | M | | | | | | | | | | | | | | | | | |
| 109 | N | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 115 | L | | | | | | | | | | | | | M | | | | | | | | | | | | | | | | |
| 120 | S | | | | | | | | | | | | | | G | | | | | | | | | | | | | | | |
| 123 | P | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 124 | N | | K | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 128 | S | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 129 | L | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 134 | A | | | | | | | | | | | | | | | S | | | | | | | | | | | | | | |
| 136 | P | | S | S | S | | | | | | | | | | | | | | | | | | | | | | | | | |
| 140 | A | | | | | | | | | | | | | | | | T | | | | | | | | | | | | | |
| 145 | R | | | | | | | | | | G | | | | | | | | | | | | | | | | | | | |
| 150 | L | | | | | | | | | | | | | | | | | I | | | | | | | | | | | | |
| 151 | I | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 154 | N | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 155 | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 156 | A | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 162 | I | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 163 | S | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 169 | R | | | | | | | | | | | | | | | | | | Q | | | | | | | | | | | |
| 170 | E | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 174 | I | | | | | | | | | | | | | | | | | | | V | | | | | | | | | | |
| 184 | A | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 183 | N | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 188 | T | | | | | | | | | | | | | | | | | | | | I | | | | | | | | | |
| 189 | N | | | | | | | | | | | | | | | | | T | | | | | | | | | | | | |
| 192 | K | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 195 | T | | | | | A | A | A | A | N | | | | | | | | | | | | | | | | | | | | |
| 198 | I | | | | | | | | | | | | | | | | | | | | | V | | | | | | | | |
| 208 | R | | | | | | | | | | | | | | | | | | | | | | K | | | | | | | |
| 209 | L | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 210 | V | | | | | | | | A | | | | | | | | | | | | | | | A | | | | | | |
| 213 | I | | | | | | | | | | | | | M | | | | | | | | | | | | | | | | |
| 214 | A | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 223 | R | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 234 | K | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 234 | K | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 236 | D | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 238 | A | | | | | | | | | | | | | | S | | | | | | | | | | E | | | | | |
| 257 | V | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 259 | K | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 268 | G | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 269 | V | | | | | | | | | | | | | | | | | | | | | | | | | L | | | | |
| 282 | V | | | | | | | | | | | | | | | | | | | | | | | | | | I | L | | |
| 285 | I | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 288 | S | | | | | | | | | | | | | | | | | | | | | | | | | | | | N | |
| 289 | M | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 294 | I | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 295 | H | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 297 | L | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 298 | T | | | | | | | | | | | | | | | | | | | | | | | | | | | | | S |
| 309 | N | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 310 | K | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 320 | S | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 322 | L | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tot Diffs | | 0 | 3 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

