# OFFLU AVIAN INFLUENZA REPORT

September 2020 to February 2021

## SCOPE

In this document we present a summary of H5, H7 and H9 avian influenza A virus events reported from September 2020 to current. (February 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 acknowledge and thank the OIE Reference laboratory and diagnostic laboratory teams at APHA, IZSVe, AAHL, FLI and NVSL for their expertise in data analyses and compiling the report.

Analyses were conducted by subtype. Reference sequences and new data added from the beginning of this reporting period (2020-09-01 until 2021-02-23) 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 consensus sequences were generated using SMOF (https://github.com/incertae-sedis/smoffor) for groups of phylogenetically similar sequences and these 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 anstisera in haemagglutination inhibition (HI) assays.

Avian influenza A virus haemagglutination inhibition (HI) assay antigenic data in this reporting period was generated by APHA-Weybridge and IZSVe using WHO-CC and OFFLU- provided ferret-origin reagents and harmonised protocols.

## Avian influenza A virus vaccination

In many AI endemic countries, H5 and other vaccination are 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 RE-14 is the H5 vaccine representative.

Currently, in China, vaccination of poultry is mandatory in all provinces (including chickens, ducks, geese, quails, pigeons and other rare birds in captivity). From September 2017, a government sponsored campaign using a bivalent H5/H7 vaccine (H5 2.3.4.4 Re-8 based on A/chicken/Guizhou/4/13(H5N1); H7N9 Re-1) has been implemented. 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. Current understanding is that RE-2 is the H7 component. 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 were due to the Goose/Guangdong (GsGD) H5 clades of HPAI. Relatively few countries reported H5 LPAI in domestic birds: H5N2 in the UK and H5N3 in South Korea both in October and H5N3 in January in France. During this reporting period there were no PCR-confirmed reports of HPAI H7 viruses and the only reports of H9 viruses were in China and India. While not officially reportable H9 viruses can significantly impact poultry.

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 were caused by H5 HPAI 2.3.4.4b. Since early 2020 small 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 and Asia which appear genetically closely related. Other distinct H5N8 2.3.4.4b events in Japan and South Korea occurred from October onwards and these Asian outbreaks appear to be closely related to outbreaks seen in European Member States in early 2020.

Throughout this reporting period H5N5 outbreaks occurred sporadically in Taiwan, Russia and subsequently in Europe from October onwards. H5N1 outbreaks have been seen periodically in Viet Nam, Laos and Germany and recently in India and Senegal. Sporadic cases of H5N6 have been seen in the first half of the year in China and continuously in Viet Nam. Phylogenetic analysis shows that they are of the clade 2.3.4.4h and are closely related to previously circulating strains in that region. H5N1 strains seen in January 2021 in Cambodia appear to be genetically similar to circulating strains of the 2.3.2.1c clade seen since throughout 2019.

# Regional geographic summary and context

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

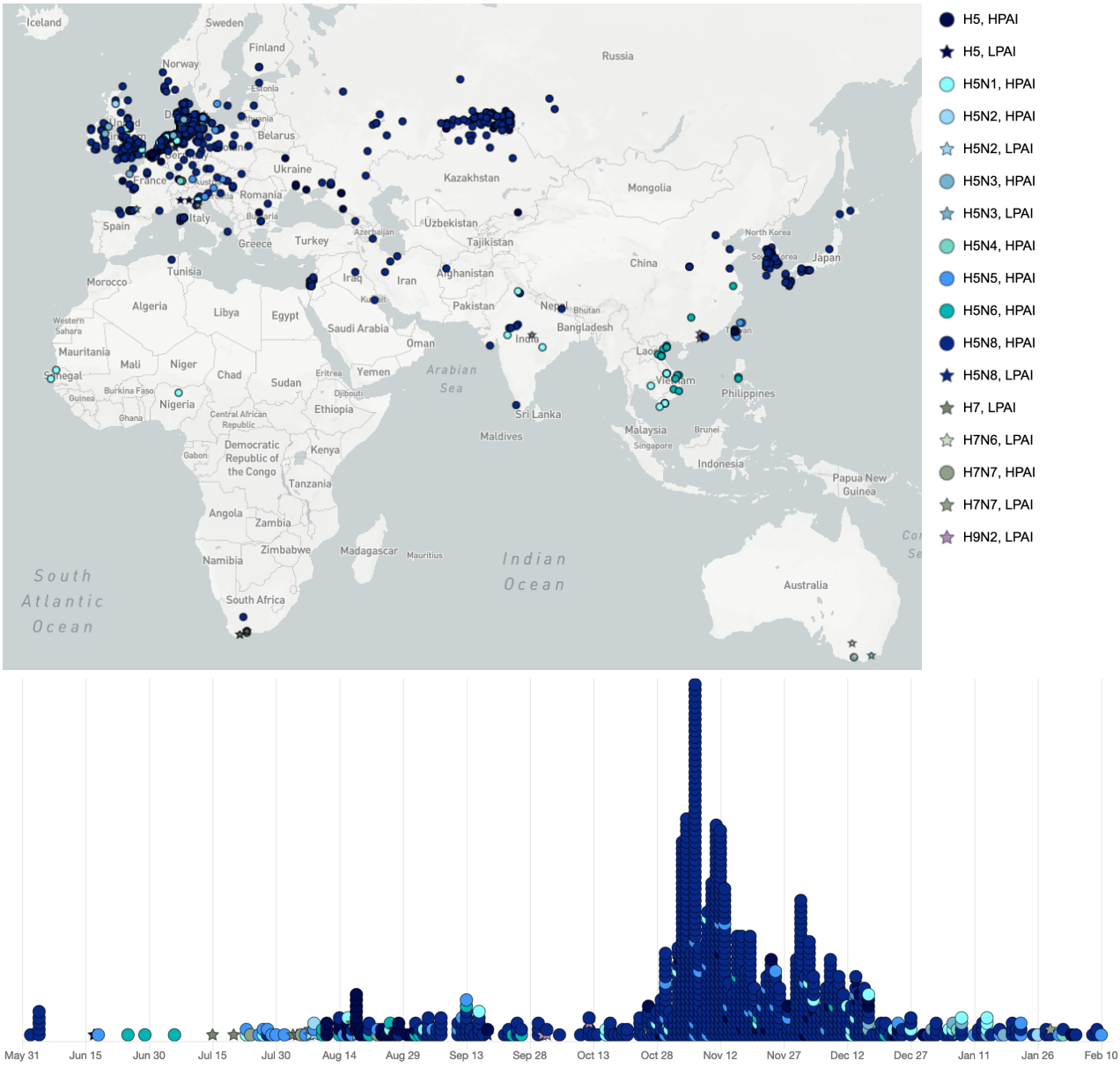



*Figure 1: MicroReact map of H5, H7 and H9 events for the timeperiod – 1 June 2020 to 22 February 2021 (N=961). Points are coloured by subtype. Shape denotes low pathogenic (star) or highly pathogenic (circle) avian influenza A viruses. Collection data is represented along the timeline. Geographic centroid coordinates were used for reports where no precise geolocation was available.*

**HPAI ACTIVITY WITHIN REPORTING PERIOD (SEPTEMBER 2020 TO FEBRUARY 2021)**

**EUROPE:**

H5

- Russia in September
- Netherlands in October
- Italy and France in November
- Ukraine, Germany and Belgium in December

H5N1

- Netherlands in October
- Italy in November
- The UK and Germany in December

H5N2

- Scotland in January

H5N3

- Germany in December
- UK and Ireland and France in January

H5N4

- Germany in January
- Switzerland in February

H5N5

- Russia in September
- Denmark and Germany in October
- Italy, UK and Sweden in November
- France and Slovenia in December

H5N8

- Russia from September onwards
- Georgia from September onwards
- UK and Ireland, the Netherlands and Germany from October onwards
- France, Denmark, Norway, Sweden, Belgium, Spain, Poland, Slovenia, Croatia in November.
- Italy and Lithuania in December onwards
- Hungary, Czech Republic, Finland, Romania and Slovakia in January
- Austria and Ukraine, Bulgaria and Latvia and Estonia in February

**ASIA:**

H5N1

- Lao and Viet Nam from September
- Cambodia and India in January

H5N8

- Kazakhstan and the Republic of Georgia in September
- Israel, Republic of Korea and Japan in October
- Kuwait in December
- India, Hong Kong, Iraq, China and Afghanistan in January

H5N5

- Taiwan in September

H5N6

- Viet Nam in September
- China in November

Philippines: Although just outside the reporting period, recently shared sequence data show that the H5N6 2.3.4.4e clade has continued to be maintained in Philippines poultry systems since first incursion back in July 2017 (following the initial emergence of A/Hyogo/1/2016(H5N6)-like viruses in Japan and ROK in 2016).

**AFRICA:**
- Senegal and Nigeria reported H5N1 in January
- Algeria reported H5N8 in January

**AMERICAS (US)/ OCEANIA (AUSTRALIA):**
No reports

**LPAI ACTIVITY:**
**EUROPE:**
H5
- Italy in November
- Belgium and the Netherlands in December

H5N2
- UK in October

H5N3
- France in January

H5N8
- Germany in September

H7N7
- Italy in January

**ASIA:**
H5
- Korea in November

H5N3
- Korea in October

H9N2
- China and India in October

**AFRICA/AMERICAS (US)/OCEANIA (AUSTRALIA)**
No reports

Data for 43 H5 and 13 H9 sequences were contributed to OFFLU by animal health laboratories in countries representing Europe, Asia, Africa, Oceania, and the Americas to which 150 H5 and 3 H9 sequences from Genbank and GISAID were added.

