



OFFLU SWINE INFLUENZA REPORT

JANUARY 2024 TO JUNE 2024

SCOPE

In this document we present a summary of H1 and H3 swine influenza A virus evolution.

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Swine influenza A viruses: Acknowledgements and Methods

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Brief Methods for Genetic and Antigenic Analyses

Analyses were conducted by subtype and lineage or clade. Reference sequences and new data (deposited on or after January 1, 2024) downloaded from GISAID or GenBank incorporated into the USDA-ARS NADC octoFLUdb and were subsequently aligned with MAFFT (Katoh and Standley, 2013) using default settings. Alignments for each segment were inspected manually and trimmed to the start and stop codon. Phylogenetic inference was conducted with FastTree (Price et al., 2009) or IQ-Tree (Minh et al., 2020). Tabular comparisons between current CVVs or human seasonal vaccine strains and new swine data were generated using the NADC IAV bioinformatic toolkit flutite (<https://github.com/flu-crew>). A clade representative was selected using PARNAS (Markin et al., 2023: <https://github.com/flu-crew/parnas>) and the best matched available strain was selected for testing against reference ferret antisera in hemagglutination inhibition (HI) assays. All HI assays were performed with ferret anti-sera and guinea pig red blood cells. H3N2 assays were performed with the addition of oseltamivir. Phylogenies were subsampled and visualized using smot (Arendsee et al., 2022: <https://github.com/flu-crew/smot>).

Global influenza A events in swine Epidemiology

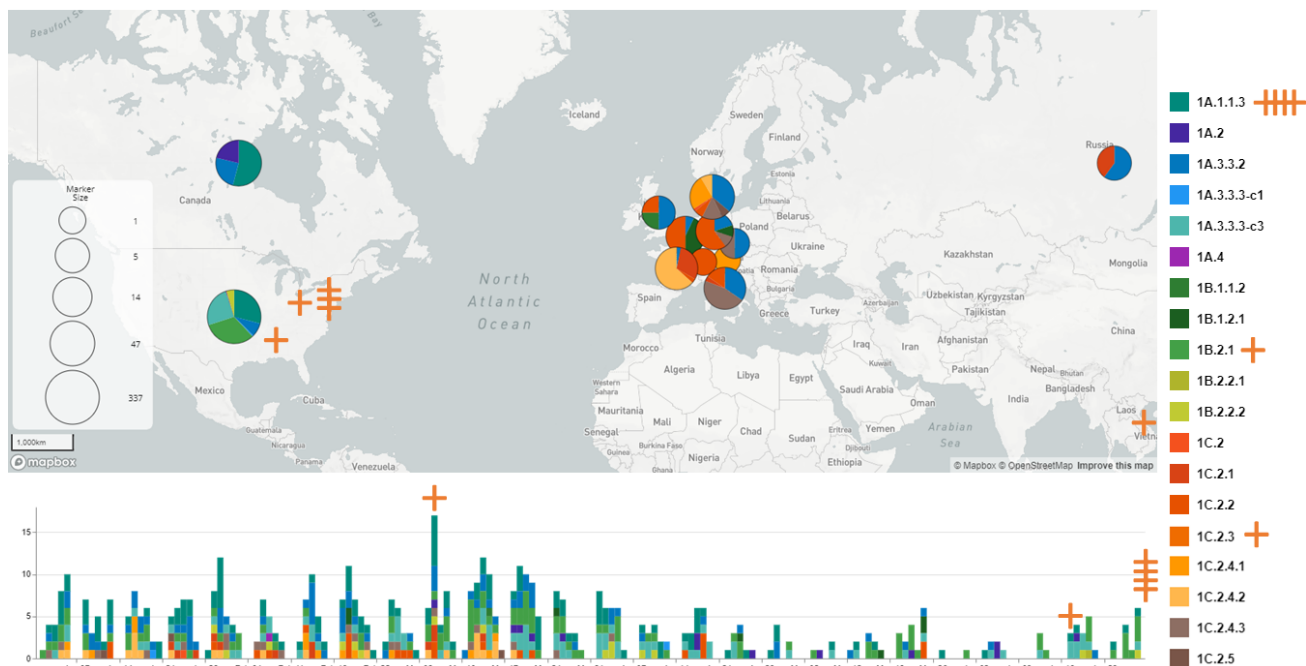


Figure 1. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank on or after January 1, 2024 (n=538); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions. Clades containing variants, location, and date of detection are marked with orange crosses.

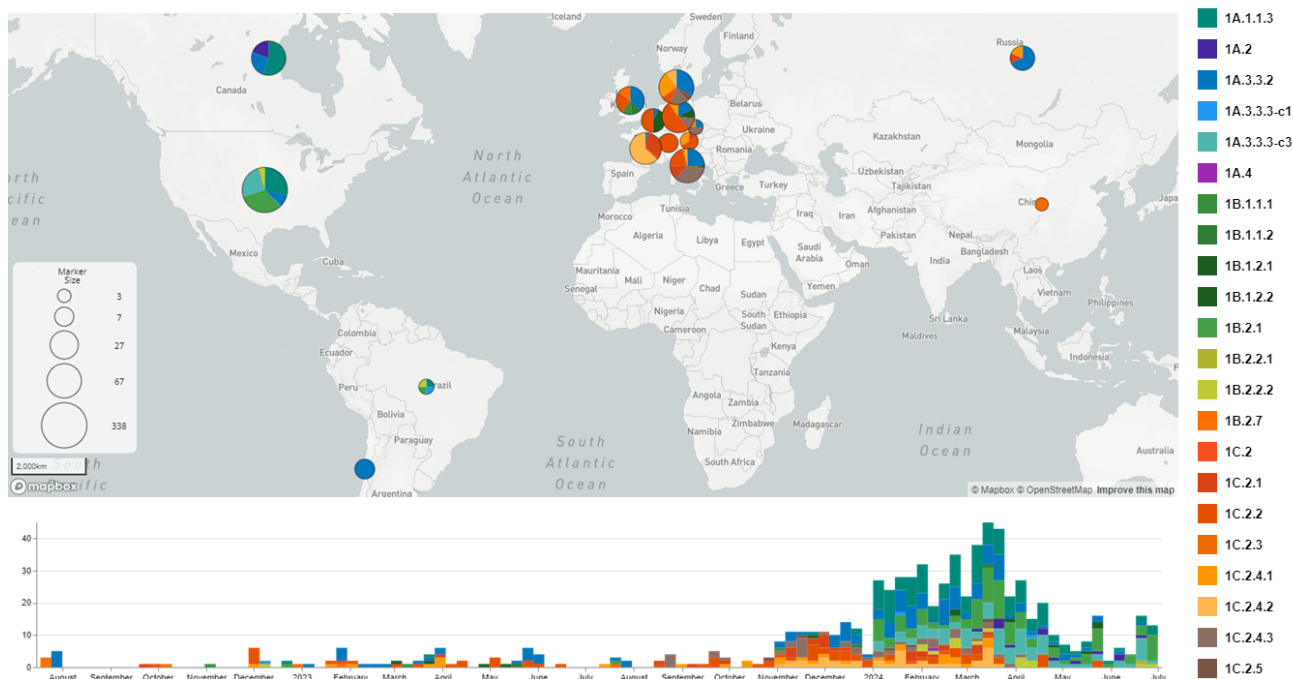


Figure 2. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank on or after July 1, 2022 (n=732); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions.

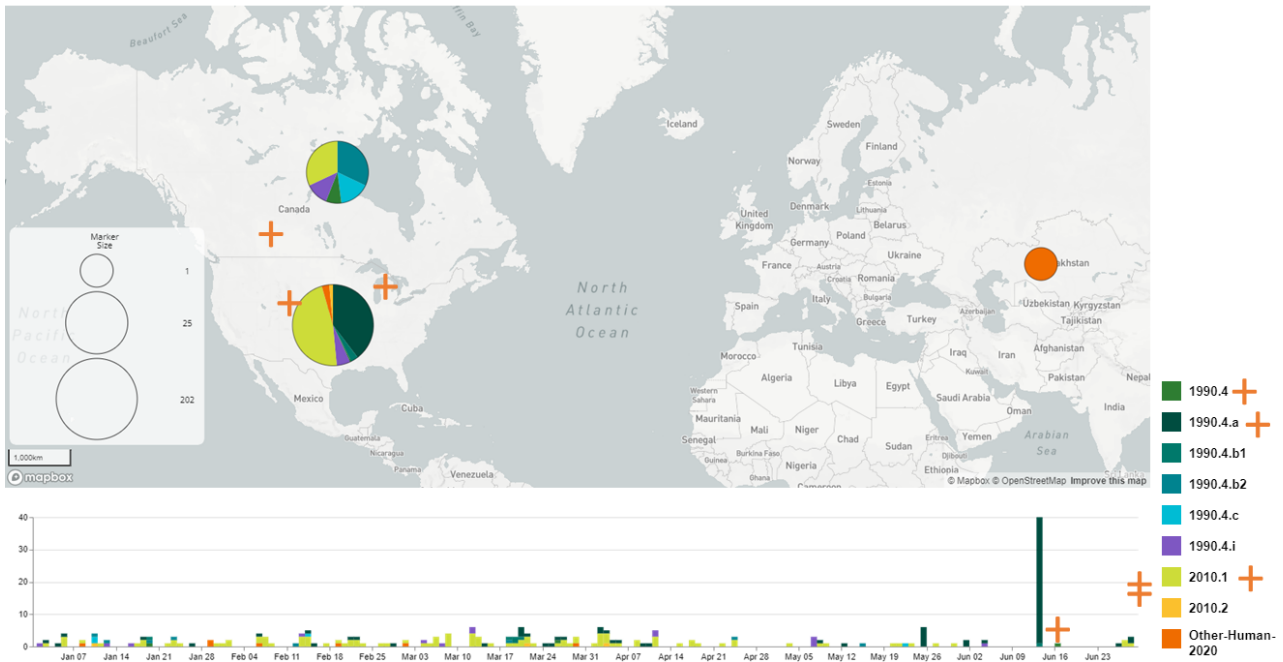


Figure 3. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank on or after January 1, 2024 (n=228). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions. Clades containing variants, location, and date of detection are marked with orange crosses.

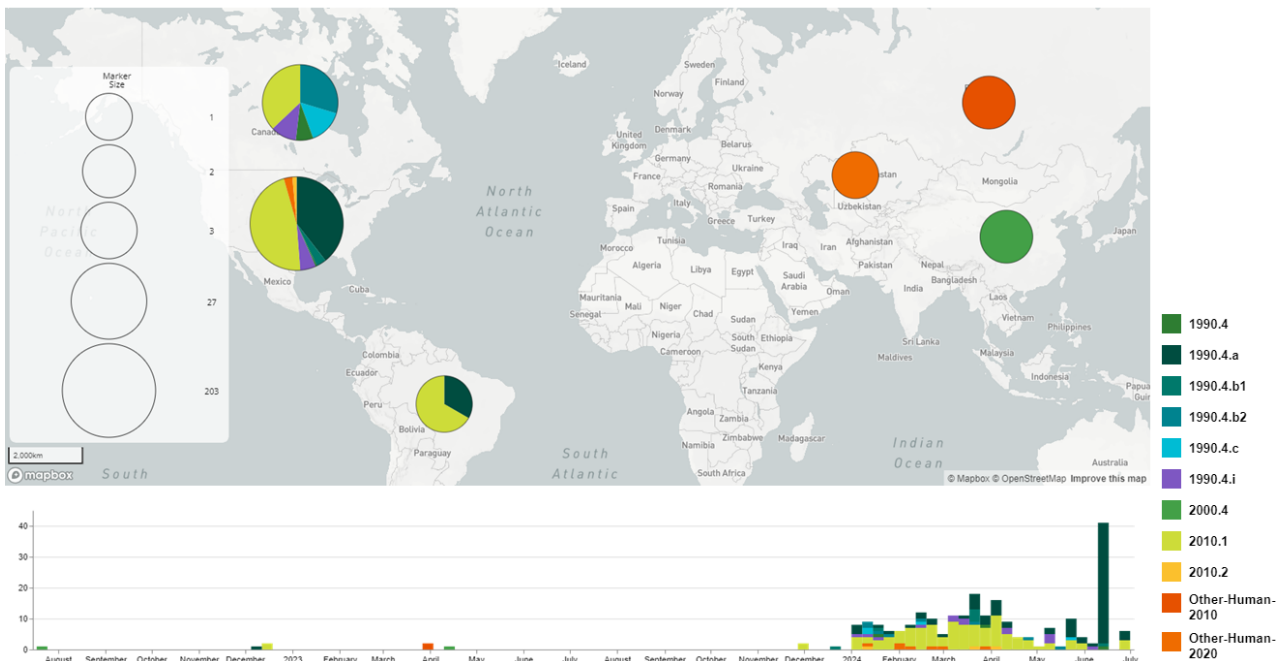
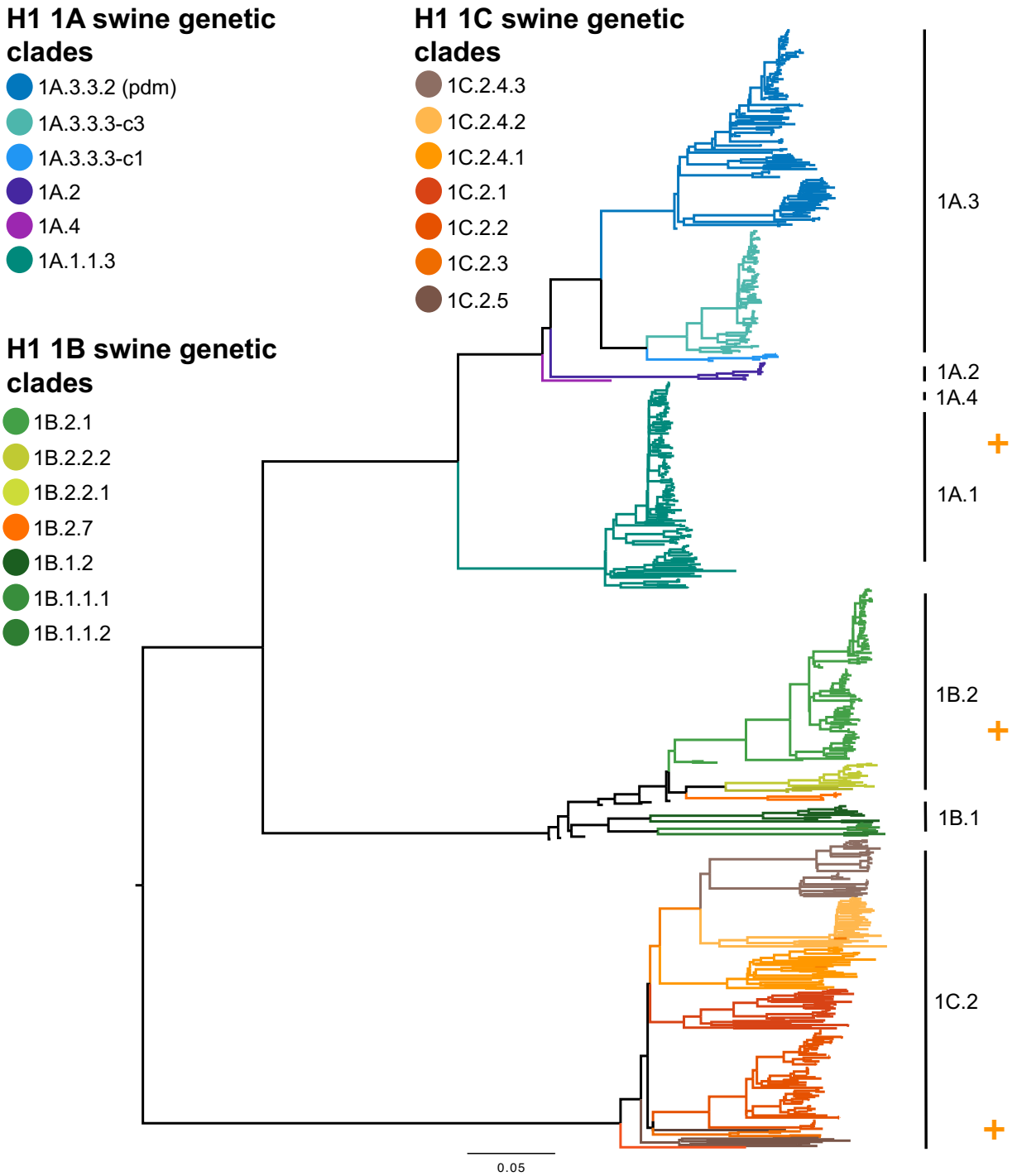