| site | Hu_A_Astrakhan_3212_2020_H5N8_20201212HA | Gisaid_EPI_ISL_1399236_A_chicken_Czech_Republic_4756_2021_H5N8 | Gisaid_EPI_ISL_2113493_A_mute_swan_Poland_MB268_2021_H5N8 | SubS2021_A_chicken_GermanySN_AI03496_2021_H5N8_20210324 | SubS2021_A_turkey_GermanyNI_AI03606_2020_H5N8_20201226 | SubS2021_A_turkey_GermanyBB_AI01980_2021_H5N8_20210228 | SubS2021_A_chicken_GermanyBY_AI01355_2021_H5N8_20210207 | SubS2021_A_greater_rhea_GermanySN_AI02351_2021_H5N8_20210302 | SubS2021_A_chicken_Romania_12448_21VIR37343_2021_H5N8_20210505 | Gisaid_EPI_ISL_1941351_A_chicken_Czech_Republic_76829_2021_H5N8 | Gisaid_EPI_ISL_1697195_A_duck_Czech_Republic_5466_2021_H5N8 | Gisaid_EPI_ISL_1941580_A_duck_Czech_Republic_665315_2021_H5N8 | Gisaid_EPI_ISL_1399241_A_chicken_Czech_Republic_4526_2021_H5N8 | SubS2021_A_knot_wader_Ireland_472_21VIR2956-1_2021_H5N3_2021-01 | SubS2021_A_red_knot_GermanySH_AI03424_2020_H5N3_20201216 | SubS2021_A_yellow-legged_gull_Switzerland_15-0039_21VIR3035_2021 | SubS2021_A_avian_Mali_263_21VIR34625_2021_H5N1 | SubS2021_A_avian_Mali_221_21VIR34622_2021_H5N1 | SubS2021_A_avian_Mali_269_21VIR34626_2021_H5N1 | SubS2021_A_avian_Togo_902_21VIR51154_2021_H5N1 | SubS2021_A_avian_Togo_903_21VIR51155_2021_H5N1 | SubS2021_A_chicken_Nigeria_VRD2198_21VIR22886_2021_H5N1_20210212 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| country | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟨 | 🟨 | 🟨 | 🟨 | 🟨 | 🟨 |
| HI strain | | | | | | | | | | | | | | 🟨 | | 🟨 | | | | | | |
| 16 | V | | | | | | | | | | | | | | | | | | | | | |
| 27 | T | | | | | | | | | | | | | | | | | | | | | |
| 35 | K | | | | | | | | | | | | | | | | | | | | | |
| 36 | T | | | | | | | | | | | | | | | | | | | | | |
| 51 | I | | | | | | | | | | | | | | | | | | | | | |
| 53 | K | | | | | | | | | | | | | | | | | | | | | |
| 71 | I | | | | | | | | | | | V | | | | | | | | | | |
| 72 | R | | | | | | | | | | | | | | | | | | | | | |
| 73 | V | | | | | | | | | M | M | M | | | | | | | | | | |
| 82 | R | | | | | | | | | | | | | | | | W | | | | | |
| 83 | A | | | | | | | | | | | | | | | | | | | | | |
| 84 | N | | | | | | | | | | | | | | | | | | | | | |
| 86 | A | | | | | | | | | | | | | | | V | | | | | | |
| 97 | D | | N | N | | | | | | | | | | | | | | | | | | |
| 104 | L | | | | | | | | | | | | | | | | | | | | | |
| 109 | N | | | | | | | | | | | | | | | | | | | | | |
| 115 | L | M | M | M | M | M | M | M | M | M | M | M | M | | | | | | | | | |
| 120 | S | | | | | | | | | | | | | | | | | | | | | |
| 123 | P | | | | | | | | | | | | | Q | Q | | | | | | | |
| 124 | N | | | | | | | | | | | | | | | | | | | | | |
| 128 | S | | | | | | | | | | | | | | | | | | | | | |
| 129 | L | | | | | | | | | | | | | | | | | | | I | | |
| 134 | A | | | | | | | | | | | | | | | | | | | | | |
| 136 | P | | | | | | | | | | | | | | | | | | | | | |
| 140 | A | | | | | | | | | | | | | | | | | | | | | |
| 145 | R | | | | | | | | | | | | | | | | | | | | | |
| 150 | L | | | | | | | | | | | | | | | | | | | | | |
| 151 | I | | | | | | | | | | | | | | | | | | | | | |
| 154 | N | | | | | | | | D | D | D | D | | | | | | | | | | |
| 155 | D | | | | | | | | | | | | | | | | | | | | | |
| 156 | A | | | | | | | | | | | | | | | T | T | T | T | S | S | S |
| 162 | I | | | | | | | V | | | | | | | | | | | | | | |
| 163 | S | | | | | | | | | | | | | | | | | | | | | |
| 169 | R | | | | | | | | | | | | | | | | | | | | | |
| 170 | E | | | | | | | | | | | | | | | | | | | | | |
| 174 | I | | | | | | | | | | | | | | | | | | | | | |
| 184 | A | | | | | | | | | | | | | | | | | | | | | |
| 183 | N | | | | | | | | | | | | | | | | | | | | | |
| 188 | T | | | | | | | | | | | | | | | | | | | | | |
| 189 | N | | | | | | | | | | | | | | | | | | | | | |
| 192 | K | | | | | | | | T | | | | | | | | | | | | | |
| 195 | T | | | | | | | | | | | | | | | | | | | | | |
| 198 | I | | | | | | | | | | | | | | | | | | | | | |
| 208 | R | | | | | | | | | | | | | | | | | | | | | |
| 209 | L | | | | | | | | | | | | | | F | | | | | | | |
| 210 | V | | | | | | | | | | | | | | | | | | | | | |
| 213 | I | | | | | | | | | | | | | | | | | | | | | |
| 214 | A | | | | | | | | | | | | | | | | | | V | | | |
| 223 | R | | | | | | | | | | | | | | | | | | | | | |
| 234 | K | | | | | | | | | | | | | | | | | | | | | |
| 234 | K | | | | | | | | | | | | | | | | | | | | | |
| 236 | D | | | | | | | | | | | | | | | | | | | | | |
| 238 | A | | | | | | | | | | | | | | | | | | | | | |
| 257 | V | | | I | | I | I | I | I | I | I | I | I | | | | | | | | | |
| 259 | K | | | | | | | | | | | | | | | E | | | | | | |
| 268 | G | | | | | | | | E | | | | | | | | | | | | | |
| 269 | V | | | | | | | | | | | | | | | | | | | | | |
| 282 | V | | | | | | | | | | | | | | | | | | | | | |
| 285 | I | | | | | | | V | | | | | | | | | | | | | | |
| 288 | S | | | | | | | | | | | | | | | | | | | | | |
| 289 | M | | | | | | | | | | | | | | | | | | | | | |
| 294 | I | | | | | | | | | | | | | | | | | | | | | |
| 295 | H | | | | | | | | | | | | | | | | | | | | | |
| 297 | L | | | | | | | | | | | | | | | | | | | | | |
| 298 | T | | | | | | | | | | | | | | | | | | | | | |
| 309 | N | | | | | | | | | | | | | | | | | | | | | |
| 310 | K | | | | | | | | | | | | | | | | | | | | | R |
| 320 | S | | | | | | | | | | | | | | | | | | | | | |
| 322 | L | | | | | | | | | | I | | | | | | | | | | | |
| Tot Diffs | | 1 | 2 | 3 | 1 | 2 | 2 | 4 | 5 | 4 | 5 | 5 | 2 | 1 | 2 | 3 | 2 | 1 | 2 | 2 | 1 | 2 |