# Activity Table (H5) viruses

*Table 1: 01/09/2020 to 12/02/2021 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 | unknown (H5N8) |
| Algeria | Poultry | unknown (H5N8) |
| Austria | Wild Birds | unknown (H5N8) |
| Bangladesh | Wild bird<br>Poultry | 2.3.4.4h (H5N6)<br>2.3.2.1a (H5N1) |
| Belgium | Wild bird<br>Poultry | unknown (H5) 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N8) unknown (H5N5) |
| Bulgaria | Poultry | unknown (H5N8) |
| Cambodia | Poultry | 2.3.2.1c (H5N1) |
| China | Human (5)*<br>Poultry/environmental<br>Wild birds | 2.3.4.4h, unknown (H5N6)<br>2.3.4.4h (H5N6), 2.3.2.1f (H5N1)<br>unknown (H5N6), 2.3.4.4h (H5N8) |
| Chinese Taipei | Poultry<br>Wild Birds | unknown (H5N2/5)<br>unknown (H5) |
| Croatia | Poultry | 2.3.4.4b (H5N8) |
| The Czech Republic | Poultry<br>Wild Birds | 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N8) |
| Denmark | Wild birds<br>Poultry | 2.3.4.4b (H5N5/N8)<br>2.3.4.4b (H5N8) |
| Estonia | Wild Birds | unknown (H5N8) |
| Finland | Wild Birds | unknown (H5N8) |
| France | Wild birds<br>Poultry | 2.3.4.4b (H5N8) unknown (H5N3)<br>2.3.4.4b (H5N5/8) |
| Germany | Wild bird<br>Poultry | unknown (H5N/x/1/3/4), 2.3.4.4b (H5N5/8)<br>2.3.4.4b (H5N5/8) |
| Hong Kong | Wild birds | unknown (H5N8) |
| Hungary | Wild birds<br>Poultry | unknown (H5N8)<br>unknown (H5N8) |
| India | Poultry<br>Wild Birds | unknown (H5N1/8)<br>2.3.4.4b(H5N8) 2.3.4.4b(H5N1) |
| The Islamic Republic of Iran | Wild bird<br>Poultry | unknown (H5N8)<br>unknown (H5N8) |
| Republic of Iraq | Poultry | 2.3.4.4b (H5N8) |
| Ireland | Wild birds<br>Poultry | unknown (H5N3), 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N8) |
| Israel | Wild birds<br>Poultry | unknown (H5N8)<br>unknown (H5N8) |
| Italy | Wild birds<br>Poultry | 2.3.4.4b (H5N1/5/8)<br>unknown (H5N8) |
| Japan | Wild birds<br>Poultry | 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N8) |
| Kazakhstan | Wild birds<br>Poultry | 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N8) |
| Kuwait | Poultry | 2.3.4.4b (H5N8) |
| Lao People's Democratic Republic | Human (1)<br>Poultry | 2.3.2.1c (H5N1)<br>unknown (H5N1) |
| Latvia | Wild Birds | unknown (H5N8) |
| Lithuania | Wild birds<br>Poultry | unknown (H5N8)<br>unknown (H5N8) |
| Nepal | Poultry | unknown (H5N8) |
| Netherlands | Wild birds<br>Poultry | 2.3.4.4b (H5N1/5/8)<br>2.3.4.4b (H5N1/8) |
| Nigeria | Poultry | unknown (H5N1) |
| Norway | Wild birds<br>Poultry | 2.3.4.4b (H5N8)<br>unknown (H5N8) |
| Poland | Poultry<br>Wild birds | 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N5/8) |
| Republic of Georgia | Wild birds | unknown (H5N8) |
| Republic of Korea | Wild birds<br>Poultry | 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N8) |
| Romania | Poultry<br>Wild birds | unknown (H5N8)<br>unknown (H5N8) |
| Russian Federation | Wild bird<br>Poultry<br>Human | 2.3.4.4b (H5N8)<br>2.3.4.4b (H5N5/8)<br>2.3.4.4b (H5N8) |
| Saudi Arabia | Poultry | unknown (H5N8) |
| Senegal | Poultry<br>Wild birds | unknown (H5N1)<br>unknown (H5N1) |
| Slovakia | Poultry<br>Wild birds | unknown (H5N5/8)<br>unknown (H5N8) |
| Slovenia | Wild birds | unknown (H5N5/8) |
| Spain | Wild birds | unknown (H5N8) |
| Switzerland | Wild Birds | unknown (H5N4) |
| Sweden | Wild birds<br>Poultry | 2.3.4.4b (H5N5/8)<br>2.3.4.4b (H5N5/8) |
| United Kingdom of Great Britain and Northern Ireland | Wild birds<br>Poultry | 2.3.4.4b (H5N1/2/3/5/8)<br>2.3.4.4b (H5N1/8) |
| Ukraine | Poultry | unknown (H5/N8) |
| Viet Nam | Poultry | unknown (H5N1) 2.3.4.4h (H5N6) |

# H5 phylogenetic tree overview:

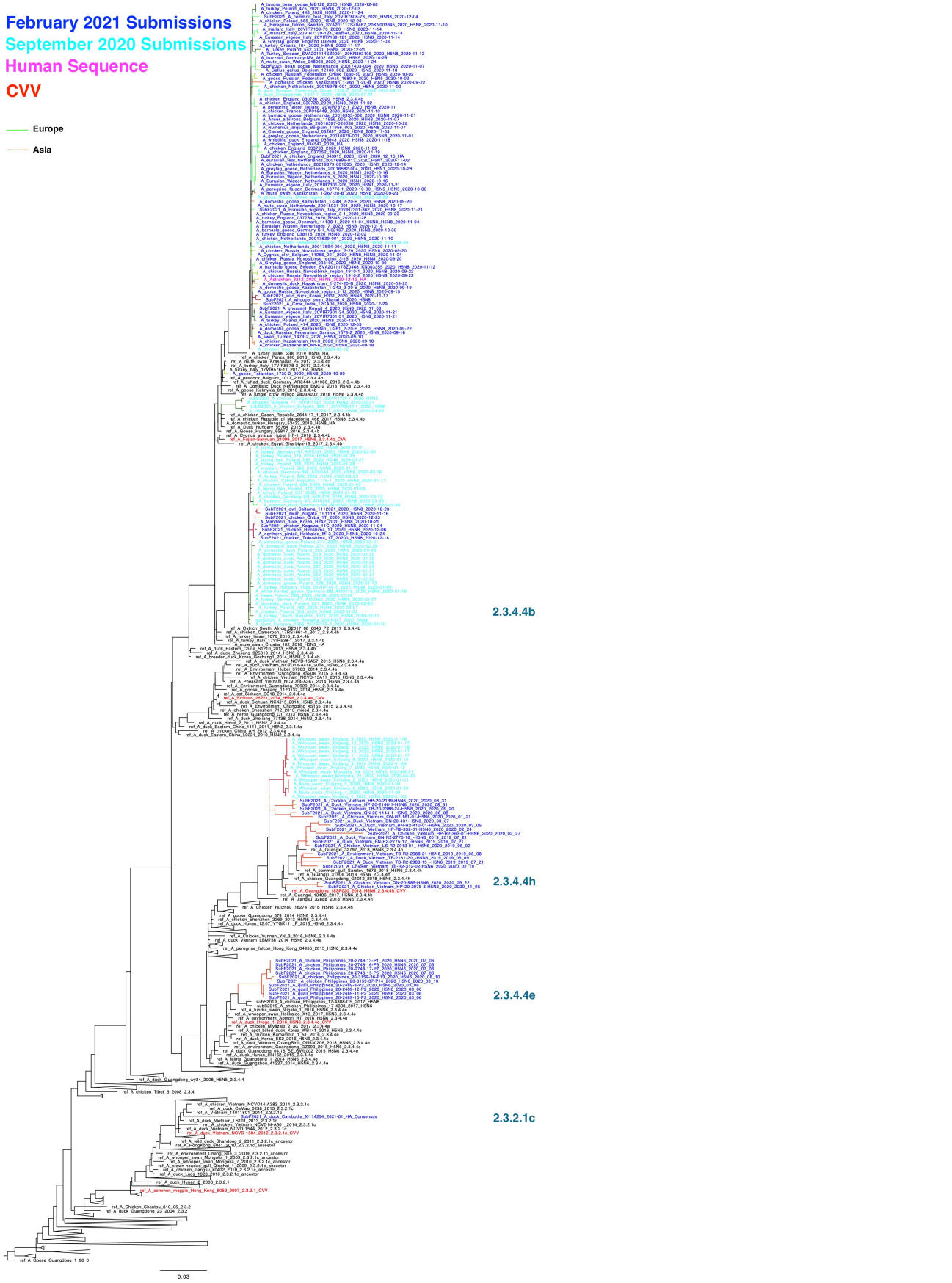



*Figure 2: Avian H5 global summary maximum likelihood phylogenetic tree annotated by clades. Analyses were conducted with reference sequences and data downloaded from GISAID or shared by the OFFLU network. Branches are coloured by geographic location (Europe: green and Asia: orange). New data acquired between September 2020 and February 2021 is coloured in dark blue and data from between February and September 2020 coloured in light blue. Candidate Vaccine strains (CVV) are shown in red and human cases in pink.*