Figure 4. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank on or after July 1, 2022 (n=238). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions.

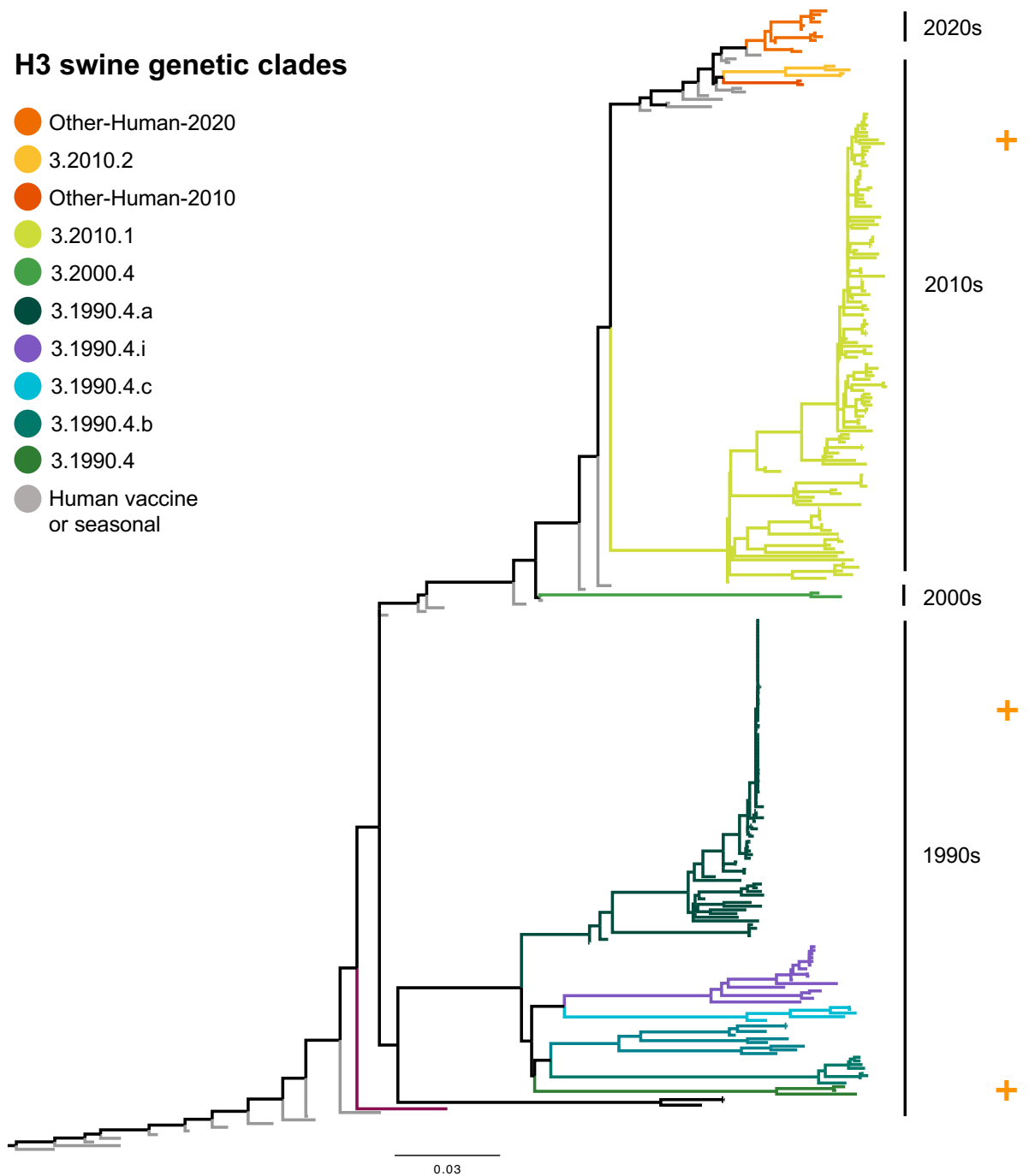
Contemporary Global H1 swine IAV: genetic diversity



n=766, data deposited within last 6 months.

Figure 5. Global swine H1 phylogenetic tree ($n=766$) with reference strains ($n=77$) colored by clade and annotated by global H1 lineage nomenclature. Analyses were conducted with reference sequences and data (all data deposited on or after January 1, 2024) from GISAID or shared via the OFFLU swine IAV working group.

Contemporary Global H3 swine IAV: genetic diversity



n=245, data deposited within last 6 months, and n=62 reference genes.

Figure 6. Global swine H3 phylogenetic tree ($n=245$) with reference strains ($n=62$) colored by clade and annotated by decade of introduction from human seasonal H3. Analyses were conducted with reference sequences and data (all data deposited on or after January 1, 2024) from GISAID or shared via the OFFLU swine IAV working group.

Regional geographic summary

Phylogenetic analysis identified 32 genetic clades from H1 and H3 IAV in swine in sequence data deposited on or after January 1, 2024.

21 clades were H1 subtype, with detections from: 1A classical swine lineage (6 clades); 1B human-seasonal lineage (7 clades); and 1C Eurasian avian lineage (7 clades).

- The 1A classical swine lineage viruses have global detection: 1A.1.1.3 in USA and Canada; 1A.2 in Canada; 1A.3.3.3-c1 in USA; 1A.3.3.3-c3 in USA. The 1A.3.3.2 (pdm09) circulated in all countries that deposited sequence data during this 6-month period (9 countries).
- 1B.1 human seasonal lineage was only in Europe, and the 1B.2 human seasonal lineage were detected in the Americas and Europe.
- The 1C.2 Eurasian avian lineage was detected in Europe and Asia.
- A new pre-2009 human-to-swine spillover was detected circulating in Northern Ireland: 1B.2.7.
- Diversity associated with unique amino acid deletion patterns and detection frequency in swine warranted new nomenclature: 1C.2.4.1, 1C.2.4.2, and 1C.2.4.3.

10 H3 clades were detected in swine within 5 distinct lineages derived from human seasonal H3 virus spillovers grouped by the decade of introduction (1990.4; 2000.4; 2010.1; 2010.2; 2020.1) an additional clade of novel human seasonal spillover was detected without evidence for onward circulation (Other-Human-2010). The 1990.4 lineage diversified into 6 co-circulating genetic clades.

- Most lineages exhibited regional circulation: the 2000.4 in China; and the 2010.2 and 2020.1 lineages were detected in the USA.
- The 1990.4 and the 2010.1 lineages were detected in the USA and Canada.
- Novel human-to-swine spillovers were detected in Russia (Other-Human-2010).
- A human-to-swine spillover was detected in the USA associated with the 2022-23 influenza season and demonstrated sustained transmission in pigs: 2020.1

Global Variant Cases:

During the reporting period (January 1 – June 30, 2024), 4 variant cases were reported and included with the swine analyses when sequence data were available:

- **Canada: H3N2v (1990.4)**
- **USA: H1N2v (2x 1A.1.1.3)**
- **Vietnam: H1N1v (1C.2.3)**

Additional variant cases were detected outside of this period, when sequence data were available these were included in analyses:

- **USA: H1N2v (2x 1A.1.1.3 – August 5, August 7 2024; 1x 1B.2.1 – July 2023), H3N2v (1990.4.a – July 24 2024), H3N2v (2010.1 – July 9, 2024)**

1A classical swine lineage

- ★ H1v candidate vaccine virus
- A/Victoria/4897/2022
- A/Wisconsin/67/2022
- H1N1pdm seasonal vaccines
- Reported H1v cases
- + New variant(s)
- # HI tested viruses

H1 1A swine genetic clades

- 1A.3.3.2/pdm (n=132)
- 1A.3.3.3-c3 (n=89)
- 1A.3.3.3-c1 (n=3)
- 1A.2 (n=13)
- 1A.4 (n=1)
- 1A.1.1.3 (n=148)

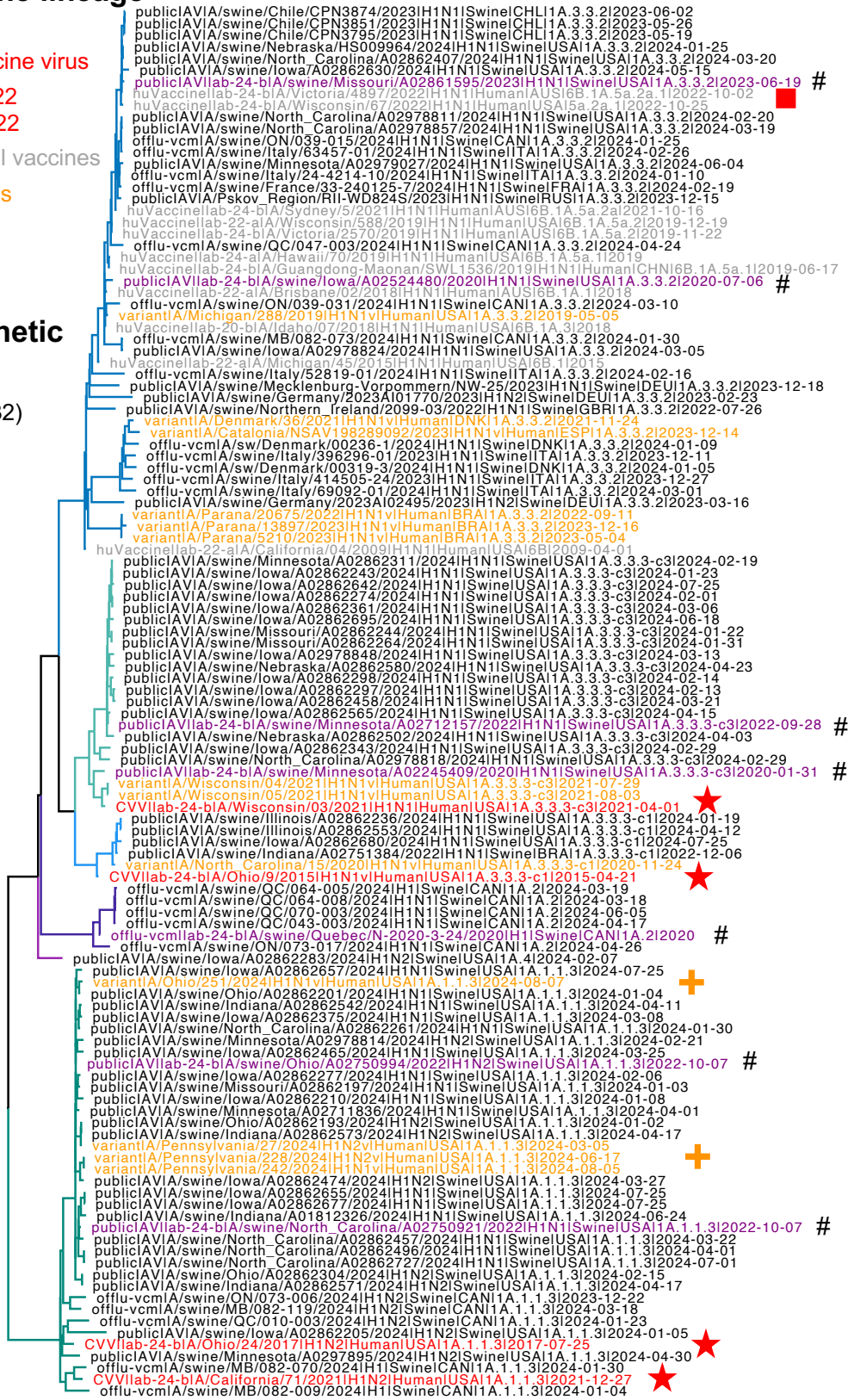


Figure 7. Swine H1 HA genes of the 1A lineage (tree was proportionally downsampled to 91 swine HA genes, excluding references). Number of detections of each clade from data deposited on or after January 1, 2024 are presented adjacent to the clade name in the figure key.

Antigenic analysis: Swine 1A Lineage

Table 1. USA Data: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine 1A lineage strains selected to represent clade consensus.