| A_chicken_Northern_Ireland_2020-17671_21VIR113-11_2020 | SubS2021_A_herring_gull_GermanyHH_AI02182_2020_H5N8_20201102 | SubS2021_A_muscovy_duck_Slovakia_Pah1_21VIR1086-1_2021 | SubS2021_A_duck_Kazakhstan_38_2020_H5N8 | SubS2021_A_chicken_Nigeria_VRD21-100_21VIR2370-423_2021_H5N8_2021-0 | SubS2021_A_chicken_Nigeria_VRD21102_21VIR2370424_2021_H5N1_2021030 | A_peregrine_falcon_Ireland_20VIR7872-1_2020_H5N8_2020-11 | A_Gallus_gallus_Belgium_12168_002_2020_H5N5_2020-11-18 | EPI_ISL_3090099_Hu_A_Sichuan_06681_2021_A_H5N6 | SubS2021_A_Grebe_Ningxia_SD001_2021_H5N8 | SubF2021_A_Crow_India_12CA06_2020_H5N8_2020-12-29 | SubS2021_A_pheasant_Kuwait_5_2020_H5N8_2020-11-10 | A_chicken_Czech_Republic_1566_2021_A_H5N8_20210122 | SubS2021_A_mute_swan_Slovenia_163920_21VIR9591_2020_H5N8_20201117 | A_domestic_turkey_Hungary_53433_2016_H5N8_HA | A_mute_swan_Croatia_102_2016_H5N5_HA | SubS2021_VN21-21AS308 A_CK_VN_CaoBang_TP.CaoBang_21AS308_2021 | SubS2021_VN21-21AD200 A_Goose_VN_HoaBinh_YenThuy_21AD200_2021 | SubS2021_A_whitetailed_eagle_Hokkaido_20210127001_2021_H5N8_2021020 | SubS2021_A_eastern_buzzard_Toyama_160213T_2021_H5N8_20210210 | SubF2021_swan_Niigata_151118_2020_H5N8_20201116 | SubS2021_A_whooper_swan_Miyagi_0402B001_2021_H5N8_20210217 | SubS2021_A_whooper_swan_Fukushima_0701B002_2021_H5N8_2021-02-08 | SubS2021_A_peregrine_falcon_Hokkaido_4801Z001_2021_H5N8_20210128 | CVV_A_Fujian-Sanyuan_21099_2017_H5N6_2.3.4.4b | Hu_A_Astrakhan_3212_2020_H5N8_20201212HA | site | Annotations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 🟩 | 🟩 | 🟩 | 🟧 | 🟨 | 🟨 | 🟩 | 🟩 | 🟥 | 🟥 | 🟧 | 🟧 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟩 | | |
| 🟨 | | 🟨 | | 🟨 | | 🟨 | 🟨 | | | | 🟨 | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | V | 16 | |
| | A | | | | | | | | | | | | | | | | | | | | | | | | T | 27 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | K | 35 | |
| | | | | | | | | | | | | | | | | | | A | A | A | A | A | A | | T | 36 | |
| | | | | | | | | | | | | | | | | | | V | V | V | V | V | V | | I | 51 | |
| | | | | | | | | | | | | | | | | | | N | N | N | N | N | N | | K | 53 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | I | 71 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | R | 72 | Antigenic Site E |
| | | | | | | | | | | | | | | | | | | | | | | | | | V | 73 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | R | 82 | Antigenic Site E |
| | | | | | | | | | | | | | | | | | | T | T | T | T | T | T | | A | 83 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | N | 84 | Antigenic Site E |
| | | | | | | | | | | | S | S | P | | | | | | | | | | | | A | 86 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | D | 97 | |
| | | | | | | | | | | | | | | | | | | | | | M | | | | L | 104 | |
| | | | | | | | | | | | | | | | | | | K | K | K | K | K | K | | N | 109 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | L | 115 | Antigenic Site A |
| | | | | | | | | G | | | | | | | | | | | | | | | | | S | 120 | |
| | | | | | | | Q | | | | | | | | | | | | | | | | | | P | 123 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | | | | | | | | | N | 124 | Antigenic Site B |
| | | A | A | | | | | | | | | | | | | | | | | | | | | | S | 128 | Antigenic Site A |
| | | | | | | | | | | | | | | | | | | | | | | | | | L | 129 | Antigenic Site A |
| | | | | | | | | | | | | | | | | | | | | | | | | | A | 134 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | P | 136 | Antigenic Site A |
| | | | | | | | | | | | | | | | T | | | T | T | T | T | T | T | T | A | 140 | Antigenic Site A |
| | | | | | | | | | | | | | | | | | | | | | | | | | R | 145 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | L | 150 | |
| | | | | | | V | V | V | V | V | | | | | | | | | | | | | | | I | 151 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | | | | | | | | | N | 154 | Antigenic Site B |
| | | | | | | | | | | | | | | | | N | N | | | | | | | | D | 155 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | | | | | | | | | A | 156 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | | | | | | | | | I | 162 | |
| | | | | | | | | | | | | | | | | | | N | N | N | N | N | N | | S | 163 | Antigenic Site D |
| | | | | | | | | | | | | Q | | | L | | | | | | Q | | | | R | 169 | |
| | | | | | | | | | | V | | | | | | | | | | | | | | | E | 170 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | I | 174 | |
| | | | | | | | | | | | | | | | | | | E | E | E | E | E | E | | A | 184 | Antigenic Site B |
| | | | | | | | | | | | | | | | S | | | | | | | | | | N | 183 | Antigenic Site B |
| | | | | | | | | I | I | | | | | | | I | I | | | | I | | | | T | 188 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | | | | | | | | | N | 189 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | | | | | | | | | K | 192 | |
| | | | A | | | | | | | | | | | | | | | | | | | | | | T | 195 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | | | | | | | | | I | 198 | Antigenic Site B |
| | | | | | | | | | | | | | | | | | K | | | | | | | | R | 208 | |
| | | | | | | | | | | | | | | | | | | M | | | | | | | L | 209 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | V | 210 | Antigenic Site D |
| | | | | | | | | | | | | | | | | | | | | | | | | | I | 213 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | A | 214 | Antigenic Site D |
| | | | | S | S | | | | | | | | | | | | | | | | | | | | R | 223 | Antigenic Site D |
| | | | | | N | | | | | | | | | | | | | | | | | | | | K | 234 | |
| | | | | | | | | | | | | | | | | | | | | N | | | | | K | 234 | |
| | | | | | | | | | | | | | | N | N | | | N | N | N | N | N | N | N | D | 236 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | A | 238 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | V | 257 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | K | 259 | |
| | | | | | | | | | | | | | | | E | | | E | E | E | E | E | E | E | G | 268 | Antigenic Site C |
| | | | | | | | | | | | | | | | | | M | | | | | | | | V | 269 | |
| | | | | | | | | I | | | | | | | | | | | | | | | | | V | 282 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | I | 285 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | S | 288 | |
| | | | | | | | | | | | | | | | | | | L | | | | | | | M | 289 | |
| | | | | | | | | | | | | | | | | | | | V | | | | | | I | 294 | |
| | | | | | | | | | | | | | | | | | | | | | | | Y | | H | 295 | |
| | | | | | | | | | | | | | | | | | | | | | | | C | | L | 297 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | T | 298 | |
| | | | T | | | | | | | | | | | | | | | | | | | | | | N | 309 | |
| | | | | | | | | | | | | | | | | | | | | | | | Q | | K | 310 | |
| N | N | | | | | | | | | | | | | | | | | | | | | | N | | S | 320 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | L | 322 | |
| 1 | 2 | 1 | 3 | 1 | 2 | 1 | 2 | 4 | 2 | 2 | 1 | 2 | 1 | 1 | 5 | 2 | 4 | 12 | 11 | 11 | 13 | 10 | 14 | 3 | | | |

# H5 antigenic analysis

*Table 4: Antigenic analysis IZSVe and APHA reference lab data: Haemagglutination inhibition assay data using reference antigen ferret antisera against H5 lineage strains selected across clades to represent currently circulating viruses. Fold differences between the homologous and antigen titres are highlighted from grey to red. Amino acid differences relative to CVV strains are annotated on the right hand side. Changes in putative antigenic sites are marked in bold. Diversity in amino acid changes for groups represented by a surrogate are noted on the far right hand side.*

| | |
|---|---|
| ▨ | Homologous |
| ▨ | 2 fold |
| ▨ | 4 fold |
| ▨ | 8 fold |
| ▨ | 16 fold |
| ▨ | < 32 fold |