## H5 2.3.4.4b Phylogenetic tree coloured by geographic location

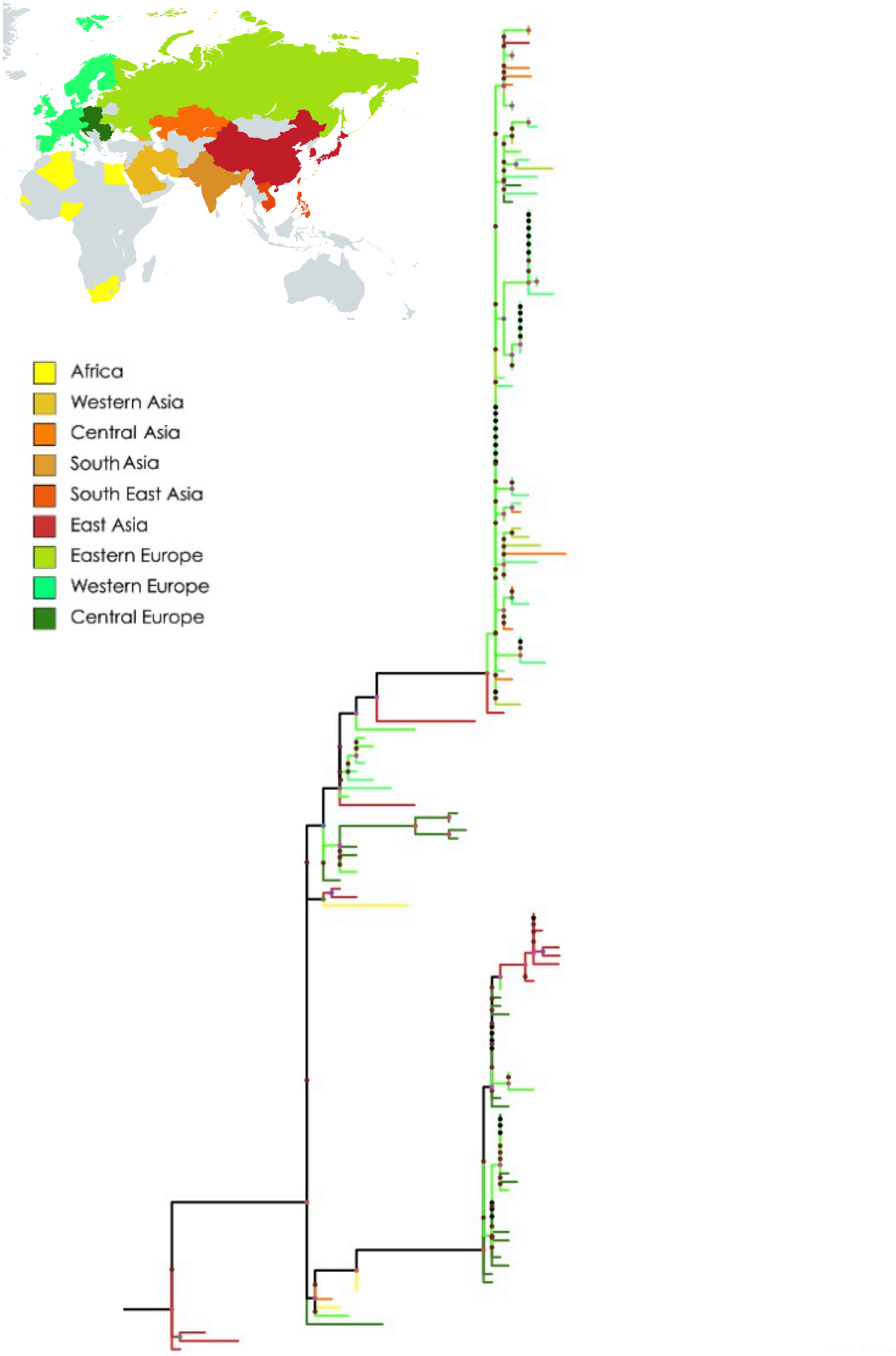



*Figure 3: H5-2.3.4.4b phylogenetic tree with labels removed coloured by geography as according to EMIS subregion nomenclature*

# H5 2.3.4.4b Phylogenetic analysis

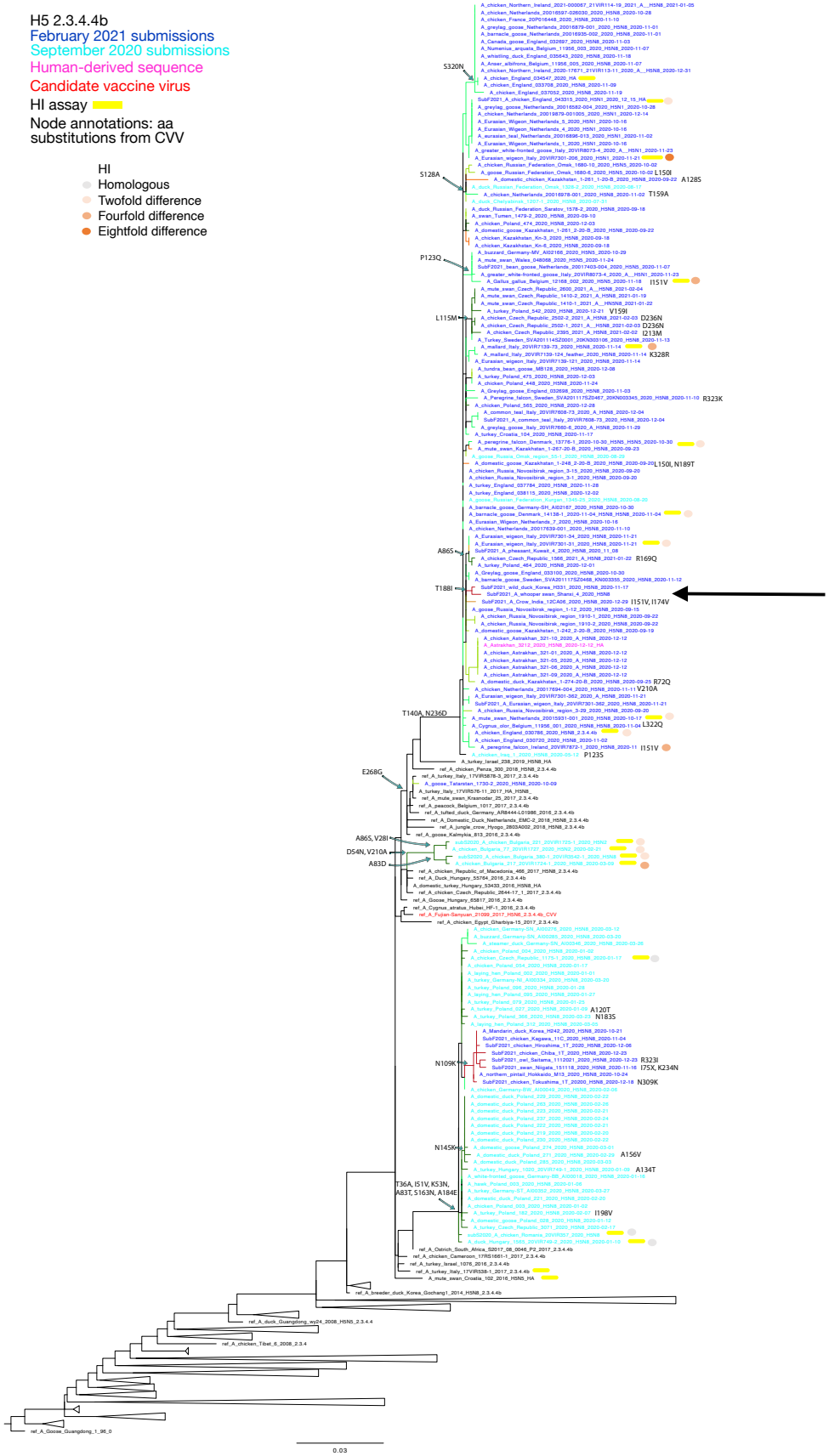
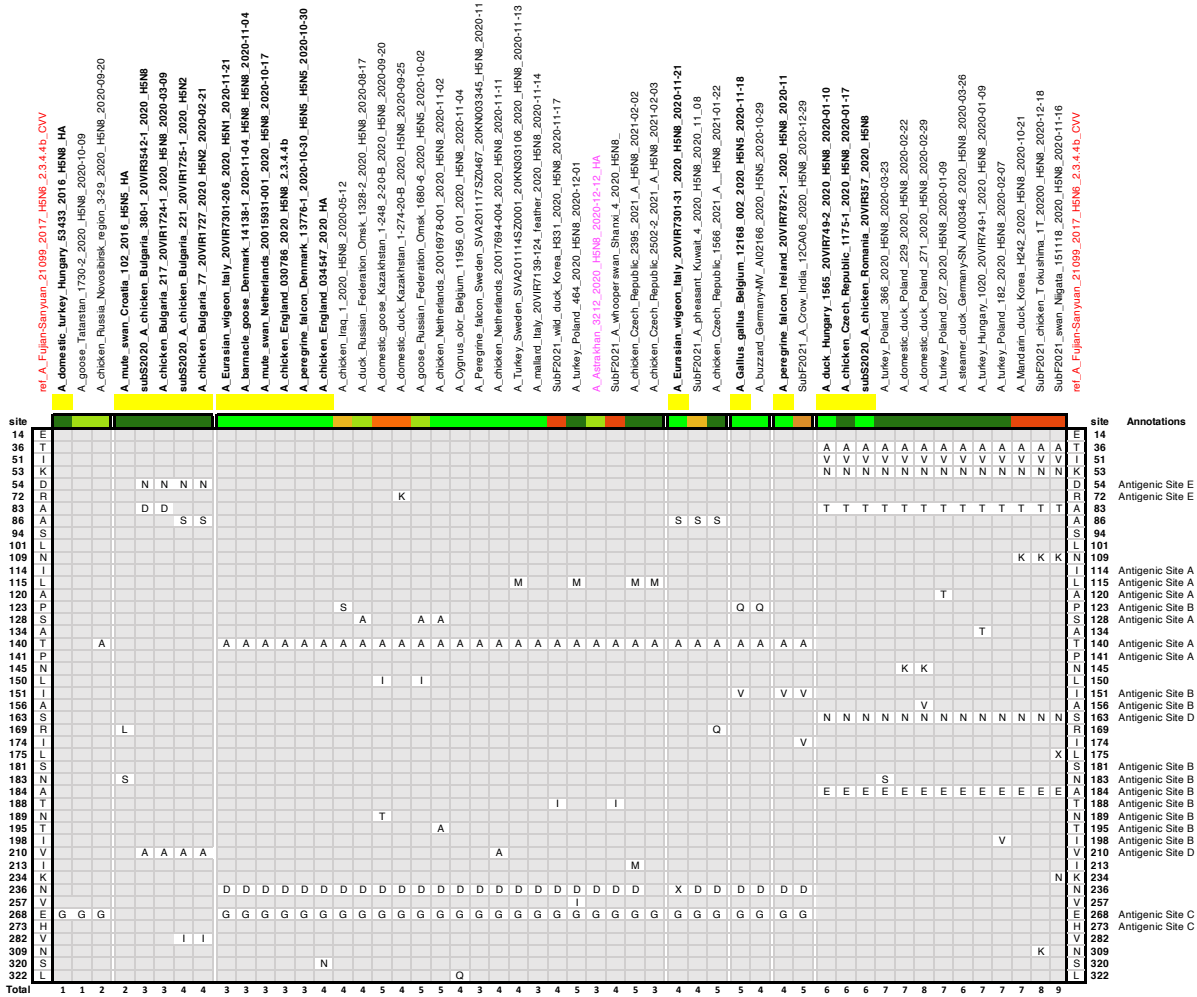