- > 8 fold reduction
- 8 fold reduction
- 4 fold reduction

| | Global Clade | US Clade | IDCDC-RG59 A/Ohio/24/2017-like CVV | A/California/71/2021 | A/swine/North_Carolina/A02750921/2022 #61 | A/swine/North_Carolina/A02750921/2022 #61 | A/Victoria/4897/2022 | A/Wisconsin/67/2022 | A/swine/Iowa/A02524480/2020 #30 | A/swine/Iowa/A02524480/2020 #14 | A/Wisconsin/3/2021-like CVV RG76C | A/swine/Minnesota/A02245409/2020 #28 | A/swine/Minnesota/A02245409/2020 #12 | Human Pooled Serum |
|---------------------------------------|-------------------|-----------|------------------------------------|----------------------|---|---|----------------------|---------------------|---------------------------------|---------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|--------------------|
| IDCDC-RG59 A/Ohio/24/2017 CVV | 1A.1.1.3 | alpha-del | 640 | 20 | 160 | 320 | 20 | <10 | 160 | 320 | 20 | 20 | 20 | 80 |
| A/California/71/2021 | 1A.1.1.3 | alpha-del | <10 | 5120 | 160 | 320 | 10 | <10 | <10 | <10 | <10 | <10 | <10 | 160 |
| A/swine/North_Carolina/A02750921/2022 | 1A.1.1.3 | alpha-del | <10 | 20 | 640 | 1280 | <10 | <10 | <10 | <10 | 20 | <10 | <10 | 80 |
| A/swine/Ohio/A02750994/2022 | 1A.1.1.3 | alpha-del | 20 | 80 | 640 | 1280 | <10 | <10 | <10 | <10 | 10 | <10 | <10 | 80 |
| A/Victoria/4897/2022 | 1A.3.3.2, 5a.2a.1 | pdm09 | <10 | <10 | <10 | <10 | 2560 | 2560 | 10 | 20 | 40 | <10 | <10 | 320 |
| A/Wisconsin/67/2022 | 1A.3.3.2, 5a.2a | pdm09 | <10 | <10 | <10 | <10 | <10 | 2560 | 320 | 40 | 80 | <10 | <10 | 160 |
| A/swine/Iowa/A02524480/2020* | 1A.3.3.2 | pdm09 | 20 | <10 | 10 | 10 | 80 | 10 | 640 | 2560 | 40 | 20 | 20 | 160 |
| A/swine/Missouri/A02861595/2023 | 1A.3.3.2 | pdm09 | <10 | <10 | <10 | <10 | 20 | 10 | 10 | 10 | 80 | <10 | <10 | 80 |
| IDCDC-RG76 A/Wisconsin/03/2021 CVV | 1A.3.3.3-c3 | gamma.3 | <10 | <10 | <10 | <10 | 20 | <10 | <10 | 10 | 5120 | 40 | 40 | 160 |
| A/swine/Minnesota/A02245409/2020* | 1A.3.3.3-c3 | gamma.3 | <10 | <10 | 40 | 80 | 40 | <10 | 40 | 40 | 640 | 1280 | 1280 | 160 |
| A/swine/Minnesota/A02712157/2022* | 1A.3.3.3-c3 | gamma.3 | <10 | <10 | 20 | 40 | 20 | <10 | 20 | 20 | 320 | 320 | 160 | 80 |
| A/swine/Quebec/N-2020-3-24/2020 | 1A.2 | beta | <10 | <10 | <10 | <10 | <10 | <10 | | | 80 | <10 | 20 | 80 |

Reference CVV in red, seasonal vaccines in dark red, new swine in bold. Homologous titers highlighted in gray. Human sera were collected in USA with titer relative to A/Wisconsin/67/2022, pool of 2 male and 2 female adults.

- The contemporary swine 1A.1.1.3 (alpha-del) had >8-fold decrease from 1A.1.1.3 CVV A/Ohio/24/2017 and A/California/71/2021 ferret anti-sera but retained reactivity to human pooled sera.
- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine had >8-fold decrease to A/Victoria/4897/2022 5a.2a.1 and A/Wisconsin/67/2022 5a.2a vaccine strains but retained reactivity to human pooled sera.
- The contemporary swine 1A.3.3.3-c3 (gamma.3) virus had 4-8-fold decrease from the within-clade 1A.3.3.3-c3 CVV A/Wisconsin/2021 but retained reactivity to human pooled sera.
- The contemporary swine 1A.2 (beta) virus has no within clade CVV and >8-fold decrease to CVV and human seasonal vaccine ferret anti-sera but retained reactivity to human pooled sera.

Table 2. EU Study Design: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine 1A lineage strains.

- >8 fold reduction
- 8 fold reduction
- 4 fold reduction

| | Global Clade | A/California/71/21 | A/California/7/2009 | A/Guangdong-Maonan/SWL1536/2019 | A/Victoria/2570/2019 (IVR-215) | A/Victoria/4897/2022 (IVR)-238 | A/Ohio/9/2015 CVV |
|---|-------------------|--------------------|---------------------|---------------------------------|--------------------------------|--------------------------------|-------------------|
| A/California/71/21 | H1N1 1A.1.1.3 | 1280 | 20 | 160 | 160 | 0 | 40 |
| A/California/7/2009 | H1N1 1A.3.3.2 | 40 | 640 | 1280 | 320 | 320 | 0 |
| A/Guangdong-Maonan/SWL1536/2019 (IVR-215) | 1A.3.3.2, 1A.5a.1 | 40 | 2560 | 10240 | 1280 | 320 | 40 |
| A/Victoria/2570/2019 (IVR-215) | 1A.3.3.2, 1A.5a.2 | 80 | 160 | 640 | 10240 | 5120 | 160 |
| A/Victoria/4897/2022 (IVR-238) | 1A 3.3.2, 5a.2a.1 | 160 | 80 | 640 | 10240 | 10240 | 320 |
| A/swine/Italy/63457-01/2024 | 1A.3.3.2 | 80 | 80 | 1280 | 10240 | 5120 | 80 |
| A/Ohio/9/2015 CVV | H1N1 1A.3.3.3 | 20 | 40 | 160 | 80 | 20 | 320 |

Reference CVV in red, reference seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted gray.

- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine retained reactivity with A/Victoria/4897/2022 5a.2a.1 vaccine strain ferret anti-sera.

Table 3. Amino acid substitutions between representative swine 1A.1.1.3 clade strains compared to the within-clade CVVs (A/California/71/2021 and A/Ohio/24/2017) and recent 1A.1.1.3 variants.

| site | A/California/71/2021 | A/Ohio/24/2017 | A/swine/Ohio/A02750994/2022 | A/swine/North_Carolina/A02750921/2022 | A/Ohio/251/2024 | A/Pennsylvania/228/2024 | A/Pennsylvania/242/2024 | A/Pennsylvania/27/2024 | annotations |
|--------|----------------------|----------------|-----------------------------|---------------------------------------|-----------------|-------------------------|-------------------------|------------------------|-------------|
| 48 | A | | S | S | S | S | S | S | |
| 69 | P | S | S | S | S | S | S | S | |
| 70 | I | | | | T | | | | Cb |
| 71 | P | L | L | L | L | L | L | L | Cb |
| 84 | N | | | | | | | S | |
| 97 | N | | | | | D | D | | |
| 103 | V | E | E | E | E | E | E | E | |
| 119 | T | I | I | I | I | I | I | I | |
| 124 | S | P | P | | P | P | P | A | Sa |
| 126 | H | | Y | Y | Y | Y | Y | Y | |
| 127 | E | K | K | K | K | K | K | K | |
| 128 | T | | | | | | | K | |
| 132 | A | V | E | E | E | E | E | E | RBS |
| 138 | Y | D | | | | | | | |
| 140 | G | | | E | | | | | Ca2 |
| 141 | K | A | | | | | | | |
| 142 | S | | | | N | | | G | Ca2 |
| 143 | S | | | | | G | G | | |
| 149 | M | I | | V | | | | | |
| 155 | G | | A | A | A | A | A | A | Sa |
| 156 | D | N | | | | | | | Sb |
| 157 | S | | | L | | | | | Sa |
| 160 | M | R | R | K | R | R | R | R | Sa |
| 166 | A | V | V | V | V | V | V | V | Ca1 |
| 168 | D | N | N | | N | N | N | N | |
| 170 | G | R | | | | | | | Ca1 |
| 185 | D | G | G | | G | G | G | G | |
| 186 | S | N | N | N | N | N | N | N | |
| 189 | R | Q | Q | Q | Q | Q | Q | Q | Sb, RBS |
| 195 | T | A | A | A | A | A | A | A | Sb, RBS |
| 209 | K | E | | | | | | | |
| 222 | N | G | G | G | G | G | G | G | Ca2, RBS |
| 224 | A | T | | | | | | | RBS |
| 232 | A | T | T | T | T | T | T | T | |
| 235 | E | | | | A | | | | |
| 252 | R | K | | | | | | | |
| 261 | S | | | | P | | | | |
| 272 | I | | | T | | | | | |
| 273 | H | | | | | | | Y | |
| 283 | K | | | | E | | | | |
| 286 | L | | | | | I | I | | |
| 298 | V | | | | | | | I | |
| 308 | R | K | K | K | K | K | K | K | |
| 310 | T | | R | R | R | R | R | R | |
| 311 | R | K | | | | | | | |
| aadiff | | 26 | 21 | 22 | 26 | 24 | 24 | 26 | |

Reference CVVs in red, HI swine strains in purple.

Table 4. Amino acid substitutions between representative swine 1A.2 strain compared to the A/Wisconsin/67/2022, the nearest HI-tested human vaccine.

| site | A/Wisconsin/67/2022 | A/swine/Quebec/N-2020-3-24/2020 | annotations |
|------|---------------------|---------------------------------|-------------|
| 35 | D | N | |
| 54 | Q | K | |
| 61 | I | L | |
| 71 | S | F | Cb |
| 73 | A | R | Cb |
| 83 | S | P | |
| 85 | S | P | |
| 104 | Q | H | |
| 109 | S | L | |
| 120 | T | A | |
| 121 | S | N | |
| 128 | S | T | |
| 129 | D | N | |
| 130 | N | K | |
| 138 | H | Y | |
| 142 | R | N | Ca2 |
| 156 | K | N | Sb |
| 161 | I | L | |
| 162 | N | S | Sa |
| 163 | Q | K | Sa |
| 164 | T | S | Sa |
| 168 | D | N | |
| 170 | G | K | Ca1 |
| 176 | L | I | |
| 185 | I | S | |
| 189 | E | Q | Sb, RBS |
| 190 | S | T | Sb, RBS |

cont. →

| site | A/Wisconsin/67/2022 | A/swine/Quebec/N-2020-3-24/2020 | annotations |
|--------|---------------------|---------------------------------|-------------|
| 195 | A | E | Sb, RBS |
| 203 | T | S | |
| 205 | R | K | |
| 216 | T | A | |
| 234 | V | I | |
| 235 | E | D | |
| 239 | K | T | |
| 250 | A | V | |
| 256 | T | A | |
| 259 | K | R | |
| 260 | E | G | |
| 261 | A | S | |
| 270 | T | K | |
| 273 | H | Q | |
| 274 | D | N | |
| 276 | N | T | |
| 277 | A | T | |
| 283 | E | K | |
| 285 | A | S | |
| 295 | V | I | |
| 298 | I | V | |
| 302 | K | E | |
| 308 | R | K | |
| 311 | K | N | |
| 314 | L | M | |
| 321 | V | I | |
| aadiff | | 53 | |

Reference vaccine strain in gray, HI strain in purple.

Table 5. Amino acid substitutions between representative swine 1A.3.3.2 clade strains compared to two current WHO recommended human seasonal vaccines used in HI assays (A/Wisconsin/67/2022 and A/Victoria/4897/2022) and additional HI-tested seasonal vaccine strains and CVV.

| site | A/Wisconsin/67/2022 | A/Victoria/4897/2022 | A/California/07/2009 | A/Victoria/2570/2019 | A/Guangdong-Maonan/SWL1536/2019 | A/swine/Iowa/A02524480/2020 | A/swine/Missouri/A02861595/2023 | A/swine/Italy/63457-01/2024 | annotations |
|--------|---------------------|----------------------|----------------------|----------------------|---------------------------------|-----------------------------|---------------------------------|-----------------------------|-------------|
| 47 | V | | | | | | | I | |
| 54 | Q | | K | K | K | K | | | |
| 74 | R | | S | | | | | | Cb |
| 83 | S | | P | | | | | | |
| 84 | N | | S | | | | | | |
| 96 | I | | | | | | | T | |
| 97 | N | | D | | | | | | |
| 112 | E | | | | | | D | | |
| 113 | R | | | | | K | | | |
| 120 | T | | | | | | | A | |
| 127 | D | | | | | | N | | |
| 129 | D | | N | | | N | | | |
| 130 | N | | K | | K | K | | | |
| 137 | S | | P | P | P | | | P | Ca2 |
| 142 | R | | K | K | K | K | | K | Ca2 |
| 156 | K | | N | | N | N | Q | | Sb |
| 161 | I | | L | | L | L | | | |
| 162 | N | | S | | | | | | Sa |
| 163 | Q | | K | | | | | | Sa |
| 164 | T | | S | | | | | | Sa |
| 173 | V | | | | | I | | | |
| 183 | P | | S | | | | | | |
| 185 | I | | S | | | T | | | |
| 186 | T | | A | A | A | A | | | |
| 187 | D | | | | A | | | | RBS |
| 189 | E | | Q | Q | | Q | | | Sb, RBS |
| 203 | T | | S | | | | | | |
| 216 | T | A | I | | | | A | | |
| 217 | R | | | | | | X | | |
| 223 | Q | R | | R | | | | | RBS |
| 224 | A | | E | E | E | E | | | RBS |
| 250 | A | | V | | V | V | | | |
| 256 | T | | A | | | | | | |
| 259 | K | | R | R | R | R | | | |
| 260 | E | | N | D | D | N | | D | |
| 277 | A | | T | T | T | T | | T | |
| 283 | E | | K | | | | | | |
| 295 | V | | I | | | | | | |
| 308 | R | | K | K | K | K | | | |
| 321 | V | | I | | | | | | |
| aadiff | | 2 | 30 | 11 | 14 | 17 | 5 | 7 | |

Reference vaccine strains in gray, HI strains in purple.