**REFERENCE FERRET ANTISERA**

| | | | | | | A/SICHUAN/26221/2014 | A/FUJIAN-SANYUAN/21099/2017XPR8 | A/TURKEY/ITALY/17VIR576-11/2017 | HI strain's amino acid changes from CVV *A_Astrakhan_3212_2020_H5N8 | Amino acid variation within the groups represented by HI strains |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Clade | 2.3.4.4a | 2.3.4.4b | 2.3.4.4b | | |
| | | | | | Subtype | H5N6 | H5N6 | H5N8 | | |
| | Semester | | | Clade | Ferret ID Subtype | | | | **A_Fujian-Sanyuan_21099_2017_H5N6<br>T140A, N236D, E268G | |
| IZSVe | | | **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 | 2.3.4.4 | H5N5 | 160 | 160 | 1280 | | V16I, K35R, **R72K, N84D**, L104M, **L115M**, **S120G**, **N124K**, **P136S**, **A140T**, R145G, L150I, R169Q, I174V, **T188I**, **N189T**, **T195A, T195N**, **I198V**, R208K, **V210A**, M213M, A238S, A238E, V269L, V282I, V282L, S288N, T298S |
| | | **2** | A/poultry/Niger/ET3_HALAL_21VIR2131-33/2021 | 2.3.4.4 | H5N1 | 80 | 80 | 640 | | |
| | **Feb21-Sept21** | **3** | A/chicken/Italy/21VIR1151-2/2021 | 2.3.4.4 | H5N8 | 80 | 40 | 320 | | |
| | | **4** | A/chicken/Austria/21052483_21VIR3291-1/2021 | 2.3.4.4 | H5N8 | 40 | 40 | 160 | | |
| | | **5** | A/chicken/Lithuania/4323_21VIR3369-1/2021 | 2.3.4.4 | H5N8 | 80 | 40 | 320 | | |
| | | **6** | A/chicken/Kosovo/82_21VIR5162-1/2021 | 2.3.4.4 | H5N8 | 40 | 40 | 160 | | |
| | | **7** | A/knot_wader/Ireland/472_21VIR2956-1/2021 | 2.3.4.4 | H5N3 | 320 | 160 | 1280 | ***P123Q** | L209F |
| | | **8** | A/yellow-legged_gull/Switzerland/15-0039_21VIR3035/2021 | 2.3.4.4 | H5N4 | <10 | <20 | 160 | *A86V, **A156T,** K259E | **R82W, T156S, L129I, A214V** |
| | | **9** | A/chicken/Northern_Ireland/2020-17671_21VIR113-11/2020 | 2.3.4.4 | H5N8 | 80 | 40 | 320 | *S320N | T27A |
| | | **10** | A/muscovy_duck/Slovakia/Pah1_21VIR1086-1/2021 | 2.3.4.4 | H5N8 | 160 | 80 | 640 | ***S128A** | **T195A,** N309T |
| | | | **Reference Antigen** | | | | | | | |
| | | | A/SICHUAN/26221/2014 | 2.3.4.4a | H5N6 | 320 | | | | |
| | **Feb21-Sept21** | | 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 | ***R223S** | K324N |
| | | | **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 | | V16I, K35R, **R72K, N84D**, L104M, **L115M**, S120G, **N124K**, **P136S**, **A140T**, R145G, L150I, R169Q, I174V, **T188I**, **N189T**, **T195A, T195N**, **I198V**, R208K, **V210A**, M213M, A238S, A238E, V269L, V282I, V282L, S288N, T298S |
| | **Sept20-Feb21** | **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 | *K331R | |
| | | **17** | A/eurasian_wigeon/italy/20vir7301-31/2020 | 2.3.4.4b | H5N8 | 40 | 40 | 160 | *A86S | |
| | | **18** | A/peregrine_falcon/ireland/20vir7872-1/2020 | 2.3.4.4b | H5N8 | 40 | 20 | 160 | ***I151V** | |
| | | **19** | A/gallus_gallus/belgium/12168_i20vir7627-3i/2020 | 2.3.4.4b | H5N5 | 20 | 20 | 80 | ***P123Q, I151V** | |
| APHA | | | **Reference antigen** | | | | | | | |
| | | | A/ANHUI/1/2005 | 2.3.4 | H5N1 | <10 | <10 | 10 | | |
| | | | A/SICHUAN/26221/2014 | 2.3.4.4a | H5N6 | 80 | 80 | 20 | | |
| | | | A/FUJIAN-SANYUAN/21099/2017XPR8 | 2.3.4.4b | H5N6 | 40 | 80 | 160 | | |
| | | | A/TURKEY/ITALY/17VIR576-11/2017 | 2.3.4.4b | H5N8 | 10 | 20 | 320 | | |
| | | | A/DUCK/ENGLAND/36254/2014 | 2.3.4.4c | H5N8 | 40 | 160 | 1280 | | |
| | **Feb21-Sept21** | | A/DUCK/HYOGO/1/2016 | 2.3.4.4e | H5N6 | 40 | 80 | 40 | | |
| | | | **Test antigen** | | | | | | | |
| | | **20** | A/chicken/Ukraine/1/2020 | 2.3.4.4b | H5N8 | 10 | 40 | 160 | | V16I, K35R, **R72K, N84D**, L104M, **L115M**, **S120G**, **N124K**, **P136S**, **A140T**, R145G, L150I, R169Q, I174V, **T188I**, **N189T**, **T195A, T195N**, **I198V**, R208K, **V210A**, M213M, A238S, A238E, V269L, V282I, V282L, S288N, T298S |
| | | **21** | A/turkey/Norway/FU496/2020 | 2.3.4.4b | H5N8 | 10 | 20 | 80 | | |
| | | **22** | A/chicken/England/030786/2020 | 2.3.4.4b | H5N8 | 20 | 40 | 320 | | |
| | | **23** | A/pheasant/Kuwait/5/2020 | 2.3.4.4b | H5N8 | 20 | 40 | 160 | *A86S | S86P, R169Q |
| | | **24** | A/chicken/England/034547/2020 | 2.3.4.4b | H5N8 | 10 | 20 | 160 | *S320N | **T195A, N309T** |

## Genome mutation analysis of human case A/Sichuan/06681/2021/A/H5N6

On the 3rd July 2021 samples were collected in the Sichuan Center for Disease Control and Prevention, from a non-influenza-vaccinated 51 year old (Female) (deceased). The sequence was subsequently deposited in GISAID by the WHO Chinese National Influenza Center Virology Institute.