*Figure 4: Avian H5 clade 2.3.4.4b phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. New data acquired between September 2020 and February 2021 is coloured in dark blue and data between February and September 2020 coloured in light blue, the human isolate is pink. The CVV for this clade A/Fujian-Sanyuan/21099/2017 is shown in red and amino acid differences relative to the CVV are annotated on the tree at the relevant nodes. Viral strains selected for HI testing are marked by a yellow bar. Branches are coloured by geographic location.*

# H5 2.3.4.4b Comparative amino acid substitutions to clade CVV

*Table 2: Avian H5 2.3.4.4b clade sequence comparison to the CVV A/Fujian-Sanyuan/21099/2017 which is shown in red. Human strain is shown in pink. HI strains are represented in bold and a yellow box. Tables were generated using the NADC IAV bioinformatic toolkit. Antigenic positions were annotated based on H5 numbering. Coloured bars correspond to geographical region.*



# H5 Antigenic analysis

*Table 3: 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. Differences from CVV strains * A/GYRFALCON/ WASHINGTON/41088-6/2014_CVV or **A/Fujian-Sanyuan/21099/2017_CVV ***A/DUCK/HYOGO/1/2016 are annotated on the table. Antigens whereby strains weren't available for analysis have been allocated a surrogate and any amino acid differences from the surrogate are noted on the right hand column.*

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

| | | REFERENCE FERRET ANTISERA | Clade | Ferret ID Subtype | A/ANHUI/1/2005_CVV | A/SICHUAN/26221/2014_CVV | A/DUCK/HYOGO/1/2016_CVV | A/GYRFALCON/WASHINGTON/41088-6/2014_CV | A/FUJIAN-SANYUAN/21099/2017_CVV** | A/TURKEY/ITALY/17VIR576-11/2017 | HI strain AA diff from CVV * or ** | AA diff from surrogate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Clade | | | 2.3.4 | 2.3.4.4a | 2.3.4.4e | 2.3.3.4c | 2.3.4.4b | 2.3.4.4b | | |
| | | Subtype | | | H5N1 | H5N6 IDCDC-RG42A | H5N6 SAN 18-02537 | H5N8 | H5N6 CNIC-21099 | H5N8 | | |
| Semester | | | | | | | | | | | | |
| | | Reference Antigen | | | | | | | | | | |
| | | A/ANHUI/1/2005 | 2.3.4 | H5N1 | 640 | <10 | 20 | <10 | | <10 | | |
| | | A/SICHUAN/26221/2014 | 2.3.4.4a | H5N6 | <10 | 160 | 320 | 40 | | 20 | | |
| | | A/DUCK/HYOGO/1/2016 | 2.3.4.4e | H5N6 | <10 | 80 | 640 | 320 | | 80 | | |
| | | A/DUCK/ENGLAND/36254/2014* | 2.3.4.4c | H5N8 | <10 | 160 | 20 | 1280 | | 1280 | *R119K, S181P, A185E, S223R, M269V, H273Y | |
| | Sept20-Feb21 | A/TURKEY/ITALY/17VIR576-11/2017 | 2.3.4.4b | H5N8 | <10 | 20 | <10 | 320 | | 320 | **E268G | |
| | | Test antigen | | | | | | | | | | |
| APHA | 1 | A/TURKEY/HUNGARY/53433/2016 | 2.3.4.4b | H5N8 | <10 | 40 | 20 | 640 | | 640 | **E268G | A/goose/Tatarstan/1730-2/2020_H5N8_2020-10-09 A_chicken_Russia_Novosibirsk_region_3-29_2020_H5N8_2020-09-20(T140A) |
| | 2 | A/MUTE SWAN/CROATIA/102/2016 | 2.3.4.4b | H5N5 | <10 | 80 | 20 | 320 | | 160 | **R169L, N183S | |
| | 3 | A/CHICKEN/ENGLAND/030786/2020 | 2.3.4.4b | H5N8 | <10 | 40 | 10 | 160 | | 160 | **T140A, N236D, E268G | |
| | 4 | A/CHICKEN/ENGLAND/034547/2020 | 2.3.4.4b | H5N8 | <10 | 40 | 10 | 320 | | 640 | **T140A, N236D, E268G | A_chicken_Iraq_1_2020_H5N8_2020-05-12(P123S) A_duck_Russian_Federation_Omsk_1328-2_2020_H5N8_2020-08-17(S128A) A_domestic_goose_Kazakhstan_1-248_J-20-8_2020_H5N8_2020-09-20(L150I,N189T) A_domestic_duck_Kazakhstan_1-274-20-8_2020_H5N8_2020-09-25(R72K) A_goose_Russian_Federation_Omsk_1680-6_2020_H5N5_2020-10-02(S128I,L150I) SubF2021_wild_duck_Korea_H331_2020_H5N8_2020-11-17(T188I) A_Turkey_Sweden_SVA20111452001_20KN303106_2020_H5N8_2020-11-13(L115M) A_chicken_Netherlands_20017694-004_2020_H5N8_2020-11-11(V210A) A_Peregrine_falcon_Sweden_SVA20111752047_20KN03345_H5N8_2020-11-10(R323K) A_Cygnus_olor_Belgium_11956_001_2020_H5N8_2020-11-04(L322Q) A_chicken_Netherlands_20016978-001_2020_H5N8_2020-11-02(S128A,I198A) A_turkey_Poland_664_2020_H5N8_2020-12-01(I115M,V257I) A_Astrakhan_3212_2020_H5N8_2020-12-12_HA SubF2021_A_whooper swan_Shanxi_4_2020_H5N8_(T188I) |
| | | Reference Antigen | | | | | | | | | | |
| | | A/SICHUAN/26221/2014 | 2.3.4.4a | H5N6 | <10 | 160 | 320 | 40 | 80 | 40 | | |
| | Sept20-Feb21 | A/FUJIAN-SANYUAN/21099/2017XPR8 | 2.3.4.4b | H5N6 | <10 | 40 | 20 | 40 | 80 | 40 | ** | |
| | | Test antigen | | | | | | | | | | |
| | 5 | A/CHICKEN/ENGLAND/030786/2020 | 2.3.4.4b | H5N8 | <10 | 10 | <10 | 80 | 20 | 80 | **T140A, N236D, E268G | |
| | 6 | A/CHICKEN/ENGLAND/43315/2020 | 2.3.4.4b | H5N1 | 80 | 10 | <10 | 20 | 20 | 20 | **T140A, N236D, E268G | |
| | | Reference antigen | | | | | | | | | | |
| | | A/SICHUAN/26221/2014 | 2.3.4.4a | H5N6 | | 320 | | | | | | |
| | | A/FUJIAN-SANYUAN/21099/2017XPR8 | 2.3.4.4b | H5N8 | | | | | 160 | | ** | |
| | | A/TURKEY/ITALY/17VIR576-11/2017 | 2.3.4.4b | H5N8 | | | | | | 640 | **E268G | |
| | | Test antigen | | | | | | | | | | |
| | 7 | A/CHICKEN/BULGARIA/380-1_20VIR3542-1/2020 | 2.3.4.4b | H5N8 | | 40 | | | 40 | 160 | **D54N, A83D, V210A | |
| | Feb20-Sept20 8 | A/CHICKEN/BULGARIA/217_20VIR1724-1/2020 | 2.3.4.4b | H5N8 | | 20 | | | 20 | 80 | **D54N, A83D, V210A | |
| | 9 | A/CHICKEN/BULGARIA/201_20VIR1723-1/2020 | 2.3.4.4b | H5N2 | | 40 | | | 40 | 80 | | |
| | 10 | A/CHICKEN/BULGARIA/221_20VIR1725-1/2020 | 2.3.4.4b | H5N2 | | 40 | | | 40 | 80 | **D54N, A86S, V210A, V282I | |
| | 11 | A/CHICKEN/BULGARIA/77_20VIR1727/2020 | 2.3.4.4b | H5N2 | | 20 | | | 40 | 80 | **D54N, A86S, V210A, V282I | |
| | 12 | A/CHICKEN/ROMANIA/20VIR357/2020 | 2.3.4.4b | H5N8 | | 320 | | | 320 | 640 | **T36A, I51V, K53N, A83T, S163N, A184E | A_turkey_Poland_366_2020_H5N8_2020-03-23(N183S) A_domestic_duck_Poland_229_2020_H5N8_2020-02-22(N145K) A_domestic_duck_Poland_271_2020_H5N8_2020-02-29(N145K,A156V) A_turkey_Poland_027_2020_H5N8_2020-01-09(A120T) A_steamer_duck_Germany-SN_AI00346_2020_H5N8_2020-03-26(R323I) A_turkey_Hungary_1020_20VIR749-1_2020_H5N8_2020-01-09(A132T) A_turkey_Poland_182_2020_H5N8_2020-02-07(I198V) A_Mandarin_duck_Korea_H242_2020_H5N8_2020-10-21(N109K) SubF2021_chicken_Tokushima_17_20200_H5N8_2020-12-18(N109K,N309K) SubF2021_swan_Niigata_151118_2020_H5N8_2020-11-16(N109K,L175K,K234N) |
| | 13 | A/DUCK/HUNGARY/1565_20VIR749-2/2020 | 2.3.4.4b | H5N8 | | 160 | | | 160 | 320 | **T36A, I51V, K53N, A83T, S163N, A184E | |
| | 14 | A/CHICKEN/CZECH_REPUBLIC/1175-1_20VIR465-1/2020 | 2.3.4.4b | H5N8 | | 160 | | | 160 | 640 | **T36A, I51V, K53N, A83T, S163N, A184E | |
| IZSVe | | Reference Antigen | | | | | | | | | | |
| | | A/SICHUAN/26221/2014 | 2.3.4.4a | H5N6 | | 320 | | | | | | |
| | | A/FUJIAN-SANYUAN/21099/2017XPR8 | 2.3.4.4b | H5N8 | | | | | 160 | | ** | |
| | | A/TURKEY/ITALY/17VIR576-11/2017 | 2.3.4.4b | H5N8 | | | | | | 1280 | **E268G | |
| | | Test antigen | | | | | | | | | | |
| | 15 | A/PEREGRINE_FALCON/DENMARK/13776-1_I20VIR7282-1/2020 | 2.3.4.4b | H5N5 | | 40 | | | 40 | 320 | **T140A, N236D, E268G | |
| | Sept20-Feb21 16 | A/MUTE_SWAN/NETHERLANDS/20015931-001/20 | 2.3.4.4b | H5N8 | | 40 | | | 40 | 320 | **T140A, N236D, E268G | |
| | 17 | A/BARNACLE_GOOSE/DENMARK/14138-1_I20VIR7282-2I/2020 | 2.3.4.4b | H5N8 | | 40 | | | 40 | 160 | **T140A, N236D, E268G | |
| | 18 | A/MALLARD/ITALY/20VIR7139-73/2020 | 2.3.4.4b | H5N8 | | 40 | | | 20 | 160 | **T140A, N236D, E268G | |
| | 19 | A/EURASIAN_WIGEON/ITALY/20VIR7301-206/2020 | 2.3.4.4b | H5N1 | | 20 | | | 10 | 40 | **T140A, N236D, E268G | |
| | 20 | A/EURASIAN_WIGEON/ITALY/20VIR7301-31/2020 | 2.3.4.4b | H5N8 | | 40 | | | 40 | 160 | **A86S, T140A, N236X, E268G | |
| | 21 | A/PEREGRINE_FALCON/IRELAND/20VIR7872-1/2020 | 2.3.4.4b | H5N8 | | 40 | | | 20 | 160 | **T140A, I151V, N236D, E268G | |
| | 22 | A/GALLUS_GALLUS/BELGIUM/12168_I20VIR7627-3I/2020 | 2.3.4.4b | H5N5 | | 20 | | | 20 | 80 | **P123Q, T140A, I151V, N236D, E268G | |
| | | Reference Antigen | | | | | | | | | | |
| | | A/DUCK/HYOGO/1/2016 | 2.3.4.4e | H5N6 | | | 80 | | | | *** | |
| AAHL | Sept20-Feb21 | Test antigen | | | | | | | | | | |
| | 23 | A/quail/Philippines/20-2489-8-P2/2020 | 2.3.4.4e | H5N6 | | | 40 | | | | ***T158P | |
| | 24 | A/chicken/Philippines/20-2748-15-P5/2020 | 2.3.4.4e | H5N6 | | | <10 | | | | ***T158L, N188D, N182S, I255V | |