Table 6. Amino acid substitutions between representative 1A.3.3.3-c3 swine strains compared to nearest HI-tested CVVs (A/Wisconsin/03/2021 and A/Ohio/9/2015).

| site | A/Wisconsin/03/2021 | A/Ohio/9/2015 | A/swine/Minnesota/A02245409/2020 | A/swine/Minnesota/A02712157/2022 | annotations |
|--------|---------------------|---------------|----------------------------------|----------------------------------|-------------|
| 2 | T | K | | | |
| 3 | L | I | | | |
| 35 | T | N | N | N | |
| 36 | R | K | | | |
| 45 | K | R | R | R | |
| 48 | A | X | | | |
| 56 | N | | | S | |
| 71 | S | A | | | Cb |
| 74 | S | | | R | Cb |
| 83 | S | | P | | |
| 84 | N | S | | K | |
| 86 | E | N | | | |
| 113 | R | K | | | |
| 120 | T | | A | | |
| 127 | E | | D | D | |
| 129 | D | N | N | N | |
| 130 | K | | R | | |
| 137 | P | | S | | Ca2 |
| 142 | R | N | N | N | Ca2 |
| 146 | R | K | | | |
| 149 | V | I | | | |
| 153 | Q | K | | | Sb |
| 155 | G | E | | | Sa |
| 161 | V | I | | I | |
| 163 | T | I | K | K | Sa |
| 166 | I | T | | | Ca1 |
| 168 | N | | | D | |
| 169 | K | R | R | | |
| 170 | E | G | | | Ca1 |
| 171 | K | | | R | |
| 183 | S | P | | | |
| 186 | A | T | | | |
| 189 | R | Q | Q | Q | Sb, RBS |
| 193 | K | Q | | | Sb, RBS |
| 195 | E | A | A | A | Sb, RBS |
| 196 | D | N | | | |
| 197 | A | S | | | |
| 205 | R | | K | K | |
| 222 | D | G | | | Ca2, RBS |
| 224 | S | A | A | A | RBS |
| 250 | A | V | | | |
| 252 | R | | | I | |
| 262 | G | | | E | |
| 269 | D | E | | | |
| 270 | A | T | T | T | |
| 271 | S | P | | | |
| 278 | T | | | A | |
| 295 | I | | | V | |
| 302 | E | | | K | |
| 326 | S | | | T | |
| aadiff | | 34 | 16 | 23 | |

Reference CVVs in red, HI swine strains in purple.

1B human-like lineage

- ★ H1v candidate vaccine virus
- A/Brisbane/59/2007
- Previous seasonal H1N1 vaccine
- Reported H1v cases
- + New variant(s)
- # HI tested viruses
- Requested/pending HI

H1 1B swine genetic clades

- 1B.2.1 (n=120)
- 1B.2.2.2 (n=16)
- 1B.2.2.1 (n=1)
- 1B.2.7 (n=6)
- 1B.1.2.1 (n=11)
- 1B.1.2.2 (n=1)
- 1B.1.1.1 (n=3)
- 1B.1.1.2 (n=3)

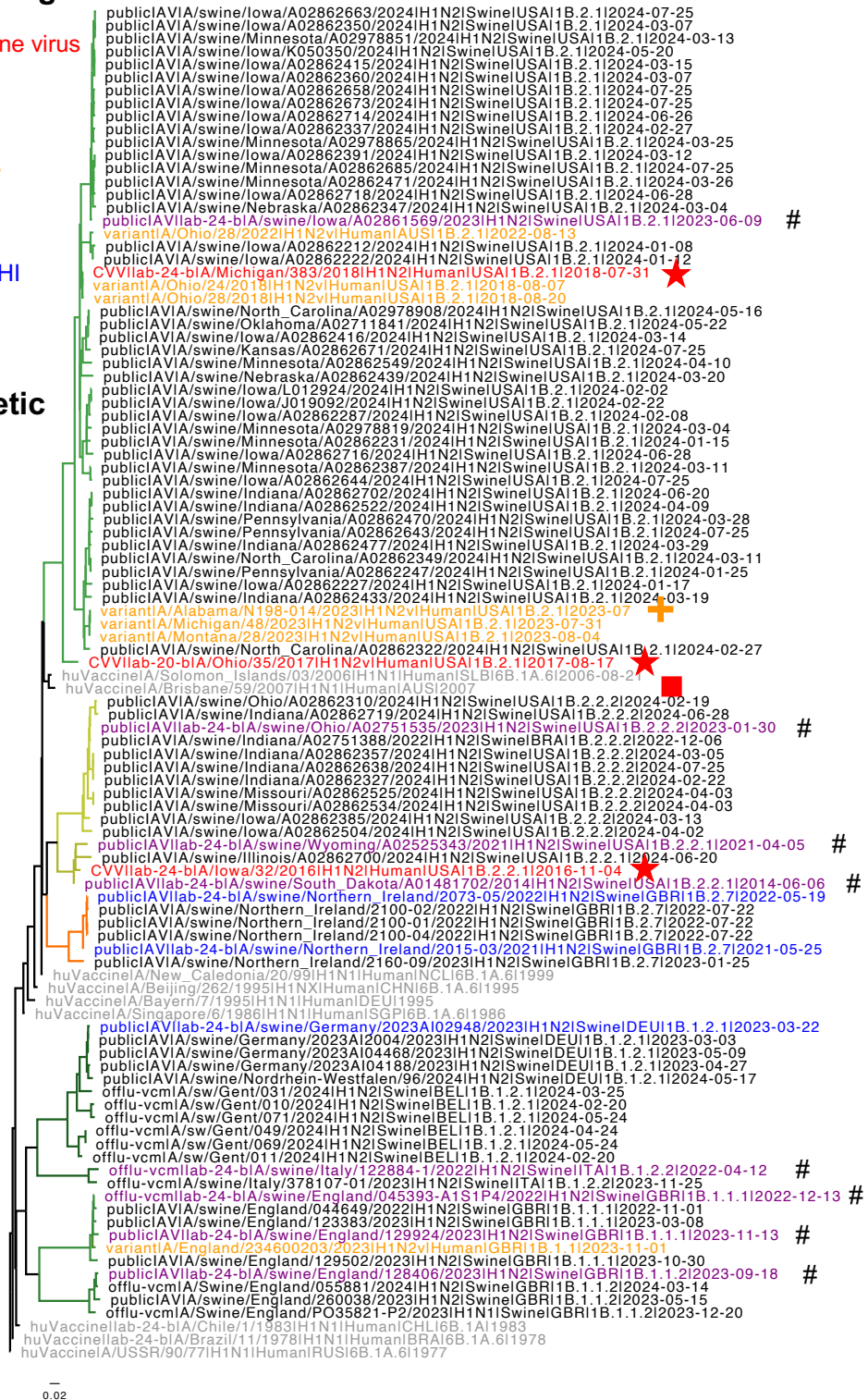


Figure 8. Swine H1 HA genes of the 1B lineage (tree was proportionally down sampled to 85 swine HA genes, excluding references). Number of detections of each clade from data deposited on or after January 1, 2024 are presented adjacent to the clade name in the figure key.

Antigenic analysis: Swine 1B Lineage

Table 7. USA Data: Hemagglutination inhibition of CVV vaccine ferret antisera against contemporary swine 1B lineage strains selected to represent clade consensus.

- > 8 fold reduction
- 8 fold reduction
- 4 fold reduction

| | Global Clade | US Clade | A/Michigan/383/2018 RG58A | A/Iowa/32/2016 | A/swine/Wyoming/A02525343/2021** #15 | A/swine/Wyoming/A02525343/2021* #16 | Human Pooled Sera |
|-------------------------------------|--------------|----------|---------------------------|----------------|--------------------------------------|-------------------------------------|-------------------|
| A/Michigan/383/2018 RG58A | 1B.2.1 | delta-2 | 160 | <10 | <10 | <10 | 80 |
| A/swine/Iowa/A02861569/2023 | 1B.2.1 | delta-2 | 160 | 40 | 10 | 10 | 40 |
| A/Iowa/32/2016 | 1B.2.2.1 | delta-1a | <10 | 160 | 40 | 20 | 80 |
| A/swine/Wyoming/A02525343/2021 | 1B.2.2.1 | delta-1a | <10 | 20 | 160 | 80 | 80 |
| A/swine/South_Dakota/A01481702/2014 | 1B.2.2.1 | delta-1a | <10 | 10 | 20 | <10 | 80 |
| A/swine/Ohio/A02751535/2023* | 1B.2.2.2 | delta-1b | <10 | 10 | <10 | <10 | 20 |

Reference CVV in red, new swine strains in bold. Homologous titers highlighted gray. Human sera were collected in USA with titer relative to A/Wisconsin/67/2022, pool of 2 male and 2 female adults.

- The 1B.2.1 (delta-2) clade swine representative had no loss of titer to the within clade CVV and had a 4-fold loss in cross-reactivity to human pooled sera.
- The 1B.2.2.1 (delta-1a) clade swine representative had 8-fold reduction from the within clade CVV A/Iowa/32/2016 but retained reactivity to human pooled sera.
- The contemporary swine 1B.2.2.2 (delta-1b) does not have a within-clade CVV and had a >8-fold decrease from CVV A/Iowa/32/2016 and had an 8-fold loss in cross-reactivity to human pooled sera.

Table 8. EU Study Design: Hemagglutination inhibition of CVV vaccine ferret antisera against contemporary swine 1B lineage strains selected to represent clade consensus.

| Global Clade | | A/Chile/1/1983 | A/Brazil/11/1978 | A/Michigan/383/2018 CVV |
|--|-------------|----------------|------------------|-------------------------|
| A/Chile/1/1983 | H1N1 1B | 640 | 640 | 20 |
| A/Brazil/11/1978 | H1N1 1B | 320 | 2560 | 40 |
| A/swine/England/045393-A1S1P4/2022* | 1B.1.1.1 | 10 | 80 | 40 |
| A/Swine/England/129924/2023* | 1B.1.1.1 | 20 | 320 | 80 |
| A/Swine/England/128406/2023* | 1B.1.1.2 | 320 | 5120 | 160 |
| A/swine/Italy/122884-1/2022 H1N2* | 1B.1.2.2 | 80 | 320 | 40 |
| A/Michigan/383/2018 CVV | H1N2 1B.2.1 | 160 | 640 | 2560 |
| A/swine/Northern Ireland/2015-03/2021 | 1B.2.7 | 40 | 40 | 80 |
| A/swine/Northern Ireland/2073-05/2022 | 1B.2.7 | 40 | 40 | 80 |

- >8 fold reduction
- 8 fold reduction
- 4 fold reduction

*Previously tested in report 2024a. Reference CVV in red, reference seasonal vaccine strains in dark red. New swine strains are bolded. Homologous titers highlighted gray.

- There are no within-clade CVVs for 1B lineages from Europe and the 1B.2.1 CVV has >8-fold loss in reactivity with 1B.1 representative swine strains.
- The swine 1B.1.1.1 had >8-fold loss in cross-reactivity with A/Chile/1/1983 and A/Brazil/11/1978.
- The swine 1B.1.1.2 strain retained cross-reactivity with the ancestral human seasonal reference strains.
- The contemporary swine 1B.1.2.2 had >8-fold loss in cross-reactivity against the human seasonal reference strains.
- The swine 1B.2.7 clade viruses had no within-clade CVV and >8-fold loss in cross-reactivity with A/Chile/1/1983 and A/Brazil/11/1978.

Table 9. Amino acid substitutions between representative swine 1B.1.x strains compared to most similar HI-tested vaccine strains (A/Chile/1/1983 and A/Brazil/11/1978).

| site | A/Chile/1/1983 | A/Brazil/11/1978 | A/swine/England/045393-A1S1P4/2022 | A/swine/England/128406/2023 | A/swine/England/129924/2023 | A/swine/Italy/122884-1/2022 | annotations |
|------|----------------|------------------|------------------------------------|-----------------------------|-----------------------------|-----------------------------|-------------|
| 14 | D | | E | | E | | |
| 19 | V | | | I | | | |
| 35 | D | | | N | | | |
| 36 | N | S | S | S | S | | |
| 43 | K | R | | | | | |
| 44 | L | | | M | | | |
| 47 | I | | | | | V | |
| 48 | A | | P | | P | | |
| 54 | K | | T | | T | R | |
| 66 | E | | K | | K | | |
| 70 | L | | | S | | | Cb |
| 71 | F | | | | | L | Cb |
| 73 | K | | | R | | | Cb |
| 74 | K | | N | | N | | Cb |
| 82 | T | | L | | S | A | |
| 85 | S | | P | P | P | A | |
| 89 | T | | I | I | I | L | |
| 94 | Y | | H | | H | H | |
| 96 | A | | S | S | S | S | |
| 111 | F | | | I | | I | |
| 116 | I | | | M | | | |
| 120 | E | | D | | D | | |
| 121 | S | R | | | | | |
| 125 | K | | G | N | G | | Sa |
| 127 | N | | | | | S | |
| 128 | V | I | | | | I | |
| 129 | T | | | | | - | |
| 130 | K | R | | R | | - | |
| 132 | V | | | K | L | A | RBS |
| 134 | A | | | V | | | RBS |
| 135 | A | S | S | S | S | S | RBS |
| 137 | S | | P | | P | P | Ca2 |
| 138 | H | | | | | K | |
| 139 | K | | D | N | D | Q | |
| 141 | K | | | | | R | |
| 146 | R | | | K | | T | |
| 149 | L | | V | | V | | |
| 153 | E | | G | | G | | Sb |
| 155 | N | | | D | | | Sa |
| 156 | G | | N | | N | | Sb |
| 157 | S | | L | L | L | | Sa |
| 161 | L | | V | I | V | M | |
| 162 | S | | N | | N | T | Sa |
| 163 | K | | | N | | M | Sa |
| 164 | S | | | R | | | Sa |
| 166 | V | | | | | M | Ca1 |
| 168 | N | | | | | D | |
| 170 | E | | | | G | R | Ca1 |
| 171 | K | | | | | R | |

cont.

| site | A/Chile/1/1983 | A/Brazil/11/1978 | A/swine/England/045393-A1S1P4/2022 | A/swine/England/128406/2023 | A/swine/England/129924/2023 | A/swine/Italy/122884-1/2022 | annotations |
|--------|----------------|------------------|------------------------------------|-----------------------------|-----------------------------|-----------------------------|-------------|
| 176 | L | | | | I | | |
| 181 | H | | | | N | | |
| 185 | I | | | | M | | |
| 189 | K | | | | R | | R |
| 190 | T | | A | A | A | A | Sb, RBS |
| 193 | R | | H | | H | | Sb, RBS |
| 194 | K | | N | | N | | RBS |
| 196 | N | | | T | | T | |
| 197 | A | | T | | T | | |
| 202 | V | | | | | | M |
| 205 | H | N | | | | | |
| 207 | N | | | | | | S |
| 208 | R | | | | K | K | |
| 209 | R | | K | | K | K | |
| 215 | A | | | E | | | |
| 216 | K | | R | | R | | |
| 220 | V | | I | I | I | I | RBS |
| 222 | N | G | | | | D | Ca2, RBS |
| 237 | G | | D | S | D | S | Ca1 |
| 238 | D | | E | E | E | | |
| 239 | T | | E | I | E | | |
| 248 | L | | | F | | | |
| 252 | W | | Q | | L | | |
| 258 | S | | | | | N | |
| 260 | G | | S | | S | | |
| 261 | F | | | L | | | |
| 262 | G | | | R | | E | |
| 267 | T | | I | I | I | V | |
| 270 | A | | T | | T | V | |
| 272 | M | | | L | | | |
| 273 | D | | | G | | | |
| 276 | D | | N | | N | N | |
| 277 | A | T | T | T | T | | |
| 278 | K | | | E | | T | |
| 283 | Q | | | | | R | |
| 287 | N | | | | T | | |
| 288 | S | | | N | | N | |
| 295 | V | | | | | I | |
| 298 | V | | | | | I | |
| 299 | T | | | A | | | |
| 305 | K | | | R | | | |
| 307 | V | | | I | | | |
| 308 | R | | K | K | K | K | |
| 310 | T | | | | K | | |
| 311 | K | | | R | | | |
| 313 | R | | | | | K | |
| 315 | V | | | | | A | |
| 324 | I | | | V | | | |
| aadiff | | 9 | 39 | 49 | 44 | 50 | |

Reference human seasonal vaccines in gray, HI swine strains in purple.