We have analysed the genome sequence data of A/Sichuan/06681/2021 - a clade 2.3.4.4b H5N6 virus, using CDC's (Atlanta) H5N1 genetic changes inventory, Suttie et al., (2019) and general literature searches to identify genetic mutations that determine viral phenotypic characteristics of importance that may increase virulence, signal adaptation to mammalian species or alter susceptibility to existing antivirals.

HA1 Mutations are listed in the table below (H5 numbering) comprising of

- 7 substitutions compared to HA1 of CVV A/Fujian-Sanyuan/21099/2017/H5N6/2.3.4.4b. Of which four of these are in antigenic sites.
- 4 substitutions relative to HA1 of current CVV A/Astrakhan/3212/2020/H5N8/2020-12-12. Of which two are found in putative antigenic sites.
- I151V is situated in antigenic site B and is present in some contemporary circulating strains.
- T188I also situated in antigenic site B and has been reported in to increase alpha 2-6 binding as determined by resialylated hemagglutinin assays (Yang *et al.,* 2007). This change is also noted in few other contemporary circulating strains.

*Table 5: Amino acid differences relative to current CVV A/Fujian-Sanyuan/21099/2017/H5N6/2.3.4.4b. Also included are recent human case and proposed CVV A/Astrakhan/3212/2020/H5N8/2020-12-12 and closest HI strain A/peregrine/falcon/Ireland/20VIR7872-1/2020/H5N8/2020-1.*

| **site** | **Ref_A_Fujian-Sanyuan_21099_2017_H5N6_2.3.4.4bCVV** | **Hu_A_Astrakhan_3212_2020_H5N8_2020-12-12CVV** | **Hu_A_Sichuan_06681_2021_H5N6** | **A_peregrine_falcon_Ireland_20VIR7872-1_2020_H5N8_2020** | **Annotations** |
|---|---|---|---|---|---|
| **120** | S |  | G |  |  |
| **140** | T | A | A | A | Antigenic Site A |
| **151** | I |  | V | V | Antigenic Site B |
| **188** | T |  | I |  | Antigenic Site B |
| **236** | N | D | D | D |  |
| **268** | E | G | G | G | Antigenic Site C |
| **282** | V |  | I |  |  |
| Total |  | 3 | 7 | 4 |  |

# H5 2.3.2.1a and 2.3.2.1c phylogenetic tree (nucleotide)

**2021 SEPTEMBER REPORTING PERIOD**
**OFFLU SUBMISSIONS**
**2021 OTHER SUBMISSIONS**
**2020 SUBMISSIONS**
**CVV**
**HUMAN VARIANTS**
**LOW PATHOGENIC STRAINS**

*Figure 7: Avian H5 clade 2.3.2.1a and 2.3.2.1c maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU.*

# H5 2.3.2.1a comparative amino acid substitutions to CVV

*Table 9: Avian H5 2.3.2.1a clade sequence comparison to the CVV shown in red. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering.*

| site | CVV_A_duck_Bangladesh_19097_2013_2.3.2.1a | SubS2021_A_crow_India_03CA24_2021_H5N1_2021-03-02 | Annotations |
|---|---|---|---|
| **94** | N | S | |
| **107** | R | K | |
| **120** | D | E | |
| **124** | D | E | Antigenic Site B |
| **127** | A | V | Antigenic Site B |
| **141** | S | P | Antigenic Site A |
| **154** | D | N | Antigenic Site B |
| **183** | D | N | Antigenic Site B |
| **187** | Q | H | |
| **189** | R | N | Antigenic Site B |
| **217** | S | P | |
| **234** | K | N | |
| Tot Diffs | | 12 | |

# NA-N8 Influenza A viruses

*Figure 8: Avian N8 maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. New data acquired since February 2021 is coloured in purple.*