# H5 2.3.4.4b Amino Acid Phylogenetic Tree

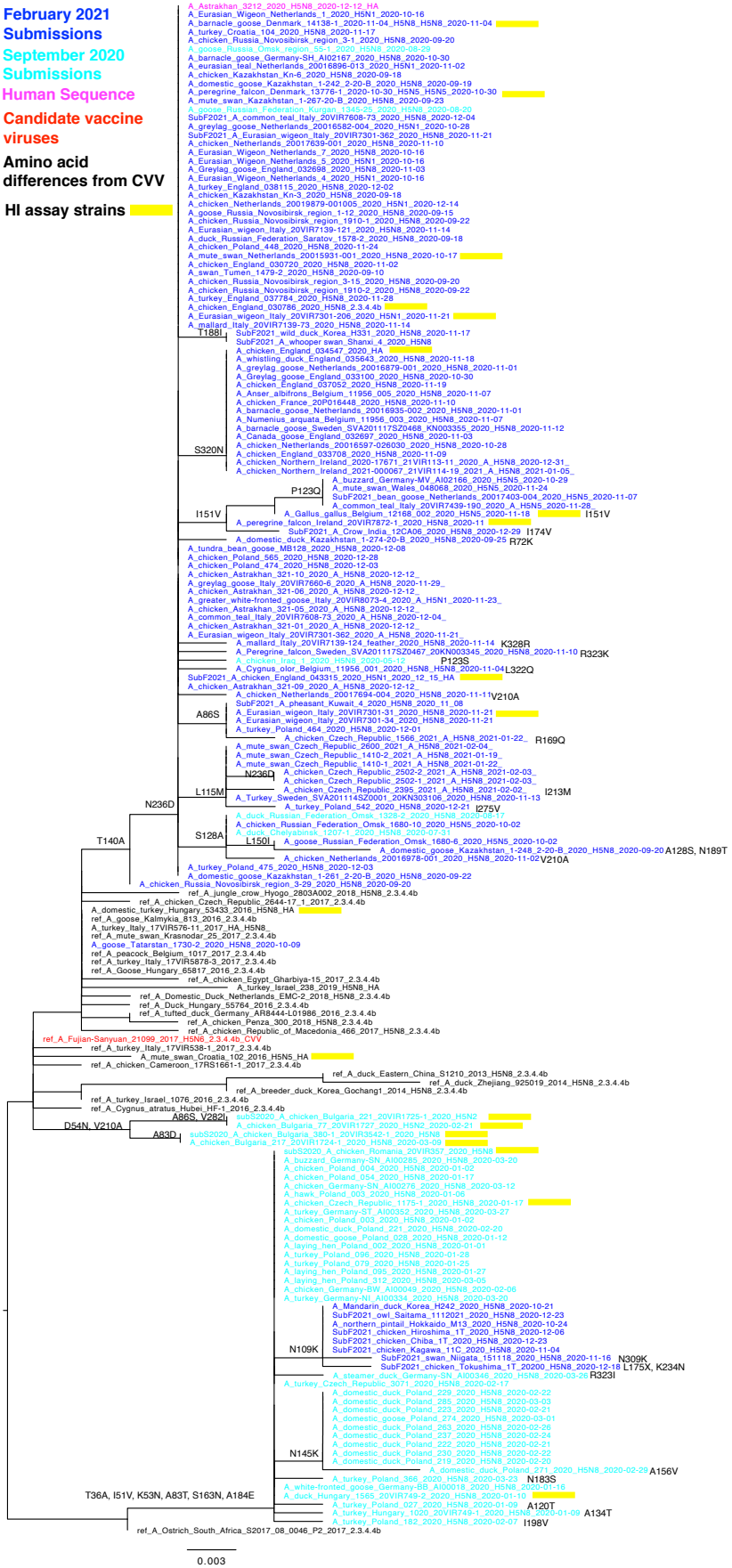



*Figure 5: Phylogenetic tree inferred from HA1 amino acid sequences has been included to complement the HI table and amino acid difference table. HI strains are shown in yellow. Amino acid annotations are added at the relevant node. The tree is midpoint rooted.*

# H5 Genome mutation analysis

## H5 2.3.4.4b mutational analysis

Full genome sequence data from A/Astrakhan/3212/2020(H5N8-2020) was compared with the CDC (Atlanta) H5N1 genetic changes inventory and Suttie et al. 2019 to identify genetic mutations that determine viral phenotypic characteristics of importance that might cause increase virulence, adaptation to mammalian species or alter susceptibility to existing antivirals.
16 unique amino acid differences were found. Phenotypic consequences where available are shown in Table 4. Further in-silico analyses indicate likely oseltamivir sensitivity and there are no changes present indicating baloxavir resistance as summarised in Table 5.

*Table 4: Analysis of genetic changes present in A/Astrakhan/3212/2020/H5N8/2020-12-12 that may increase virulence, signal adaptation to mammalian species or alter susceptibility to existing antivirals.*

| Protein | Amino acid position/motif | Phenotypic consequences | Reference |
|---|---|---|---|
| PB2 | N556S | Mutation carried in second- and third-wave viruses of study. These variants were found to replicate more rapidly in human airway epithelial (HAE) cells | Elderfield et al. 2014 |
| | E677G | Mutation was studied however no effect to virulence was observed | Herfst et al. 2009 |
| PB1 | S216N | Genomic signature of pandemic H1N1 | Chen and Shih 2009 |
| | E614D | No result | |
| PA | S115D | No result | |
| | R319G | No result | |
| | N436D | No result | |
| | S599N | No result | |
| | M620L | No result | |
| | D681N | No result | |
| HA | No mutations found | | |
| NP | G16S | No result | |
| | S129A | No result | |
| NA (aligned against H5N8 2020-2021 sequences) | S28N | No result | |
| | V50I | Mutation observed in H3N2 canine influenza | Ou, Lu, Jia and Li 2020 |
| | M295I | Mutation observed in H5N1 viruses isolated in Vietnam in 2012 | Xuan, Chi and Quang 2014 |
| MP | No mutations found | | |
| NS1 and NS2 | S3P | AA substitution observed in HPAI H5N1 in Thailand 2004-2005 | Suwannakhon et al 2008 |

## H5 2.3.4.4b In-silico risk antiviral resistance assessment

*Table 5: Analyses of A/Astrakhan/3212/2020/H5N8/2020-12-12 for any changes which indicate antiviral resistance.*

| Protein | Amino acid position/motif | A/Astrakhan/3212/2020 (H5N8) |
|---|---|---|
| **PA** | I38T | Not present |
| | E23K | Not present |
| | A37T | Not present |
| | A36V | Not present |