Table 10. Amino acid substitutions between representative 1B.2.1 swine strain compared to the within-clade CVV (A/Michigan/383/2018 RG58A) and a recent 1B.2.1 variant.

| site | A/Michigan/383/2018 | A/swine/Iowa/A02861569/2023 | A/Alabama/N198-014/2023 | annotations |
|--------|---------------------|-----------------------------|-------------------------|-------------|
| 5 | V | | I | |
| 16 | V | | I | |
| 69 | L | | M | |
| 71 | N | T | I | Cb |
| 82 | T | | K | |
| 83 | S | A | P | |
| 89 | T | I | A | |
| 112 | K | E | | |
| 113 | K | | R | |
| 119 | K | | E | |
| 120 | K | E | | |
| 141 | N | E | | |
| 168 | K | N | T | |
| 169 | E | | K | |
| 170 | G | | E | Ca1 |
| 173 | V | I | I | |
| 185 | M | | I | |
| 193 | H | R | N | Sb, RBS |
| 224 | E | | G | RBS |
| 237 | G | | K | Ca1 |
| 256 | E | K | | |
| 258 | S | N | | |
| 260 | S | | G | |
| 277 | T | | A | |
| 310 | A | | T | |
| aadiff | | 11 | 20 | |

Reference CVV strain in red, HI swine strain in purple, variant in orange.

Table 11. Amino acid substitutions between representative swine 1B.2.2.1 strains compared to the within-clade CVV (A/Iowa/32/2016).

| site | A/Iowa/32/2016 | A/swine/Wyoming/A02525343/2021 | A/swine/South_Dakota/A01481702/2014 | annotations |
|--------|----------------|--------------------------------|-------------------------------------|-------------|
| 19 | L | V | V | |
| 72 | S | P | | |
| 74 | K | E | E | Cb |
| 96 | T | A | A | |
| 106 | S | N | | |
| 130 | T | D | | |
| 132 | K | T | V | RBS |
| 141 | E | | K | |
| 157 | L | R | | Sa |
| 166 | K | | E | Ca1 |
| 168 | E | D | D | |
| 175 | I | V | | |
| 208 | R | K | | |
| 215 | T | A | | |
| 222 | D | N | | Ca2, RBS |
| 241 | I | V | | |
| 287 | N | T | | |
| 310 | T | R | | |
| aadiff | | 16 | 7 | |

Reference CVV strain in red, HI swine strains in purple.

Table 12. Amino acid substitutions between representative swine 1B.2.2.2 strain compared to the nearest tested CVV (A/Iowa/32/2016) in USA HI assays and the most similar human vaccine (A/Michigan/2/2003).

| Relative to HI | | | | Relative to most similar vaccine | | | |
|----------------|----------------|-----------------------------|-------------|----------------------------------|-------------------|-----------------------------|-------------|
| site | A/Iowa/32/2016 | A/swine/Ohio/A02751535/2023 | annotations | site | A/Michigan/2/2003 | A/swine/Ohio/A02751535/2023 | annotations |
| 19 | L | V | | 36 | S | N | |
| 36 | S | N | | 50 | L | I | |
| 50 | L | I | | 69 | L | S | |
| 82 | A | T | | 74 | E | K | Cb |
| 85 | S | P | | 94 | Y | D | |
| 86 | D | E | | 129 | V | T | |
| 94 | Q | D | | 132 | V | K | RBS |
| 96 | T | A | | 141 | K | E | |
| 119 | R | K | | 146 | R | K | |
| 129 | V | T | | 149 | L | V | |
| 142 | G | S | Ca2 | 153 | G | E | Sb |
| 146 | R | K | | 162 | I | S | Sa |
| 149 | I | V | | 166 | A | K | Ca1 |
| 153 | V | E | Sb | 168 | N | D | |
| 168 | E | D | | 175 | I | V | |
| 170 | G | E | Ca1 | 176 | L | I | |
| 175 | I | V | | 184 | N | D | |
| 176 | L | I | | 186 | G | E | |
| 183 | S | P | | 187 | D | N | RBS |
| 184 | N | D | | 193 | H | R | Sb, RBS |
| 186 | G | E | | 194 | T | K | RBS |
| 187 | D | N | RBS | 202 | V | A | |
| 193 | H | R | Sb, RBS | 208 | R | E | |
| 194 | T | K | RBS | 222 | D | N | Ca2, RBS |
| 202 | M | A | | 228 | N | K | |
| 208 | R | E | | 236 | P | A | |
| 222 | D | N | Ca2, RBS | 244 | A | S | |
| 228 | N | K | | 249 | I | V | |
| 236 | P | A | | 256 | A | T | |
| 244 | T | S | | 261 | F | L | |
| 249 | I | V | | 271 | P | S | |
| 256 | A | T | | 273 | D | N | |
| 269 | K | N | | 274 | E | Q | |
| 271 | P | S | | 277 | A | T | |
| 273 | D | N | | aadiff | | 34 | |
| 274 | E | Q | | | | | |
| 277 | A | T | | | | | |
| 283 | K | Q | | | | | |
| 289 | N | S | | | | | |
| 310 | T | A | | | | | |
| aadiff | | 40 | | | | | |

Reference CVV in red, reference human seasonal vaccine in gray, HI swine strain in purple.

Table 13. Amino acid substitutions between representative swine 1B.2.7 strains compared to the nearest tested CVV (A/Iowa/32/2016) in HI assays and the most similar human vaccine (A/New_Caledonia/20/99).

| Relative to HI | | | | | Relative to most similar vaccine | | | | | |
|----------------|----------------|---------------------------------------|---------------------------------------|---------------------------------------|----------------------------------|--------|-----------------------|---------------------------------------|---------------------------------------|-------------|
| site | A/Iowa/32/2016 | A/swine/Northern_Ireland/2015-03/2021 | A/swine/Northern_Ireland/2015-03/2021 | A/swine/Northern_Ireland/2073-05/2022 | annotations | site | A/New_Caledonia/20/99 | A/swine/Northern_Ireland/2015-03/2021 | A/swine/Northern_Ireland/2073-05/2022 | annotations |
| 19 | L | V | V | V | | 24 | V | I | I | |
| 24 | V | I | I | I | | 30 | V | I | I | |
| 30 | V | I | I | I | | 43 | L | S | S | |
| 43 | L | S | S | S | | 44 | L | I | I | |
| 44 | L | I | I | I | | 68 | E | K | K | |
| 68 | E | K | K | K | | 69 | L | S | S | |
| 72 | S | P | P | P | | 72 | S | P | P | |
| 73 | K | Q | Q | Q | Cb | 73 | K | Q | Q | Cb |
| 74 | K | E | E | E | Cb | 86 | E | K | K | |
| 82 | A | T | T | T | | 89 | T | A | A | |
| 85 | S | P | P | P | | 94 | Y | H | H | |
| 86 | D | K | K | K | | 96 | A | D | D | |
| 94 | Q | H | H | H | | 112 | E | K | K | |
| 96 | T | D | D | D | | 141 | K | E | E | |
| 112 | E | K | K | K | | 142 | S | R | R | Ca2 |
| 119 | R | K | K | K | | 153 | G | E | E | Sb |
| 132 | K | V | V | V | RBS | 163 | K | M | M | Sa |
| 142 | G | R | R | R | Ca2 | 166 | V | S | S | Ca1 |
| 149 | I | L | L | L | | 173 | V | I | I | |
| 153 | V | E | E | E | Sb | 175 | V | I | I | |
| 163 | K | M | M | M | Sa | 184 | N | D | D | |
| 166 | K | S | S | S | Ca1 | 186 | G | Q | Q | |
| 168 | E | N | N | N | | 190 | A | T | T | Sb, RBS |
| 170 | G | E | E | E | Ca1 | 197 | A | T | T | |
| 173 | V | I | I | I | | 215 | A | T | T | |
| 183 | S | P | P | P | | 237 | G | E | E | Ca1 |
| 184 | N | D | D | D | | 258 | S | D | D | |
| 186 | G | Q | Q | Q | | 271 | P | S | S | |
| 187 | D | N | N | N | RBS | 273 | D | S | S | |
| 197 | A | T | T | T | | 277 | A | T | T | |
| 202 | M | V | V | V | | 289 | S | R | R | |
| 237 | G | E | E | E | Ca1 | 295 | V | I | I | |
| 244 | T | A | A | A | | 306 | Y | F | F | |
| 258 | S | D | D | D | | 321 | I | T | T | |
| 261 | L | F | F | F | | aadiff | | 32 | 33 | |
| 269 | K | N | N | N | | | | | | |
| 271 | P | S | S | S | | | | | | |
| 273 | D | S | S | S | | | | | | |
| 277 | A | T | T | T | | | | | | |
| 283 | K | Q | Q | Q | | | | | | |
| 289 | N | R | R | R | | | | | | |
| 295 | V | I | I | I | | | | | | |
| 306 | Y | F | F | F | | | | | | |
| 310 | T | A | A | A | | | | | | |
| 321 | I | T | T | T | | | | | | |
| aadiff | | 43 | 44 | | | | | | | |

Reference human seasonal vaccine strain in gray, HI swine strains in purple.

1C Eurasian avian lineage

★ H1v candidate vaccine virus

Reported H1v cases

+ New variant(s)

HI tested viruses

Requested/pending HI

H1 1C swine genetic clades

● 1C.2.4.3 (n=43)

● 1C.2.4.2 (n=37)

● 1C.2.4.1 (n=31)

● 1C.2.1 (n=28)

● 1C.2.2 (n=71)

● 1C.2.3 (n=3)

● 1C.2.5 (n=5)

● 1C.2 (n=1)

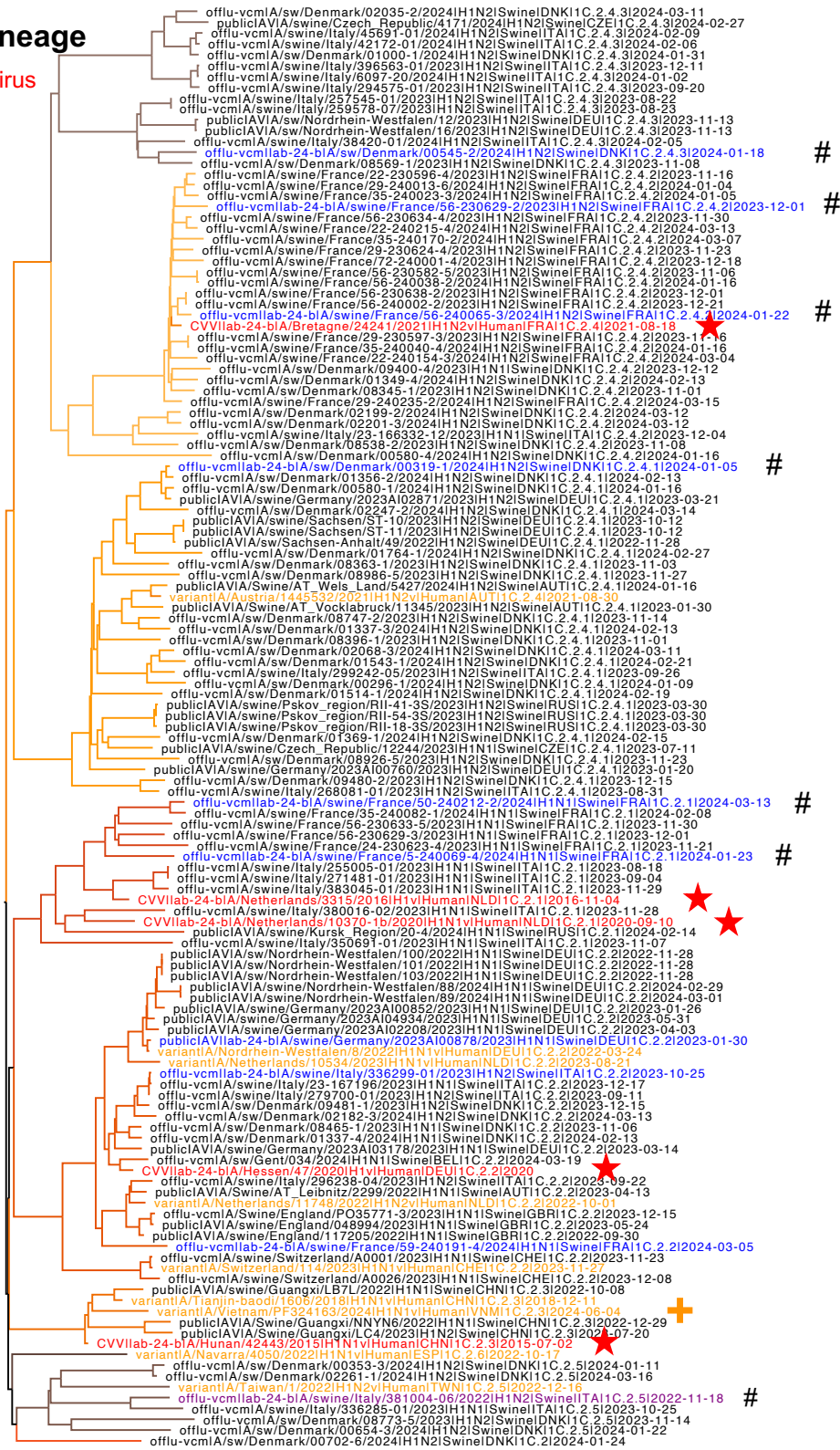


Figure 9. Swine H1 HA genes of the 1C lineage (tree was proportionately downsampled to 109 swine HA sequences, excluding references). Number of detections of each clade from data deposited on or after January 1, 2024 are presented adjacent to the clade name in the figure key.