# H9 Influenza A viruses

## H9 Phylogenetic analysis

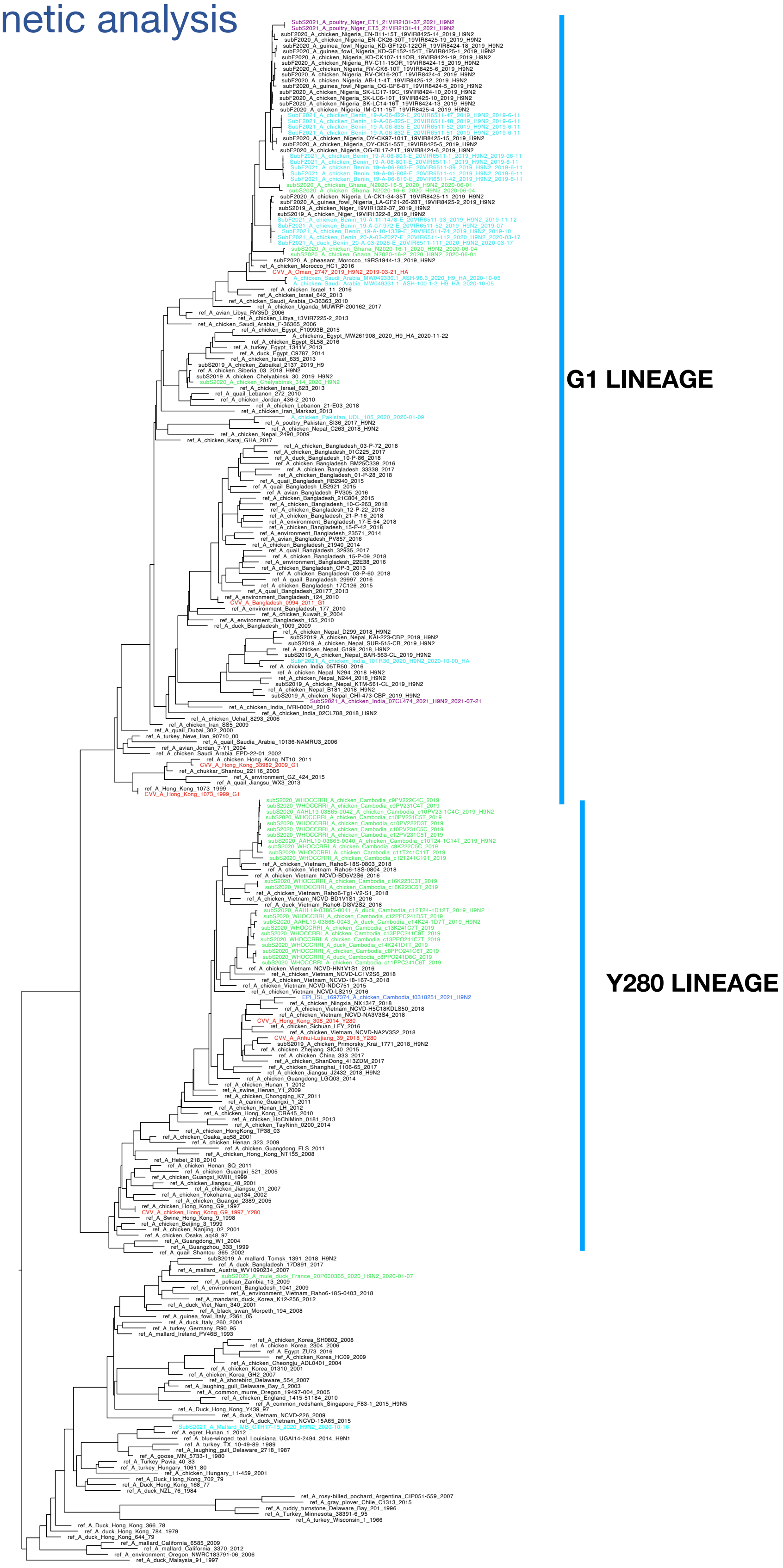


*Figure 11: Avian H9 maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. New data acquired since February 2021 is coloured in purple, data from between September 2020 and February 2021 in light blue and data from February 2021 to September 2021 is coloured in green. The CVV for this clade is shown in red.*

# H9 G1 Comparative amino acid substitutions to clade CVV

*Tables 7: Avian H9 G1 current circulating strains clade sequence comparison to the CVVs shown in red. Tables were generated using the NADC IAV bioinformatic toolkit.*

| site | CVV_A_Bangladesh_0994_2011_G1 | SubS2021_A_chicken_India_07CL474_2021_H9N2_2021-07-2 | Annotation |
|---|---|---|---|
| 22 | T | S | |
| 36 | D | E | |
| 48 | H | N | |
| 56 | N | T | |
| 57 | V | I | |
| 74 | R | K | Published escape mutation |
| 95 | V | I | |
| 109 | S | K | Published escape mutation |
| 148 | N | S | Published escape mutation |
| 150 | L | F | Published escape mutation |
| 153 | V | I | |
| 158 | F | Y | |
| 260 | K | R | |
| 288 | I | V | |
| 319 | S | K | |
| 358 | R | K | |
| 359 | V | A | |
| 363 | K | Q | |
| 365 | V | I | |
| 451 | L | M | |
| 478 | N | D | |
| 483 | Q | K | |
| 521 | M | L | |
| 523 | F | L | |
| Total | | 24 | |

| site | CVV_A_Anhui-Lujiang_39_2018_Y280 | CVV_A_Hong_Kong_308_2014_Y280 | EPI_ISL_1697374_A_chicken_Cambodia_f0318251_2021_H9N | Annotation |
|---|---|---|---|---|
| 51 | I | | V | |
| 57 | V | I | I | |
| 69 | L | | P | |
| 70 | S | L | L | |
| 71 | L | | P | |
| 73 | G | | E | |
| 114 | I | | V | |
| 127 | D | N | | Published escape mutation |
| 131 | T | K | N | Published escape mutation |
| 132 | A | | T | |
| 135 | D | G | G | Published escape mutation |
| 140 | S | | N | |
| 148 | D | N | | Published escape mutation |
| 150 | N | D | | Published escape mutation |
| 153 | T | I | V | |
| 156 | A | V | | |
| 174 | H | | N | |
| 179 | D | T | | Published escape mutation |
| 183 | N | D | | Published escape mutation |
| 221 | N | D | | |
| 242 | I | | V | |
| 248 | H | | Y | |
| 260 | K | | R | |
| 359 | D | | E | |
| 365 | I | | V | |
| 395 | T | | N | |
| 442 | V | | I | |
| 465 | N | D | D | |
| 466 | D | N | | |
| 479 | R | K | | |
| Total | | 15 | 21 | |

| site | CVV_A_Oman_2747_2019_H9N2_2019-03-21_HA | CVV_A_Bangladesh_0994_2011_G1 | SubS2021_A_poultry_Niger_ET1_21VIR2131-37_2021_H9N2 | SubS2021_A_poultry_Niger_ET5_21VIR2131-41_2021_H9N2 | Annotation |
|---|---|---|---|---|---|
| 28 | Q | H | | | |
| 36 | E | D | | | |
| 45 | N | | K | K | |
| 48 | R | H | | | |
| 56 | T | N | | | |
| 57 | I | V | | | |
| 69 | M | L | | | |
| 77 | S | | A | A | |
| 86 | V | | A | A | |
| 104 | L | | F | F | |
| 114 | V | I | | | |
| 116 | L | I | | | |
| 120 | S | T | | | Published escape mutation |
| 150 | G | L | N | N | Published escape mutation |
| 153 | I | V | | | |
| 158 | Y | F | | | |
| 187 | X | R | R | R | |
| 198 | T | N | | | |
| 246 | F | Y | | | |
| 260 | R | K | | | |
| 262 | D | N | N | N | |
| 264 | S | N | | | |
| 283 | M | L | | | |
| 295 | T | N | | | |
| 315 | H | P | | | |
| 317 | R | K | | | |
| 354 | M | I | | | |
| 363 | R | K | K | K | |
| 374 | S | N | N | N | |
| 393 | V | | I | I | |
| 411 | I | V | | | |
| 451 | M | L | | | |
| 483 | T | Q | | | |
| 496 | V | I | | | |
| 500 | A | S | | | |
| 508 | S | T | | | |
| Total | | 31 | 10 | 10 | |