# H5 2.3.4.4e Phylogenetic analyses

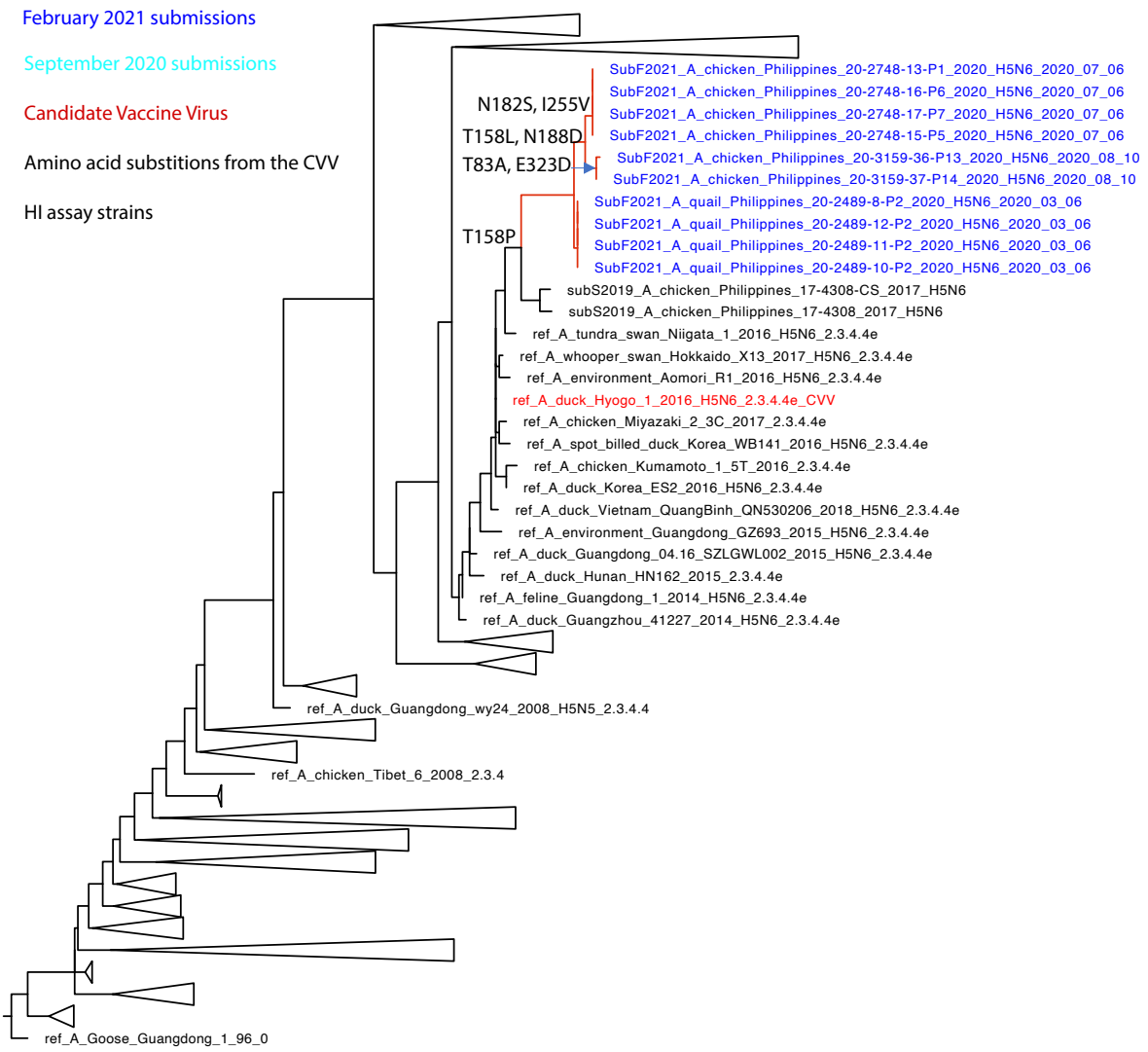



*Figure 6: Avian H5 clade 2.3.4.4e maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. New data acquired since September 2020 is coloured in dark blue and data from between February and September 2020 in light blue. The CVV for this clade is shown in red and amino acid differences relative to the CVV are annotated on the tree.*

## H5 2.3.4.4e Comparative amino acid substitutions to clade CVV

*Table 6: Avian H5 2.3.4.4e 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 | ref_A_duck_Hyogo_1_2016_H5N6_2.3.4.4e_CVV | SubS2019_A_chicken_Philippines_17-4308-CS_2017_H5N6 | SubS2019_A_chicken_Philippines_17-4308_2017_H5N6 | SubF2021_A_quail_Philippines_20-2489-8-P2_2020_H5N6_2020_03_06 | SubF2021_A_chicken_Philippines_20-2748-13-P1_2020_H5N6_2020_07_06 | SubF2021_A_chicken_Philippines_20-3159-36-P13_2020_H5N6_2020_08_10 | Annotations |
|---|---|---|---|---|---|---|---|
| 83 | T | | | | | A | |
| 96 | N | Y | Y | | | | |
| 158 | T | P | P | P | L | L | |
| 182 | N | | | | S | | |
| 188 | N | | | | D | D | Antigenic Site B |
| 255 | I | | | | V | | |
| 268 | M | I | I | | | | Antigenic Site C |
| 271 | G | | S | | | | |
| Total | | 3 | 4 | 1 | 4 | 3 | |

# H5 2.3.4.4h Phylogenetic analysis


*Figure 7: Avian H5 clade 2.3.4.4h maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. New data acquired since September 2020 is coloured in dark blue and data from between February and September 2020 in light blue. The CVV for this clade is shown in red and amino acid differences relative to the CVV are annotated on the tree. Branches are coloured by geographical region.*

# H5 2.3.4.4h Comparative amino acid substitutions to clade CVV

*Table 7: Avian H5 2.3.4.4h 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 | ref_A_Guangdong_18SF020_2018_H5N6_2.3.4.4h_CVV | A_Duck_Vietnam_TB-2181-20_H5N6_2019_2019_06_09 | A_Duck_Vietnam_BN-R2-2775-16_-H5N6_2019_2019_07_21 | A_Duck_Vietnam_BN-R2-2775-17_-H5N6_2019_2019_07_21 | A_Duck_Vietnam_TB-R2-2988-15_-H5N6_2019_2019_07_21 | A_Chicken_Vietnam_LS-R2-2913-01-H5N6_2020_2019_08_02 | A_Environment_Vietnam_TB-R2-2988-21-H5N6_2019_2019_08_0 | A_Chicken_Vietnam_QN-R2-161-01-H5N6_2020_2020_01_21 | A_Chicken_Vietnam_TB-R2-312-02-H5N6_2020_2020_02_19 | A_Duck_Vietnam_HP-R2-332-01-H5N6_2020_2020_02_24 | A_Duck_Vietnam_BN-R2-410-01-H5N6_2020_2020_03_05 | site | Annotations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | Q | R | | | | | | | | | | 2 | |
| 5 | I | F | | | | | | | | | | 5 | |
| 8 | H | Q | | | | | | | Q | | | 8 | |
| 11 | N | | | | H | | | | | | | 11 | |
| 16 | V | | | | | | | | G | | | 16 | |
| 28 | H | | | | Q | | | | | | | 28 | |
| 30 | Q | | R | R | | R | | | | R | R | 30 | |
| 39 | G | | | | | | | R | | | | 39 | |
| 51 | V | I | I | I | I | I | I | I | I | I | I | 51 | |
| 56 | S | | | | T | | | | | | | 56 | |
| 72 | S | | | | | | | | | I | | 72 | Antigenic Site E |
| 84 | N | D | | | D | | D | | D | | | 84 | Antigenic Site E |
| 93 | G | A | | | | | | | | | | 93 | |
| 94 | N | T | | | T | | T | | T | | | 94 | |
| 95 | L | | | | | | | | F | | | 95 | |
| 99 | E | | | | K | | | | | | | 99 | |
| 100 | E | | | | K | | | | | | | 100 | |
| 105 | L | | | W | W | | | | | | | 105 | |
| 111 | F | C | | | | | | | | | | 111 | |
| 124 | N | | | | S | | | | | | | 124 | Antigenic Site B |
| 132 | A | | | | | | | T | | | | 132 | |
| 135 | P | | | | | | | | | S | S | 135 | |
| 137 | Q | T | T | T | T | T | T | | T | T | T | 137 | Antigenic Site A |
| 153 | N | | | | | | | | T | | | 153 | Antigenic Site B |
| 161 | M | K | R | R | K | R | K | | K | R | R | 161 | |
| 183 | A | | | | | | | V | | | | 183 | Antigenic Site B |
| 187 | T | A | A | A | A | A | A | | A | A | E | 187 | |
| 188 | D | | | | | | | N | | | | 188 | Antigenic Site B |
| 191 | K | | | | E | | | | | | | 191 | |
| 197 | V | | | | L | | | | | | I | 197 | |
| 199 | V | | | | L | | | | | | | 199 | |
| 234 | P | R | | | R | | R | | R | | | 234 | |
| 254 | K | | | | | | | H | | Q | | 254 | |
| 265 | K | | | | | | | N | | | N | 265 | |
| 269 | E | | | | Q | | | | | | | 269 | |
| 270 | Y | | | | H | | | H | | | | 270 | |
| 276 | K | | | | | | | | | | | 276 | |
| 290 | F | | | | L | | | | | | | 290 | |
| 295 | P | | | | | | | S | | | | 295 | |
| 303 | K | | R | | | | | | | R | | 303 | |
| 308 | N | | | | | | | Y | | Y | | 308 | |
| 311 | V | | | | | | | I | | | I | 311 | |
| 317 | R | | | | | | | T | | | | 317 | |
| Total | | 12 | 6 | 6 | 20 | 5 | 7 | 12 | 11 | 10 | 9 | Total | |