Antigenic analysis: Swine 1C Lineage

Table 14. UK & EU Study Design: Hemagglutination inhibition of CVV vaccine ferret antisera against contemporary swine 1C lineage strains selected to represent clade consensus.

| Global Clade | | A/Netherlands/3315/2016 CVV | A/Netherlands/10370-1b/2020 CVV | A/Hessen/47/2020-like CVV | A/Hunan/42443/2015 CNIC-1601 CVV | A/Bretagne/24241/2021 CVV |
|---|----------|-----------------------------|---------------------------------|---------------------------|----------------------------------|---------------------------|
| A/Netherlands/3315/2016 CVV | 1C.2.1 | 640 | 640 | 1280 | 20 | 80 |
| A/Netherlands/10370-1b/2020 CVV | 1C.2.1 | 40 | 640 | 640 | 80 | 20 |
| A/swine/France/5-240069-4/2024 | 1C.2.1 | 160 | 320 | 320 | 20 | 160 |
| A/swine/France/50-240212-2/2024 | 1C.2.1 | 160 | 320 | 320 | 20 | 20 |
| A/Hessen/47/2020-like CVV | 1C.2.2 | 40 | 160 | 5120 | 640 | 20 |
| A/Hunan/42443/2015 CNIC-1601 CVV | 1C.2.3 | 160 | 320 | 5120 | 1280 | 40 |
| A/swine/Denmark/00319-1/2024 | 1C.2.4.1 | 80 | 640 | 320 | 40 | 320 |
| A/Bretagne/24241/2021 CVV | 1C.2.4.2 | 160 | 160 | 160 | 40 | 10240 |
| A/swine/France/56-230629-2/2023 | 1C.2.4.2 | 80 | 160 | 1280 | 40 | 10240 |
| A/swine/France/50-2400122-4/2024 | 1C.2.4.2 | 80 | 320 | 320 | 40 | 2560 |
| A/swine/France/56-240065-3/2024 | 1C.2.4.2 | 80 | 160 | 640 | 40 | 5120 |
| A/swine/Denmark/00545-2/2023 | 1C.2.4.3 | 20 | 160 | 640 | 20 | 40 |

- > 8 fold reduction
- 8 fold reduction
- 4 fold reduction

Reference CVV in red, new swine strains in bold. Homologous titers highlighted in gray. *Previously tested in report 2024a.

- The 1C.2.1 strain retained cross-reactivity to the 1C.2.1 CVV A/Netherlands/10370-1b/2020.
- The 1C.2.4.1 swine strain from Denmark retained cross-reactivity with a different clade CVV strain (1C.2.1: A/Netherlands/10370-1b/2020).
- The 1C.2.4.2 swine strains retained reactivity to the within-clade CVV A/Bretagne/24241/2021.
- The representative swine 1C.2.4.3 had no within-clade CVV and demonstrated a range of 4- to >8-fold loss to available 1C CVV strains.

Table 15. Amino acid substitutions between representative swine 1C.2.1 strains compared to within-clade CVVs (A/Netherlands/3315/2016 and A/Netherlands/10370-1b/2020).

| site | A/Netherlands/3315/2016 | A/Netherlands/10370-1b/2020 | A/swine/France/5-240069-4/2024 | A/swine/France/50-240212-2/2024 | annotations |
|--------|-------------------------|-----------------------------|--------------------------------|---------------------------------|-------------|
| 20 | M | | L | L | |
| 31 | N | D | | | |
| 44 | L | M | | | |
| 51 | Q | | | H | |
| 69 | L | | S | S | |
| 71 | I | L | | L | Cb |
| 74 | N | K | | | Cb |
| 80 | I | | | V | |
| 83 | S | P | | | |
| 84 | N | | K | K | |
| 96 | A | | T | T | |
| 97 | D | N | N | | |
| 120 | E | A | A | A | |
| 125 | N | | S | | Sa |
| 132 | S | T | | | RBS |
| 134 | V | | I | | RBS |
| 136 | C | | | X | |
| 137 | S | P | T | T | Ca2 |
| 141 | I | A | V | V | |
| 142 | K | N | N | X | Ca2 |
| 152 | V | | I | | |
| 155 | E | G | A | A | Sa |
| 161 | I | L | | | |
| 162 | S | | | N | Sa |
| 163 | T | K | K | E | Sa |
| 169 | K | | | R | |
| 175 | I | V | V | V | |
| 179 | V | | I | | |
| 190 | T | | | A | Sb, RBS |
| 199 | V | I | | | |
| 202 | G | E | E | E | |
| 204 | S | | P | | Ca1 |
| 208 | K | Q | | | |
| 214 | I | | | V | |
| 215 | V | A | I | | |
| 220 | V | | I | | RBS |
| 236 | Q | | | K | |
| 253 | Y | H | | | |
| 258 | N | D | | | |
| 262 | N | | | S | |
| 266 | M | | | V | |
| 267 | M | I | | | |
| 271 | N | H | | S | |
| 272 | V | | | I | |
| 273 | H | | | Q | |
| 288 | S | | | G | |
| 298 | I | | | L | |
| 311 | Q | R | | | |
| 321 | T | I | V | I | |
| 324 | V | I | I | I | |
| aadiff | | 26 | 22 | 30 | |

Reference CVVs in red, HI swine strains in purple.

Table 16. Amino acid substitutions between recent 1C.2.3 variant compared to within-clade CVV (A/Hunan/42443/2015).

| site | A/Hunan/42443/2015 | A/Vietnam/PF324163/2024 | annotations |
|--------|--------------------|-------------------------|-------------|
| 31 | N | D | |
| 47 | K | Q | |
| 54 | N | K | |
| 56 | S | N | |
| 66 | K | E | |
| 86 | K | T | |
| 107 | T | A | |
| 108 | V | I | |
| 171 | K | R | |
| 253 | H | Y | |
| 258 | K | R | |
| 302 | E | K | |
| 314 | M | L | |
| aadiff | | 13 | |

Reference CVV in red, new variant detection in orange.

Table 17. Amino acid substitutions between representative swine 1C.2.4.x strains compared to the within-clade CVV (A/Bretagne/24241/2021) and similar 1C.2.3 CVV (A/Hunan/42443/2015).

cont.

| site | A/Bretagne/24241/2021 | A/Hunan/42443/2015 | A/swDenmark/00319-1/2024 | A/swine/France/56-230629-2/2023 | A/swine/France/56-240065-3/2024 | A/swDenmark/00545-2/2024 | A/swine/France/50-240122-4/2024 | annotations |
|------|-----------------------|--------------------|--------------------------|---------------------------------|---------------------------------|--------------------------|---------------------------------|-------------|
| 5 | I | V | V | | | V | | |
| 20 | M | L | L | | | L | T | |
| 31 | N | | | | | D | | |
| 35 | T | N | | | | | | |
| 36 | N | S | S | | | | | |
| 39 | G | | E | | | | | |
| 40 | K | | | | | R | | |
| 43 | S | | | | | N | | |
| 48 | A | I | | | | | | |
| 56 | D | S | N | N | N | N | N | |
| 57 | V | | I | | | | | |
| 66 | E | K | | | | | | |
| 69 | L | | | | | I | M | |
| 71 | V | L | L | | | L | | Cb |
| 74 | N | | S | | | | | Cb |
| 80 | I | | V | | | | | |
| 83 | S | | P | | | | | |
| 84 | N | | S | | | | | |
| 86 | K | | E | | | | | |
| 89 | T | A | | | | I | | |
| 96 | A | | S | | | S | | |
| 99 | E | | | | | G | | |
| 102 | R | K | | | | | | |
| 104 | L | Q | R | | | | | |
| 105 | L | | | | | | I | |
| 111 | F | | L | | | | | |
| 119 | K | | | | | G | E | |
| 120 | - | A | A | | | A | | |
| 121 | N | T | T | | | T | | |
| 124 | P | | | | | K | | Sa |
| 125 | S | N | D | | | N | | Sa |
| 127 | E | D | | | | K | | |
| 128 | A | T | T | | | T | | |
| 129 | - | T | T | | | | | |
| 130 | - | R | Q | | | D | | |
| 134 | V | | I | | | | | RBS |
| 135 | S | A | A | | | | | RBS |
| 138 | K | H | H | R | | R | | |
| 141 | A | | | | | T | | |
| 142 | N | | K | S | | P | X | Ca2 |
| 146 | R | | | | | Q | | |
| 152 | T | V | V | | | | | |
| 153 | S | K | | | | K | | Sb |
| 155 | G | | | | | - | | Sa |
| 156 | K | N | G | | | S | | Sb |

| site | A/Bretagne/24241/2021 | A/Hunan/42443/2015 | A/swDenmark/00319-1/2024 | A/swine/France/56-230629-2/2023 | A/swine/France/56-240065-3/2024 | A/swDenmark/00545-2/2024 | A/swine/France/50-240122-4/2024 | annotations |
|--------|-----------------------|--------------------|--------------------------|---------------------------------|---------------------------------|--------------------------|---------------------------------|-------------|
| 157 | P | S | S | | | S | | Sa |
| 159 | S | P | P | | | P | | Sa |
| 161 | L | | | | | I | | |
| 162 | S | | N | | | | | Sa |
| 163 | K | | G | | | E | | Sa |
| 166 | K | T | T | | R | T | | Ca1 |
| 168 | N | | | | | D | | |
| 169 | K | | | | | R | | |
| 172 | E | | | | | K | | |
| 185 | Y | D | | | | | | |
| 186 | S | | R | | | | | |
| 194 | N | | H | | | S | | RBS |
| 200 | S | | | | | | L | |
| 201 | V | | | | | | I | |
| 202 | V | G | E | | | | | |
| 203 | S | | | | | T | | |
| 208 | Q | K | R | | | R | | |
| 216 | P | A | T | | S | D | | |
| 218 | P | | Q | | | | | RBS |
| 219 | E | K | R | | | K | | RBS |
| 220 | I | V | | | | | | RBS |
| 222 | E | | | K | | | K | Ca2, RBS |
| 224 | R | A | A | | | A | | RBS |
| 236 | Q | | | | | K | | |
| 237 | E | G | G | | | G | | Ca1 |
| 239 | T | | | | | N | | |
| 252 | R | W | W | | | | | |
| 253 | Y | H | | | | | | |
| 258 | E | K | D | D | | D | D | |
| 259 | K | | | | | M | | |
| 260 | G | | | | | S | | |
| 261 | S | | L | Y | | P | | |
| 262 | N | S | | | | S | S | |
| 266 | M | | V | | | | | |
| 267 | K | R | I | | E | | | |
| 269 | D | | E | | | | | |
| 271 | H | Q | | | | R | | |
| 288 | S | G | G | | | | | |
| 289 | D | N | N | | | N | G | |
| 290 | R | L | L | | | | | |
| 298 | I | V | | | | | | |
| 307 | V | | | | | I | | |
| 311 | Q | | R | | K | | | |
| 313 | R | | | | | K | | |
| aadiff | | 44 | 49 | 8 | 5 | 52 | 12 | |

Reference CVVs in red, HI swine strains in purple.