# H9 Antigenic analysis

*Table 8: Antigenic analysis IZSVe reference lab data: Haemagglutination inhibition assay data using reference antigen ferret antisera against H9 lineage strains selected to represent currently circulating viruses. Fold differences between the homologous and antigen titres are highlighted from grey to red.*

Homologous
2 fold
3 fold
4 fold
5 fold
< 5 fold

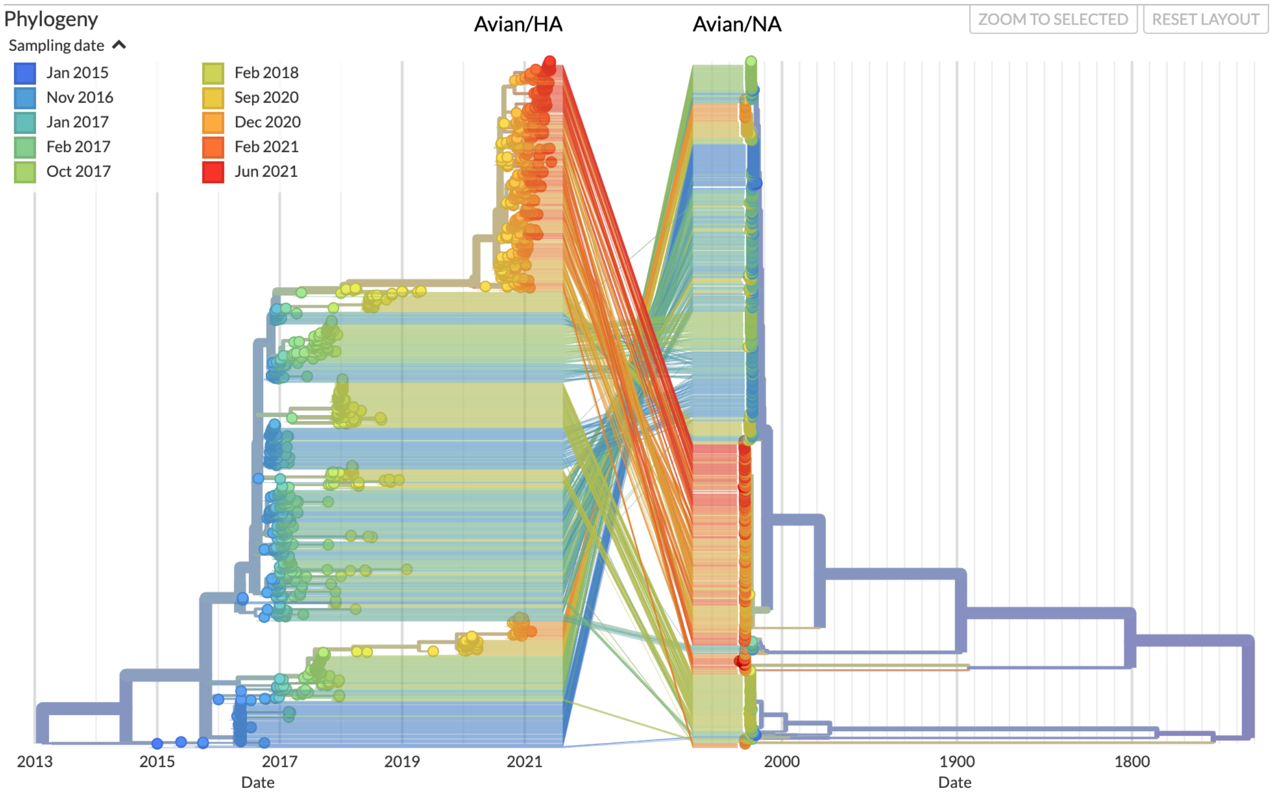
**REFERENCE FERRET ANTISERA**

| | | | | | | A/BANGLADESH/0994/2011 | A/HONG KONG/33982/2009 |
|---|---|---|---|---|---|---|---|
| | | | | | **Lineage** | G1 | G1 |
| | | | | | **Subtype** | H9N2 | H9N2 |
| | | | | | **Ferret ID** | | |
| | **Semester** | | | **Lineage** | **Subtype** | | |
| IZSVe | Sept-21 | | **Reference Antigen** | | | | |
| | | | A/BANGLADESH/0994/2011 | G1 | H9N2 | 320 | |
| | | | A/HONG KONG/33982/2009 | G1 | H9N2 | | 640 |
| | | | **Test antigen** | | | | |
| | | 1 | A/poultry/Niger/ET1_21VIR2131-37/2021 | G1 | H9N2 | 160 | 80 |
| | | 2 | A/avian/Niger/19VIR1322-37/2019 | G1 | H9N2 | 20 | 40 |