# H5 2.3.2.1c Phylogenetic analyses

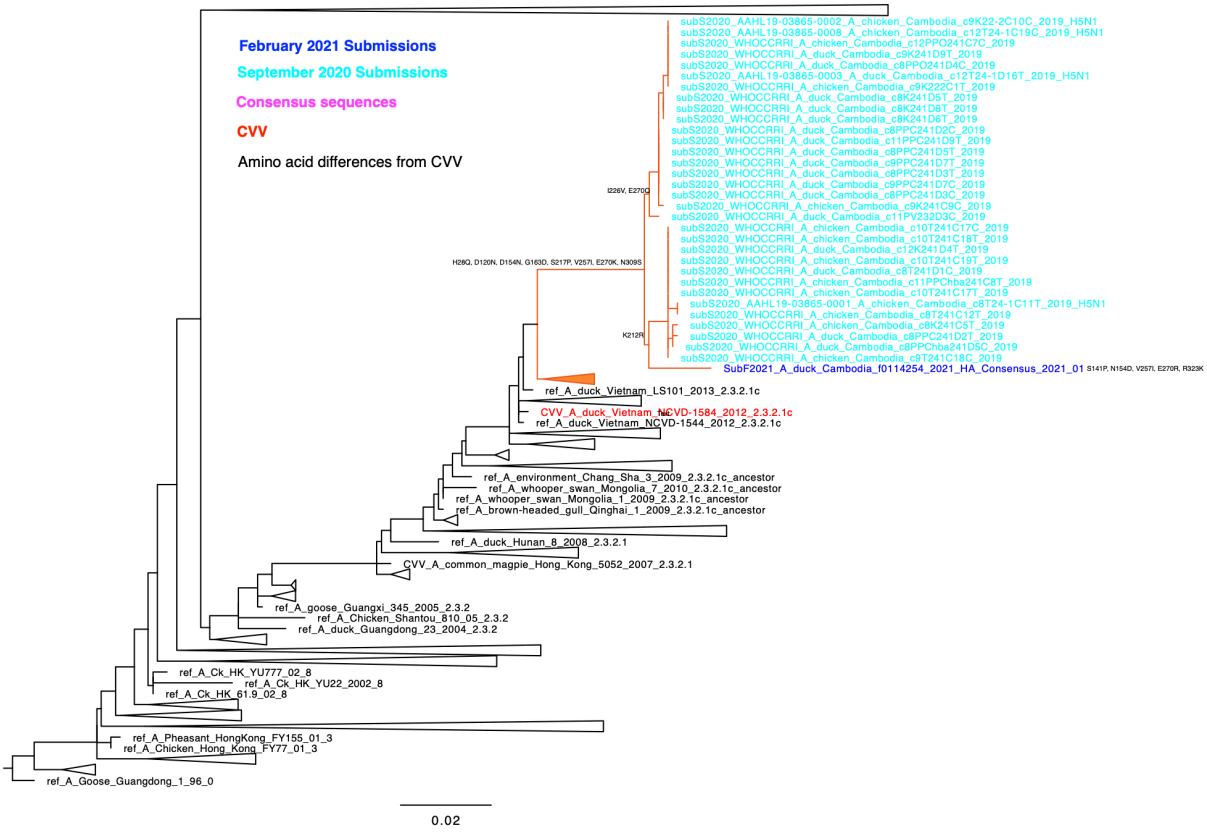



*Figure 8: Avian H5 clade 2.3.2.1c maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. New data acquired since September 2020 is coloured in dark blue and data from between February and September 2020 in light blue. The CVV for this clade is shown in red and amino acid differences relative to the CVV are annotated on the tree. Branches are coloured by geographical region.*

## H5 2.3.2.1c Comparative amino acid substitutions to CVV

*Table 8: Avian H5 2.3.2.1c 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_Vietnam_NCVD-1584_2012_2.3.2.1c | A_duck_Cambodia_f0114254_2021_HA_Consensus_2021_01 | Annotations |
|---|---|---|---|
| 28 | H | Q | |
| 120 | D | N | Antigenic Site A |
| 141 | S | P | Antigenic Site A |
| 163 | G | D | Antigenic Site D |
| 212 | K | R | |
| 217 | S | P | |
| 257 | V | I | |
| 270 | E | R | |
| 309 | N | S | |
| Total | | 9 | |

# H9 HPAI Influenza A Viruses

## H9 Phylogenetic analysis


*Figure 9: Avian H9 maximum likelihood phylogenetic tree. Analyses were conducted with reference sequences and data downloaded from GISAID/shared by OFFLU. New data acquired since September 2020 is coloured in dark blue, data from between February and September 2020 in light blue and data from September 2019 to February 2020 is coloured in green. The CVV for this clade is shown in red.*

# H9 G1 Comparative amino acid substitutions to clade CVV

*Table 9: Avian H9 G1 clade sequence comparison to the CVV 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 | SubF2021_A_chicken_India_10TR30_2020_H9N2_2020-10-00_HA gene | A_chicken_Pakistan_UDL_105_2020_2020-01-09 | SubF2021_A_duck_Benin_20-A-03-2026-E_20VIR6511-111_2020_H9N2_2020-03- | A_chicken_Saudi_Arabia_MW049330.1_ASH-98.3_2020_H9_HA_2020-10-05 | A_chicken_Saudi_Arabia_MW049331.1_ASH-100.1-2_H9_HA_2020-10-05 | site | Annotations |
|---|---|---|---|---|---|---|---|---|
| 2 | K | | I | | | | 2 | |
| 12 | S | | C | | | | 12 | |
| 22 | T | N | S | | | | 22 | |
| 24 | V | | I | | | | 24 | |
| 28 | Q | H | | | | | 28 | |
| 57 | I | | V | | | | 57 | |
| 69 | M | L | L | | | | 69 | |
| 74 | R | K | | | | | 74 | Published escape mutation, potential antigenic site |
| 77 | S | | | A | | | 77 | |
| 94 | N | H | | | | | 94 | |
| 103 | T | | M | | | | 103 | |
| 104 | L | | | F | | | 104 | |
| 108 | S | | A | | | | 108 | |
| 109 | S | | N | | | | 109 | Published escape mutation, potential antigenic site |
| 114 | V | I | I | | | | 114 | |
| 116 | L | I | I | | | | 116 | |
| 120 | S | T | A | | | | 120 | Published escape mutation, potential antigenic site |
| 127 | T | | D | | | | 127 | Published escape mutation, potential antigenic site |
| 135 | D | | N | G | | | 135 | Published escape mutation, potential antigenic site |
| 148 | N | | | D | | | 148 | |
| 150 | G | A | N | S | | | 150 | Published escape mutation, potential antigenic site |
| 152 | P | T | | | | | 152 | Published escape mutation, potential antigenic site |
| 153 | I | V | | | | | 153 | |
| 158 | Y | F | | | | | 158 | |
| 169 | V | | I | | | | 169 | |
| 177 | T | S | | | | | 177 | |
| 180 | A | | | | V | | 180 | |
| 185 | Y | | | X | | | 185 | |
| 187 | X | R | R | R | R | R | 187 | |
| 194 | V | I | | | | | 194 | |
| 198 | T | N | N | | | | 198 | |
| 246 | F | Y | Y | | | | 246 | |
| 249 | V | | | X | | | 249 | |
| 250 | L | | I | | | | 250 | |
| 259 | L | | | X | | | 259 | |
| 260 | R | K | K | | | | 260 | |
| 263 | L | | K | | | | 263 | |
| 264 | S | K | | | | | 264 | |
| 265 | S | N | | | | | 265 | |
| 266 | G | | | | S | S | 266 | |
| 267 | N | D | | | | | 267 | |
| 269 | V | L | | | | | 269 | |
| 283 | M | L | L | | | | 283 | |
| 295 | T | N | | | N | N | 295 | |
| 311 | L | | | | M | M | 311 | |
| 315 | H | P | P | | | | 315 | |
| Total | | 17 | 10 | 5 | 5 | 4 | | |

# Annex
## Additional H5 2.3.4.4b Analyses

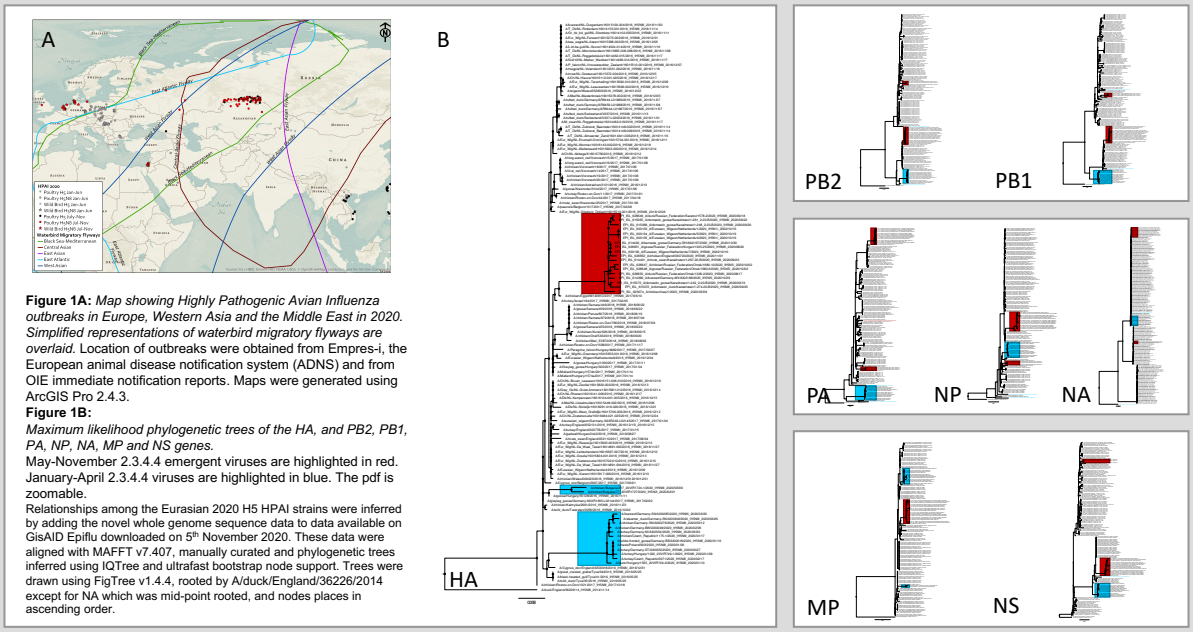



**Figure 1A:** *Map showing Highly Pathogenic Avian Influenza outbreaks in Europe, Western Asia and the Middle East in 2020. Simplified representations of waterbird migratory flyways are overlaid.* Location of outbreaks were obtained from Empres-i, the European animal disease notification system (ADNS) and from OIE immediate notification reports. Maps were generated using ArcGIS Pro 2.4.3.

**Figure 1B:**
*Maximum likelihood phylogenetic trees of the HA, and PB2, PB1, PA, NP, NA, MP and NS genes.*
May-November 2.3.4.4 emergent viruses are highlighted in red. January-April 2.3.4.4 viruses are highlighted in blue. The pdf is zoomable.
Relationships among the Eurasian 2020 H5 HPAI strains were inferred by adding the novel whole genome sequence data to data available on GisAID Epiflu downloaded on 5th November 2020. These data were aligned with MAFFT v7.407, manually curated and phylogenetic trees inferred using IQTree and ultrafast bootstrap node support. Trees were drawn using FigTree v1.4.4, rooted by A/duck/England/36226/2014 except for NA which was mid-point rooted, and nodes places in ascending order.