H3 swine lineage

★ H3v candidate vaccine virus

■ A/Massachusetts/18/2022

Previous H3N2 seasonal vaccines

Reported H3N2v cases

+ New variant(s)

HI tested viruses

H3 swine genetic clades

● Other-Human-2020 (n=8)

● 3.2010.2 (n=3)

● Other-Human-2010 (n=2)

● 3.2010.1 (n=113)

● 3.2000.4 (n=2)

● 3.1990.4.a (n=81)

● 3.1990.4.i (n=14)

● 3.1990.4.c (n=4)

● 3.1990.4.b2 (n=8)

● 3.1990.4.b1 (n=8)

● 3.1990.4 (n=2)

● Human vaccine or seasonal

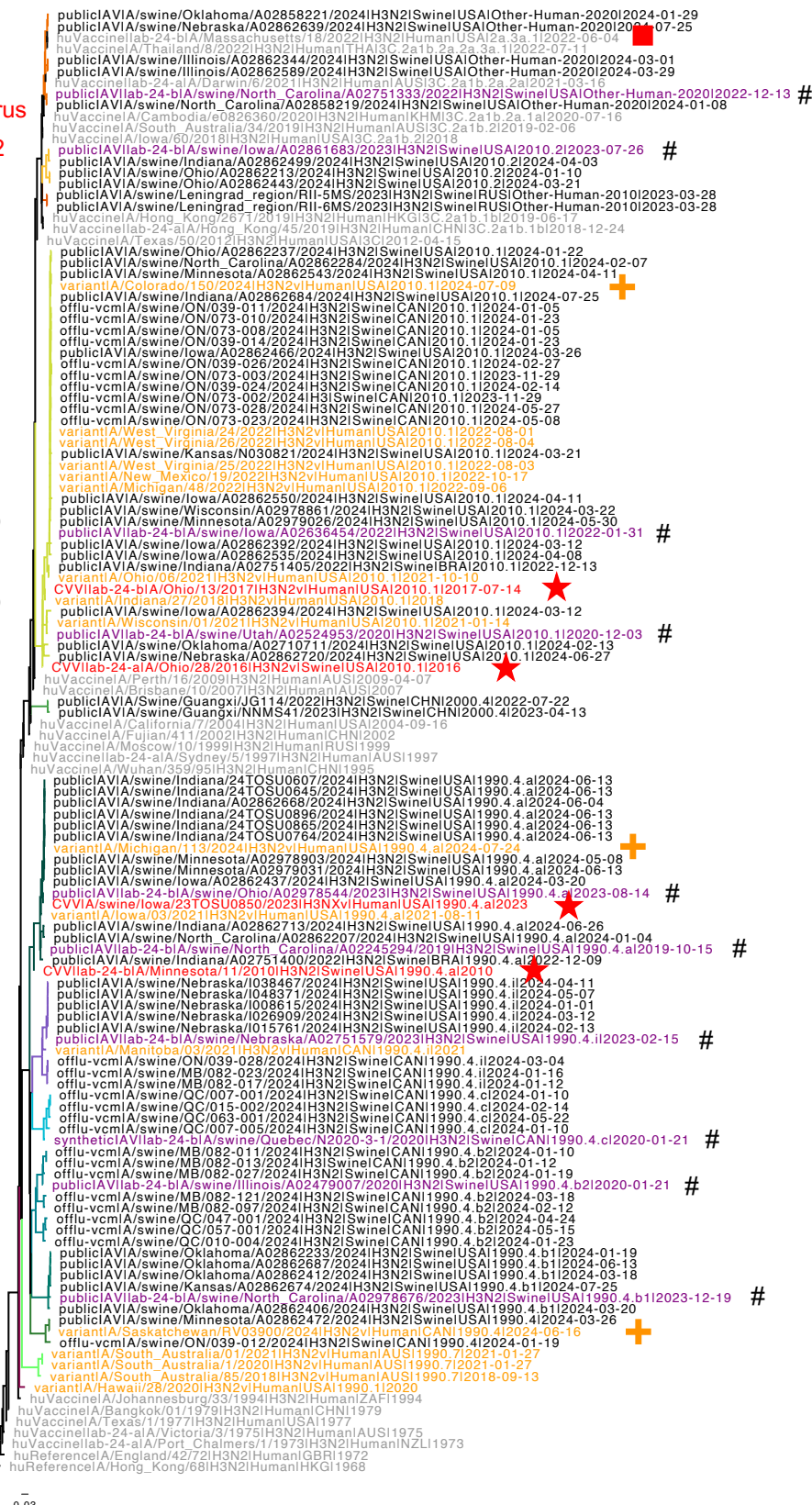


Figure 10. Swine H3 HA genes (tree was proportionally down sampled to 85 swine HA genes, excluding references). Number of detections of each clade from data deposited on or after January 1, 2024 are presented adjacent to the clade name in the figure key.

Antigenic analysis: Swine H3

Table 18. USA Data: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine H3 strains selected to represent clade consensus.

- > 8 fold reduction
- 8 fold reduction
- 4 fold reduction

| | Global Clade | A/Minnesota/11/2010 x 203 | A/swine/Iowa/23TOSU0850/2023 | A/swine/North_Carolina/A02245294/2019 (H3N2) #32 | A/swine/North Carolina/A02245294/2019 (H3N2) #18 | IDCDC-RG60A A/Ohio/13/2017 CVV | A/swine/Utah/A02524953/2020 (H3N2) #19 | A/swine/Utah/A02524953/2020 (H3N2) #20 | A/Massachusetts/18/2022 | Human pooled sera |
|---|--|---------------------------|------------------------------|--|--|--------------------------------|--|--|-------------------------|-------------------|
| | A/Minnesota/11/2010 x 203 | 1280 | 40 | 80 | 160 | 20 | 40 | 80 | nt | 40 |
| | A/swine/Iowa/23TOSU0850/2023 | 20 | 1280 | 40 | 40 | <10 | <10 | <10 | <10 | 160 |
| | A/swine/North_Carolina/A02245294/2019 | 80 | 160 | 640 | 640 | 20 | 20 | 40 | 20 | 40 |
| + | A/swine/Ohio/A02978544/2023 | 40 | 320 | 320 | 320 | 10 | 10 | 20 | 20 | 80 |
| | A/swine/North_Carolina/A02978676/2023 | 40 | 20 | 40 | 40 | 20 | 40 | 40 | 20 | 80 |
| | A/swine/Illinois/A02479007/2020 | 20 | 640 | 640 | 640 | 40 | 20 | 20 | 20 | 40 |
| | A/swine/Quebec/N2020-3-1/2020 | 40 | 80 | 40 | 40 | 20 | 40 | 40 | 20 | 10 |
| | A/swine/Nebraska/A02751579/2023 | <10 | 40 | 10 | 10 | <10 | 10 | 10 | 20 | 40 |
| | IDCDC-RG60A A/Ohio/13/2017 CVV | <10 | 80 | 20 | 80 | 1280 | 160 | 160 | 20 | 320 |
| | A/swine/Utah/A02524953/2020 | 10 | 20 | 40 | 40 | 160 | 640 | 640 | 40 | 80 |
| + | A/swine/Iowa/A02636454/2022 | 10 | <10 | 20 | 40 | 160 | 320 | 320 | 10 | 160 |
| | A/swine/Iowa/A02861683/2023 | 10 | <10 | 10 | 10 | <10 | 10 | 20 | 80 | 80 |
| | A/swine/North_Carolina/A02751333/2022 | <10 | <10 | 10 | 10 | <10 | <10 | <10 | 160 | 320 |
| | A/Massachusetts/18/2022 | <10 | <10 | 10 | 10 | 10 | <10 | 10 | 1280 | 160 |
| | A/Darwin/6/2021 | | | | | | | | | 640 |

Reference CVV in red, seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted in gray. Human sera were collected in USA with titer relative to A/Darwin/6/2021, pool of 2 male and 2 female adults. nt=not tested.

- The contemporary swine 1990.4.a had a 4-fold decrease from CVV A/swine/Iowa/23TOSU0850/2023 and had > 8-fold reduction in reactivity in adult human pooled sera.
- The contemporary swine 1990.4.b1 does not have a CVV contained within clade and demonstrated a >8-fold decrease from CVV and had >8-fold reduction in reactivity in adult human pooled sera.
- The contemporary swine 1990.4.i does not contain a within clade CVV and demonstrated a >8-fold decrease from CVV A/Minnesota/11/2010 and had >8-fold reduction in reactivity in adult human sera.
- The contemporary swine 2010.1 representative strain had an 8-fold loss to A/Ohio/13/2017 and an 8-fold loss in reactivity with adult human sera.
- The contemporary swine 2010.2 and 2020.1 representative strains do not contain CVV and demonstrated 8-fold or greater loss to the human vaccine strain. The 2010.2 strain had 8-fold loss in reactivity against adult human high responder sera.

Table 19. Amino acid substitutions between representative swine 1990.4.x strains compared to within-lineage CVVs (A/swine/Iowa/23TOSU0850/2023 and A/Minnesota/11/2010 x 203) and recent variants.

| site | A/swine/Iowa/23TOSU0850/2023 | A/Minnesota/11/2010 | A/swine/Ohio/A02978544/2023 | A/swine/North_Carolina/A02245294/2019 | A/swine/Nebraska/A02751579/2023 | A/swine/North_Carolina/A02978676/2023 | A/swine/Illinois/A02479007/2020 | A/swine/Quebec/N2020-3-1/2020 | A/Saskatchewan/RV03900/2024 | A/Michigan/113/2024 | annotations |
|------|------------------------------|---------------------|-----------------------------|---------------------------------------|---------------------------------|---------------------------------------|---------------------------------|-------------------------------|-----------------------------|---------------------|-------------|
| 1 | Q | | | | K | | | | R | | |
| 2 | K | | | | | N | | | | | |
| 5 | G | | | | | | | F | | | |
| 6 | N | S | | S | S | S | S | S | D | S | |
| 8 | N | | | | K | | | | | | |
| 9 | S | | | | | | | N | N | | |
| 10 | M | | | | T | | | | | | |
| 21 | P | | | | | | | S | | | |
| 27 | K | | | | | | R | | | | |
| 46 | S | | | | | T | | | | | |
| 48 | T | | | | | K | | | | | |
| 49 | G | | | | D | | | | | | |
| 50 | R | | | | | E | | | | | |
| 53 | N | | | | | S | S | | | | Site C |
| 54 | S | | | | | | | | G | | Site C |
| 56 | H | | | | | | Y | | | | |
| 57 | Q | | | | K | K | K | | K | | |
| 58 | I | | | | V | | | | V | | |
| 62 | K | | | | G | | | | | | |
| 78 | D | | | | | | | | G | | |
| 80 | Q | | | | E | | | | | | |
| 81 | N | | | | | D | | D | | | |
| 82 | K | | | | | R | | | | | |
| 83 | E | | | | | T | K | | | K | |
| 96 | S | N | | | N | D | N | N | N | | |
| 101 | Y | | | | | | | | F | | |
| 103 | P | | | | | | | R | | | |
| 104 | D | | | | | E | | | | | |
| 105 | Y | | | | | | | | | H | |
| 106 | A | | | | | | T | | | | |
| 107 | T | | | | S | S | S | S | S | | |
| 117 | N | | | | T | T | T | T | T | | |
| 119 | E | | | K | | | | | Q | | |
| 121 | T | | | | N | | N | | | | |
| 122 | Q | | | | P | | | P | | | Site A |
| 124 | I | S | | | N | N | S | N | V | | |
| 131 | A | | | T | G | D | | | N | | |
| 133 | D | | | | N | | | | | | Site A |
| 135 | S | | | | | A | | | | | |
| 137 | Y | | | | F | | I | F | L | | Site A |
| 138 | S | A | | | | | A | | A | | |
| 140 | R | | | | K | | K | | K | | |
| 142 | G | | | | N | | | | | K | |
| 144 | V | | | | | E | D | | N | | Site A |
| 145 | N | | | | K | | S | K | | | Site A |
| 155 | Y | | | | H | H | | | H | | Site B |
| 156 | H | N | | | | S | | | N | | |
| 158 | N | | | | | E | | | D | | |

| site | A/swine/Iowa/23TOSU0850/2023 | A/Minnesota/11/2010 | A/swine/Ohio/A02978544/2023 | A/swine/North_Carolina/A02245294/2019 | A/swine/Nebraska/A02751579/2023 | A/swine/North_Carolina/A02978676/2023 | A/swine/Illinois/A02479007/2020 | A/swine/Quebec/N2020-3-1/2020 | A/Saskatchewan/RV03900/2024 | A/Michigan/113/2024 | annotations |
|--------|------------------------------|---------------------|-----------------------------|---------------------------------------|---------------------------------|---------------------------------------|---------------------------------|-------------------------------|-----------------------------|---------------------|-------------|
| 159 | Y | | | | H | | | H | H | | |
| 163 | E | | | | A | A | A | A | A | | |
| 164 | Q | | | | | L | L | L | | | |
| 171 | N | | | | | D | | D | | | |
| 172 | E | D | | D | D | G | D | D | G | | |
| 173 | K | | | | | | | N | | | |
| 174 | F | | | | | S | | | | | |
| 188 | D | | | | | N | | | N | | Site B |
| 189 | K | | | | M | V | | | S | | Site B |
| 192 | T | | | | | I | | I | | | |
| 193 | N | | | | S | S | | S | S | | Site B |
| 196 | I | V | | V | V | A | V | A | V | | |
| 198 | A | | | | E | | | | | | |
| 199 | S | | | | | | Q | P | | | |
| 200 | G | | | | | | | E | | | |
| 201 | R | | | | | G | | | E | | Site D |
| 202 | V | | | | | I | | | | | |
| 203 | I | | | | T | T | | | T | | |
| 204 | V | | | | | | I | | | | |
| 207 | R | K | | | K | K | K | K | K | | Site D |
| 210 | Q | | | | L | | | | | | |
| 216 | N | | | | | | D | | | | |
| 217 | I | | | | | V | V | | | | Site D |
| 228 | S | | | | | G | | | | | |
| 233 | Y | | | | | | | | H | | |
| 236 | I | | | | | | V | V | | | |
| 242 | I | | | | | | M | | | | |
| 252 | I | | | | | | V | | | | |
| 260 | I | | | | M | | | | | | |
| 261 | Q | | | | R | | | | | | |
| 262 | S | | | | I | | | | | | |
| 263 | G | | | | | R | | | | | |
| 264 | K | | | | | | | | N | | |
| 269 | R | | | | K | | | | | | |
| 272 | A | | | | | S | | | V | | |
| 273 | H | | | | L | | P | P | S | | |
| 275 | D | | | | G | G | | | | | Site C |
| 276 | E | | | | N | N | N | N | N | | |
| 278 | N | | | | | K | S | | | | Site C |
| 279 | S | | | | | | | | | F | |
| 282 | I | | | | | | | | A | | |
| 289 | P | | | S | | | | | | S | |
| 299 | K | | | | R | R | R | R | R | | |
| 304 | A | | | | | | | T | | | |
| 310 | K | | | | | | | | R | | |
| 312 | N | | | | G | S | | | | | |
| 323 | V | | | I | | | | | | | |
| aadiff | | 8 | 0 | 7 | 41 | 44 | 33 | 30 | 40 | 5 | |

↓
cont.

Reference CVVs in red, HI swine strains in purple, new variant detection in orange.

Table 20. Amino acid substitutions between representative swine 2010.1 strains compared to the within-clade CVVs (A/Ohio/28/2016) and recent variant.

| site | A/Ohio/13/2017 | A/swine/Utah/A02524953/2020 | A/swine/Iowa/A02636454/2022 | A/Colorado/150/2024 | annotations |
|--------|----------------|-----------------------------|-----------------------------|---------------------|-------------|
| 25 | I | | | M | |
| 30 | T | | | M | |
| 31 | N | | D | D | |
| 45 | S | | N | N | |
| 56 | Y | H | | | |
| 62 | E | | G | G | |
| 88 | V | I | | | |
| 92 | K | R | | | |
| 96 | N | | D | D | |
| 106 | A | | V | | |
| 112 | V | A | | | |
| 122 | N | | K | K | Site A |
| 142 | R | | | K | |
| 144 | S | T | | | Site A |
| 156 | H | Q | | | |
| 158 | N | | | D | |
| 167 | T | A | | | |
| 202 | I | | T | T | |
| 209 | N | G | | | |
| 212 | A | | T | T | |
| 216 | N | | | I | |
| 223 | V | | I | I | |
| 238 | R | | K | K | |
| 264 | K | | R | R | |
| 312 | N | K | | | |
| aadiff | | 9 | 11 | 15 | |

Reference CVV in red, HI swine strains in purple, new variant detection in orange.