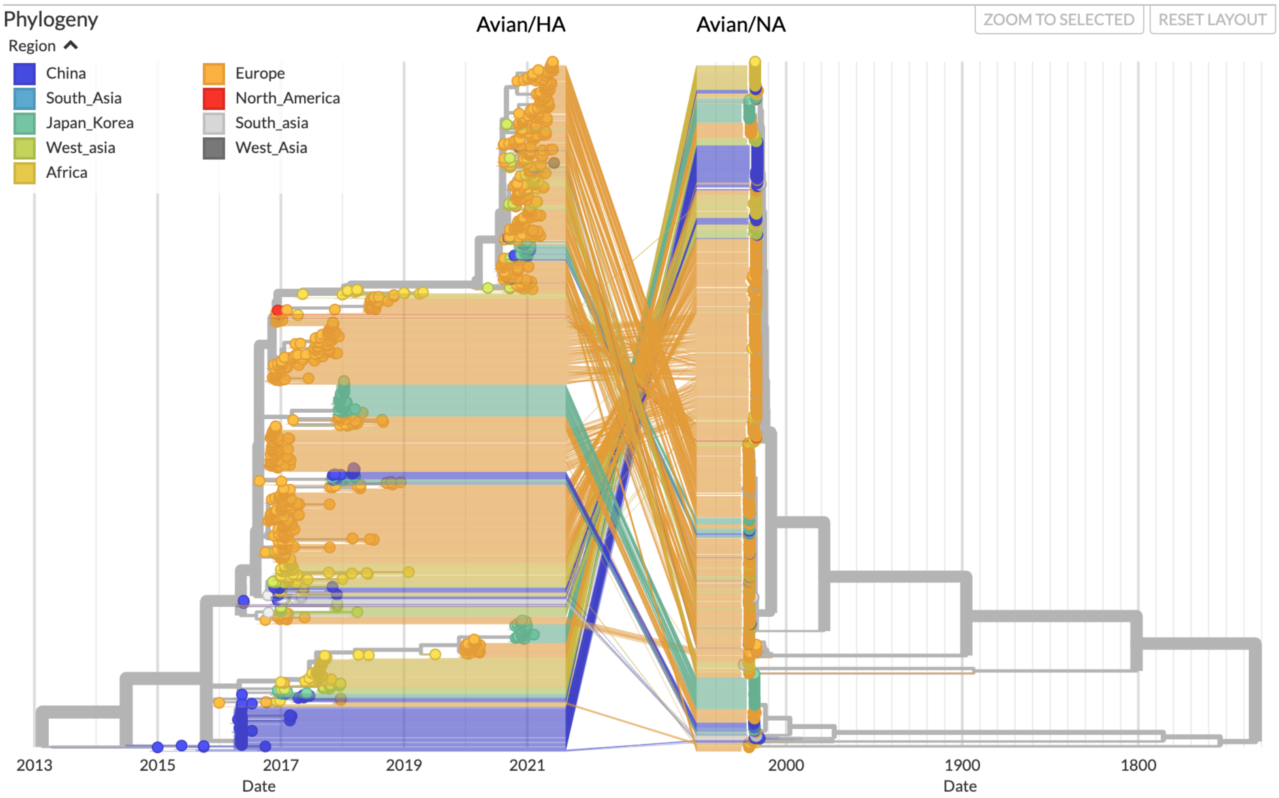
# Annex

## H5 2.3.4.4b HA and NA tangle tree

*A.*



*B.*



*C.*

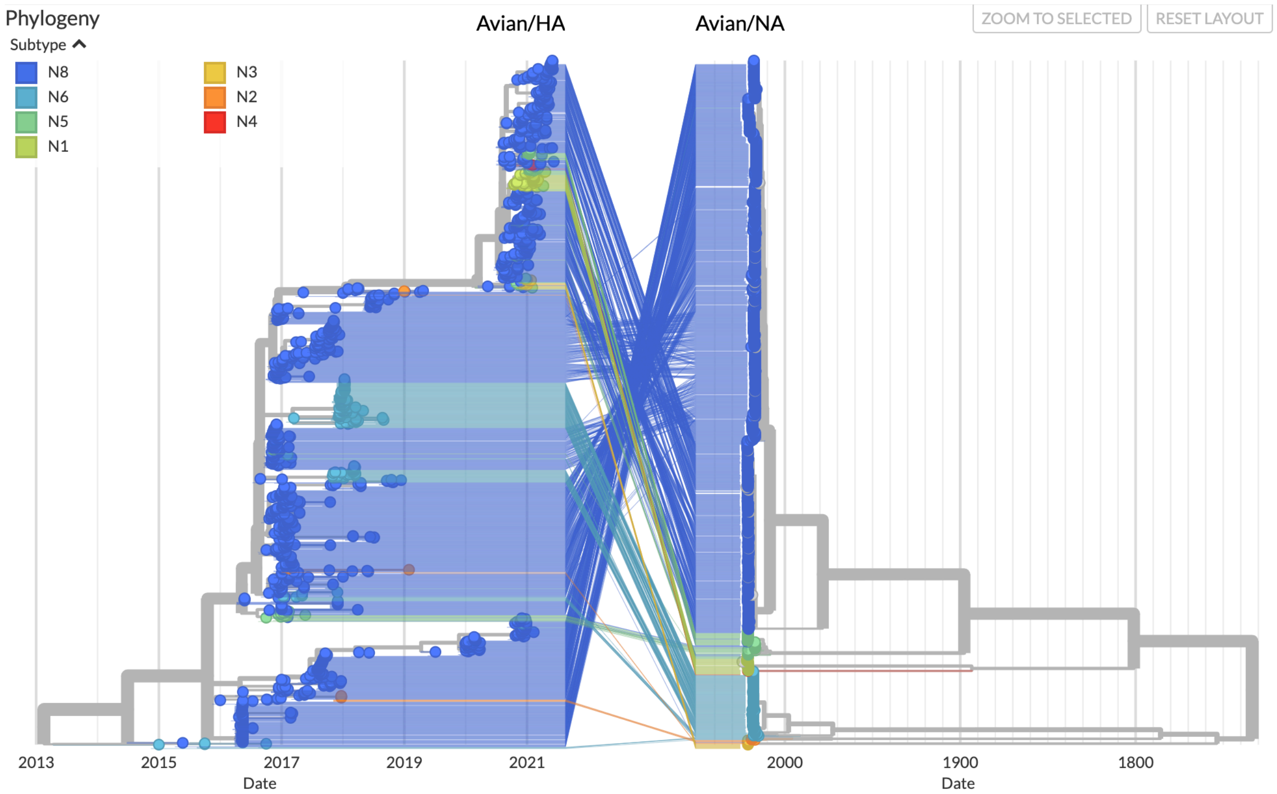


*Figure 10: Analyses conduced in Nextstrain. Avian H5 HA and NA sequences were downloaded from GISAID. FluDB clade classifier and a reference backbone sequence was used to triage and classify by clade. SMOT and SMOF were used to infer clades and keep 2.3.4.4b HA sequences. Phylogenetic analysis were conduced using the NEXTSTRAIN augur pipeline. Matching NA sequences were analysed. OFFLU sequences were subsequently added to both datasets. Results were visualised using AUSPICE. A: Nodes coloured by date; B: Nodes coloured by region; C: Nodes coloured by subtype.*

# H7 Influenza A viruses

## H7 Phylogenetic analysis

*Figure 10: Avian H7 maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU.*