*Figure 10: A/turkey/England/038115/2020 (A/H5N8) segment 6 (NA) closest blast relative to A/crow/India/12CA06/2020_H5N8_2020-12-29 taken from https://www.tandfonline.com/doi/full/10.1080/22221751.2021.1872355*

## Genome mutation analysis of A/chicken/England/030685/2020 H5N8

We have analysed the full genome sequence data of A/chicken/England/030685/2020 H5N8 HPAIV (H5N8-2020) and compared it with the CDC (Atlanta) H5N1 genetic changes inventory and Suttie et al. 2019 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. This totals 212 mutations or combinations of mutations.

We have observed 29 mutations/combinations of mutations that are listed (Table 1). It should be noted that all of the genetic changes identified were also present in representative H5N8-2016 sequences.

For the proteins of the polymerase complex, six, two and four mutations/combination of mutations were identified in PB2, PB1 and PA, respectively. The majority of these genetic changes are reported to increase polymerase activity and virulence in mammals and chickens, but there were also mutations reported to decrease virulence in mice. However, the PB2 E627K mutation was not identified. The PB1-F2 protein was found to be truncated, which has been associated with adaption to mammalian infection and increased virulence in mammals.

The five mutations identified in the HA are reported to increase binding to mammalian α2-6 receptors, with the T156A mutation being the only change that has also been observed in the Asian H5N6 viruses. However, the T156A mutation was also present in European H5N6 and H5N8 viruses. Nevertheless, all five HA mutations are not considered characteristic of enabling the binding to α2-6 receptors in the literature. Therefore, it is predicted that the HA of H5N8-2020 will bind to avian α2-3 receptors.

The two mutations identified in NP are associated with increased virulence in chickens and do not have any reported impact on mammalian adaptation.

Mutations in NA reported to affect zanamivir and oseltamivir susceptibility (Table 2) were not found.

Within M1, three mutations associated with increased virulence in mice, chickens and ducks were identified, however, no mutations reported to effect amantadine and rimantadine susceptibility in M2 (Table 2) were found.

There is a lack of a deletion in NS1 at amino acid position 80-84 that is conserved among contemporary H5 viruses, possibly decreasing the zoonotic potential of the H5N8 virus. However, six mutations reported to increase virulence and decrease antiviral responses in mammals and chickens were identified.

**In conclusion, no strong correlates for specific increased affinity for humans were found.**

**Table 1**. A/chicken/England/030685/2020 H5N8 (H5N8-2020): Genetic changes that may increase virulence, signal adaptation to mammalian species or alter susceptibility to existing antivirals.

| Protein | Amino acid position/motif | Phenotypic consequences | Reference |
|---|---|---|---|
| PB2 | I292V<br>[Same as 2016] | Increased polymerase activity in mammalian cell line, increased virulence in mice | Gao et al. 2019; Xiao et al. 2016 |
| | V598T<br>[Same as 2016] | Increased polymerase activity and replication in mammalian cells, increased virulence in mice | Hu et al. 2017 |
| | K627E<br>[Same as 2016] | Increased virulence in chickens | Schat et al. 2012 |
| | S715N<br>[Same as 2016] | Decreased virulence in mice | Sun et al. 2015 |
| | L89V, G308D<br>[Same as 2016] | Increased polymerase activity in mammalian cell line and increased virulence in mice | Li et al. 2009 |
| | L89V, G309D, T339K, R477G, I495V, K627E, A676T<br>[Same as 2016] | Increased polymerase activity in mammalian cell line and increased virulence in mice | Li et al. 2009 |
| PB1 | D3V[1]<br>[Same as 2016] | Increased polymerase activity and viral replication in avian and mammalian cell lines | Elgendy et al. 2017 |
| | D622G<br>[Same as 2016] | Increased polymerase activity and virulence in mice | Feng et al. 2016 |
| PB1-F2 | Truncated PB1-F2<br>[Same as 2016] | Adaptation to mammalian infection with increased virulence in mammals | Kamal et al. 2015 |
| PA | S37A<br>[Same as 2016] | Increased polymerase activity in mammalian cell line | Yamayoshi et al. 2014 |
| | P190S<br>[Same as 2016] | Decreased virulence in mice | DesRochers et al. 2016 |

| | | | |
|---|---|---|---|
| | N383D<br>[Same as 2016] | Increased polymerase activity in mammalian and avian cell lines | Song et al. 2015; Song et al. 2011 |
| | N409S<br>[Same as 2016] | Increased polymerase activity and replication in mammalian cell line | Yamayoshi et al. 2014 |
| PA-X | None found | | |
| HA (H5 numbering) | S133A<br>[Same as 2016] | Increased pseudovirus binding at a2-6 | Yang et al. 2007 |
| | S154N<br>[Same as 2016] | Increased virus binding to a2-6 | Wang et al. 2010 |
| | T156A<br>[Same as 2016] | Increased virus binding to a2-6 and increased transmission in guinea pigs | Wang et al. 2010; Gao et al. 2009 |
| | S107R, T108I<br>[Same as 2016] | Increased virulence in chickens and mice, increased pH of fusion | Wessels et al. 2018 |
| | K218Q, S223R<br>[Same as 2016] | Increased virus binding to a2-3 and a2-6 | Guo et al. 2017 |
| NP | M105V<br>[Same as 2016] | Increased virulence in chickens | Tada et al. 2011a; Tada et al. 2011b |
| | A184K<br>[Same as 2016] | Increased replication in avian cells and virulence in chickens, enhanced IFN response | Wasilenko et al. 2009 |
| NA | None found | | |
| NS1 | P42S<br>[Same as 2016] | Increased virulence and decreased antiviral response in mice | Jiao et al. 2008 |
| | L103F<br>[Same as 2016] | Increased virulence in mice | Kuo and Krug 2009; Spesock et al., 2011 |
| | I106M<br>[Same as 2016] | Increased viral replication in mammalian cells virulence in mice | Ayllon et al. 2014 |

| | | | |
|---|---|---|---|
| | C138F<br>[Same as 2016] | Increased replication in mammalian cells, decreased interferon response | Li et al. 2018 |
| | V149A<br>[Same as 2016] | Increased virulence and decreased interferon response in chickens | Li et al. 2006 |
| | L103F, I106M<br>[Same as 2016] | Increased virulence in mice | Kuo et al. 2009; Spesock et al. 2011 |
| NS2/NEP | None found | | |
| M1 | M30D<br>[Same as 2016] | Increased virulence in mice | Fan et al. 2009 |
| | I43M<br>[Same as 2016] | Increased virulence in mice, chickens and ducks | Nao et al. 2015 |
| | T215A<br>[Same as 2016] | Increased virulence in mice | Fan et al. 2009 |
| M2 | None found | | |

**Table 2**. Genetic changes demonstrated to alter antiviral susceptibility. NA mutations are numbered according to N2.

| Protein | Amino acid position/motif | Phenotypic consequences | Reference |
|---|---|---|---|
| NA | V116A | Reduced susceptibility to oseltamivir and zanamivir | Hurt et al. 2007; Boltz et al. 2010 |
| | I117T | Reduced susceptibility to oseltamivir and zanamivir | Kode et al. 2019a |
| | E119A | Reduced susceptibility to zanamivir<br>Reduced susceptibility to oseltamivir and zanamivir<br>Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir | Gubareva et al. 1997; Baek et al. 2015; Ilyushina et al. 2010; Gubareva et al. 2017 |
| | Q136L | Reduced susceptibility to oseltamivir and zanamivir | Hurt et al. 2010 |
| | V149A | Reduced susceptibility to zanamivir | Naughtin et al. 2011 |
| | R156K | Reduced susceptiblity to oseltamivir, zanamivir, and peramivir | Ilyushina 2012 |
| | D198G | Reduced susceptibility to oseltamivir and zanamivir | Hurt et al. 2009 |
| | I222M | Reduced susceptibility to oseltamivir | Hurt et al. 2009 |
| | S246N | Reduced susceptibility to oseltamivir | Boltz et al. 2010 |
| | H274Y | Reduced susceptibility to oseltamivir and peramivir<br>Reduced susceptibility to oseltamivir | Baek et al. 2015; Ilyushina et al. 2010; Gubareva et al. 2017; Hurt et al. 2009; Le et al. 2005; Nguyen et al. 2013 |
| | E277Q | Reduced susceptibility to oseltamivir | Govorkova et al. 2007 |
| | R292K | Reduced susceptibility to zanamivir<br>Reduced susceptibility to oseltamivir<br>Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir | Gubareva et al. 1997; Gillman et al. 2015; Hai et al. 2013; Sleeman et al. 2013 |
| | N294S | Reduced susceptibility to oseltamivir | Kode et al. 2019b |
| | K432T | Reduced susceptibility to zanamivir | Creanga et al. 2017 |

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|---|---|---|---|
| | I117V, I314V | Reduced susceptibility to oseltamivir | Hurt et al. 2007 |
| | E119A/D/G, H274Y | Reduced susceptibility to oseltamivir, zanamivir, and peramivir | Baek et al. 2015 |
| | I222L, S246N | Reduced susceptibility to oseltamivir | Creanga et al. 2017 |
| | I222M/V, H274Y | Reduced susceptibility to oseltamivir and peramivir | Hurt et al. 2009 |
| | K150N, I222L, S246N | Reduced susceptibility to oseltamivir | Boltz et al. 2010 |
| M2 | L26F | Increased resistance to amantadine and rimantadine | Abed et al. 2005; Lan et al. 2010; Bean et al. 1989 |
| | I/V27A/T/S I/ V27A/T/S | Increased resistance to amantadine and rimantadine | Abed et al. 2005; Lan et al. 2010; Cheung et al. 2006 Bean et al. 1989; Ilyushina et al. 2005 |
| | A30V/T/S | Increased resistance to amantadine and rimantadine | Cheung et al. 2006 Bean et al. 1989; Ilyushina et al. 2005 |
| | S31N/G | Increased resistance to amantadine and rimantadine | Lan et al. 2010; Cheung et al. 2006; Bean et al. 1989; Ilyushina et al. 2005; He et al. 2008; Puthavathana et al. 2005; Buranathai et al. 2007 |
| | G34E | Increased resistance to amantadine and rimantadine | Abed et al. 2005 |