Table 21. Amino acid substitutions between representative swine 2010.2 strain compared to current WHO recommended human seasonal vaccine and used in HI assays (A/Massachusetts/18/2022) and HI-tested CVV (A/Ohio/13/2017). A separate comparison was also made against the most similar human vaccine (A/Kansas/14/2017).

| Relative to A/Massachusetts/18/2022 | | | | |
|-------------------------------------|-------------------------|----------------|-----------------------------|-------------|
| site | A/Massachusetts/18/2022 | A/Ohio/13/2017 | A/swine/Iowa/A02861683/2023 | annotations |
| 3 | I | L | | |
| 9 | S | N | G | |
| 10 | T | M | | |
| 31 | N | | D | |
| 33 | R | Q | | |
| 45 | N | S | | |
| 46 | S | F | | |
| 48 | I | T | | |
| 50 | K | E | E | |
| 56 | H | Y | | |
| 58 | I | | V | |
| 62 | G | E | E | |
| 67 | I | | V | |
| 82 | K | N | | |
| 83 | E | K | Q | |
| 92 | R | K | | |
| 94 | N | H | Y | |
| 96 | S | N | N | |
| 106 | A | | V | |
| 121 | K | N | | |
| 131 | K | T | T | |
| 133 | N | D | | Site A |
| 135 | T | A | I | |
| 138 | A | S | S | |
| 142 | G | R | | |
| 144 | S | | K | Site A |
| 145 | S | N | | Site A |
| 156 | S | H | Q | |
| 158 | N | | D | |
| 159 | N | F | Y | |
| 160 | I | K | K | |
| 164 | Q | L | L | |
| 165 | N | E | | |
| 167 | T | | I | |
| 171 | K | N | N | |
| 174 | F | | X | |
| 186 | D | A | G | Site B |
| 189 | K | | S | Site B |
| 190 | N | D | D | |
| 192 | F | I | T | |
| 195 | F | Y | Y | |
| 197 | Q | | R | |
| 198 | S | A | | |
| 199 | S | A | | |
| 203 | T | I | I | |
| 209 | S | N | | |
| 223 | V | | I | |
| 238 | K | R | | |
| 242 | I | | T | |
| 278 | K | N | | Site C |
| 280 | E | A | | |
| 291 | D | | N | |
| 312 | S | N | | |
| 323 | V | I | | |
| aadiff | | 41 | 32 | |

| Relative to most similar vaccine | | | |
|----------------------------------|------------------|-----------------------------|-------------|
| site | A/Kansas/14/2017 | A/swine/Iowa/A02861683/2023 | annotations |
| 9 | S | G | |
| 31 | N | D | |
| 53 | D | N | Site C |
| 58 | I | V | |
| 67 | I | V | |
| 83 | K | Q | |
| 91 | N | S | |
| 92 | K | R | |
| 106 | A | V | |
| 121 | N | K | |
| 128 | A | T | |
| 135 | T | I | |
| 140 | I | K | |
| 156 | H | Q | |
| 158 | N | D | |
| 159 | S | Y | |
| 167 | T | I | |
| 174 | F | X | |
| 189 | K | S | Site B |
| 190 | N | D | |
| 192 | I | T | |
| 197 | Q | R | |
| 203 | T | I | |
| 242 | I | T | |
| 246 | X | N | |
| 291 | D | N | |
| 326 | R | K | |
| aadiff | | 27 | |

Reference vaccine strain in gray, CVV in red, HI swine strain in purple.

Table 22. Amino acid substitutions between representative swine 2020.1 strain compared to the A/Massachusetts/18/2022, the nearest HI-tested human vaccine and most similar human vaccine.

| site | A/Massachusetts/18/2022 | A/swine/North_Carolina/A02751333/2022 | annotations |
|--------|-------------------------|---------------------------------------|-------------|
| 53 | N | D | Site C |
| 79 | F | V | |
| 96 | S | N | |
| 156 | S | H | |
| 192 | F | I | |
| 223 | V | I | |
| aadiff | | 6 | |

Reference vaccine in gray, HI swine strain in purple.

Summary and Risk Assessment

Global Variant Cases:

During the reporting period (January 1 – June 30, 2024), 4 variant cases were reported and included with the swine analyses when sequence data were available:

- Canada: H3N2v (1990.4)
- USA: H1N2v (2x 1A.1.1.3)
- Vietnam: H1N1v (1C.2.3)

Additional variant cases were detected outside of this period, when sequence data were available these were included in analyses:

- USA: H1N2v (2x 1A.1.1.3 – August 5, August 7 2024; 1x 1B.2.1 – July 2023), H3N2v (1990.4.a – July 24 2024), H3N2v (2010.1 – July 9, 2024)

Europe Swine:

- H1 1A: The contemporary swine 1A.3.3.2 retained cross-reactivity with recent human seasonal H1 vaccines.
- H1 1B: There were no within-clade CVVs tested for 1B lineages from Europe and the 1B.2.1 CVV has significant loss in reactivity with the 1B.1 representative swine strains. The swine 1B.1.1.1 had significant loss in cross-reactivity with A/Chile/1/1983 and A/Brazil/11/1978. The swine 1B.1.1.2 strain retained cross-reactivity with the ancestral human seasonal reference strains. The contemporary swine 1B.1.2.2 had significant decrease in cross reactivity against the human seasonal reference strains. A newly detected group of viruses, 1B.2.7, had no within-clade CVV and >8-fold loss in cross reactivity to ancestral human seasonal reference strains.
- The 1C.2.1 strain retained cross-reactivity to the 1C.2.1 CVV A/Netherlands/10370-1b/2020. The 1C.2.4.1 swine strain retained cross-reactivity with a different clade CVV strain (1C.2.1: A/Netherlands/10370-1b/2020). The 1C.2.4.2 swine strains retained reactivity to the within-clade CVV A/Bretagne/24241/2021. The representative swine 1C.2.4.3 had no within-clade CVV and demonstrated a range of 4- to >8-fold loss to available 1C CVV strains.

North America Swine:

- H1 1A: The contemporary swine 1A.1.1.3 had >8-fold decrease from within clade CVVs but retained reactivity to human pooled sera. The contemporary swine 1A.3.3.2 had >8-fold decrease to seasonal vaccine strains but retained reactivity to human pooled sera. The contemporary swine 1A.3.3.3-c3 virus had 4-8-fold decrease from the within-clade CVV but retained reactivity to human pooled sera. The contemporary swine 1A.2 virus has no within clade CVV and >8-fold decrease to CVV and human seasonal vaccine ferret anti-sera but retained reactivity to human pooled sera.
- H1 1B: The 1B.2.1 swine representative had no loss of titer to the within clade CVV and had a 4-fold loss in cross-reactivity to human pooled sera. The 1B.2.2.1 clade swine representative had 8-fold reduction from the within clade CVV but retained reactivity to human pooled sera. The contemporary swine 1B.2.2.2 does not have a within-clade CVV and had a >8-fold decrease from the 1B.2.2.1 CVV and had an 8-fold loss in cross-reactivity to human pooled sera.
- H3: The contemporary swine 1990.4.a had a 4-fold decrease from the new CVV and had > 8-fold reduction in reactivity in adult human pooled sera. The contemporary swine 1990.4.b1 does not have a within clade CVV and demonstrated a >8-fold decrease from 1990.4.a CVV and had >8-fold reduction in reactivity in adult human pooled sera. The contemporary swine 1990.4.i does not contain a within clade CVV and demonstrated a >8-fold decrease from 1990.4.a CVV and had >8-fold reduction in reactivity in adult human sera. The contemporary swine 2010.1 representative strain had an 8-fold loss to the 2010.1 CVV and an 8-fold loss in reactivity with adult human sera. The contemporary swine 2010.2 and 2020.1 representative strains do not contain CVV and demonstrated \geq 8-fold loss to the human vaccine strain. The 2010.2 strain had 8-fold loss in reactivity against adult human high responder sera.

Annex 1. Geographic Distribution of swine HA phylogenetic clades by country

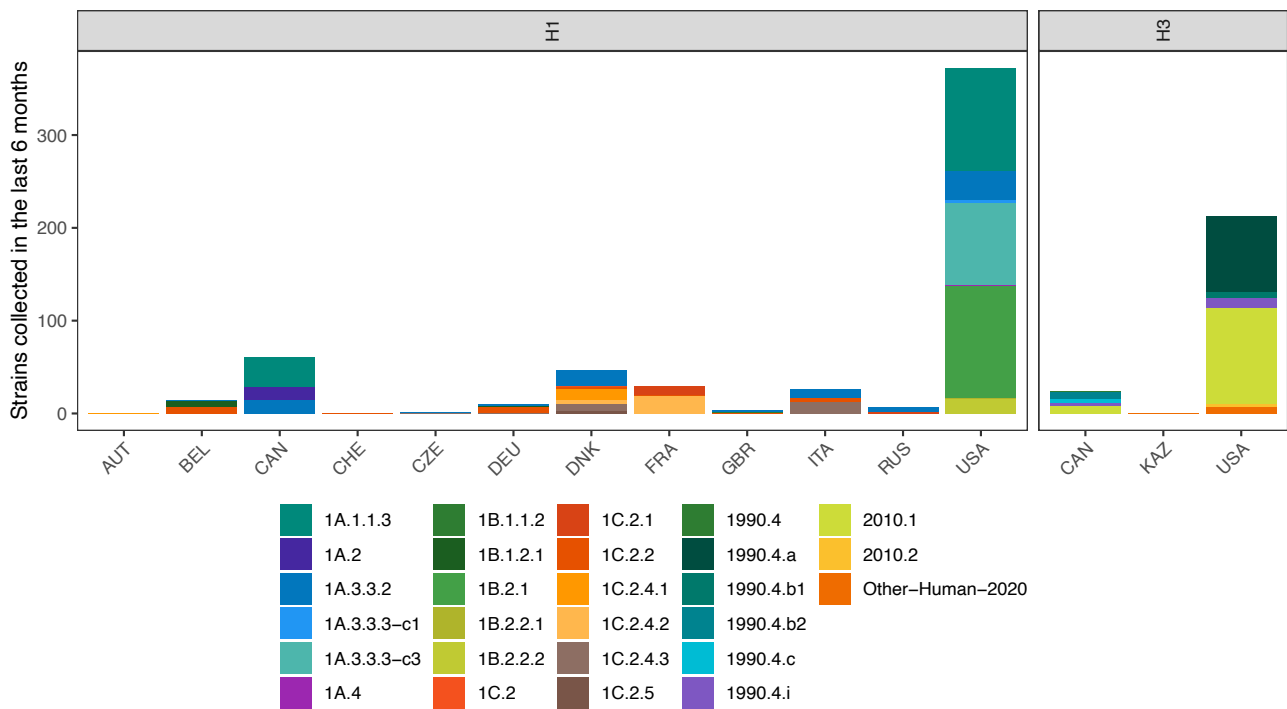


Figure A1. Summary of swine HA genes colored by phylogenetic clade for sequences deposited on or after January 2024 and truncated to those collected within the last 6 months (n = 813).

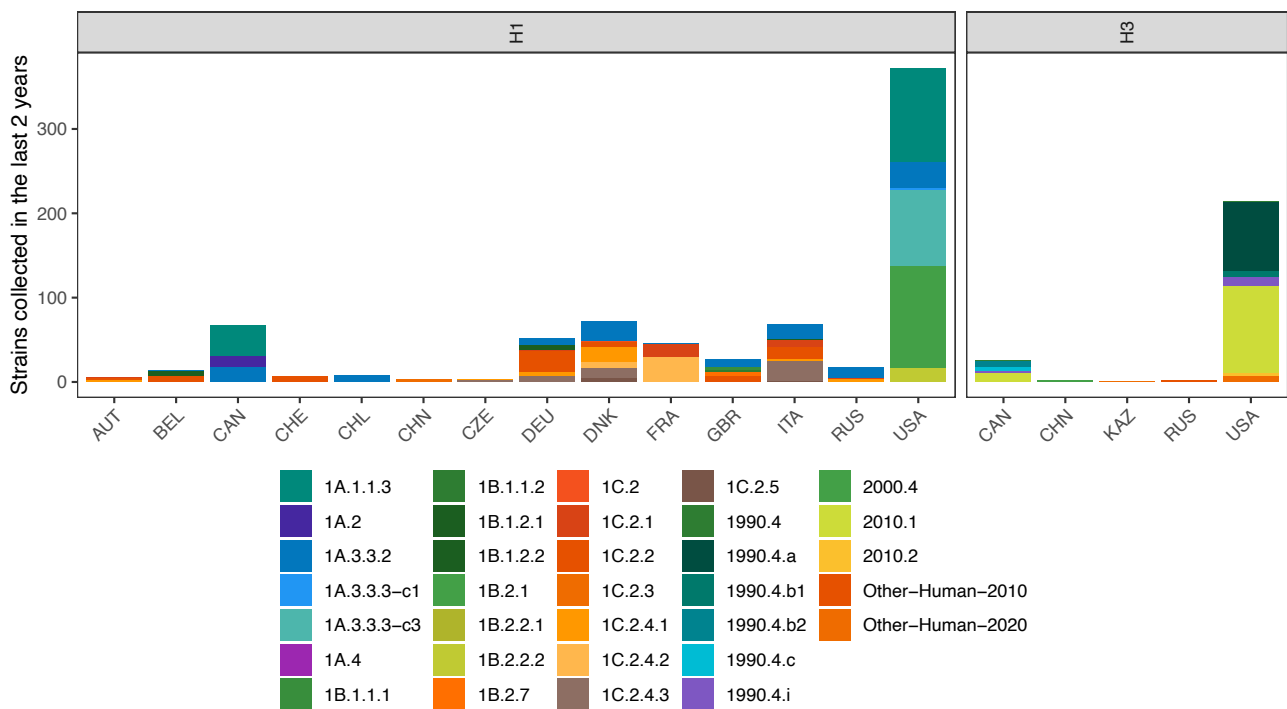


Figure A2. Summary of swine HA genes colored by phylogenetic clade. Expanded collection date criteria for non-USA sequences to a 24-month window, post June 2022 (n = 1009).

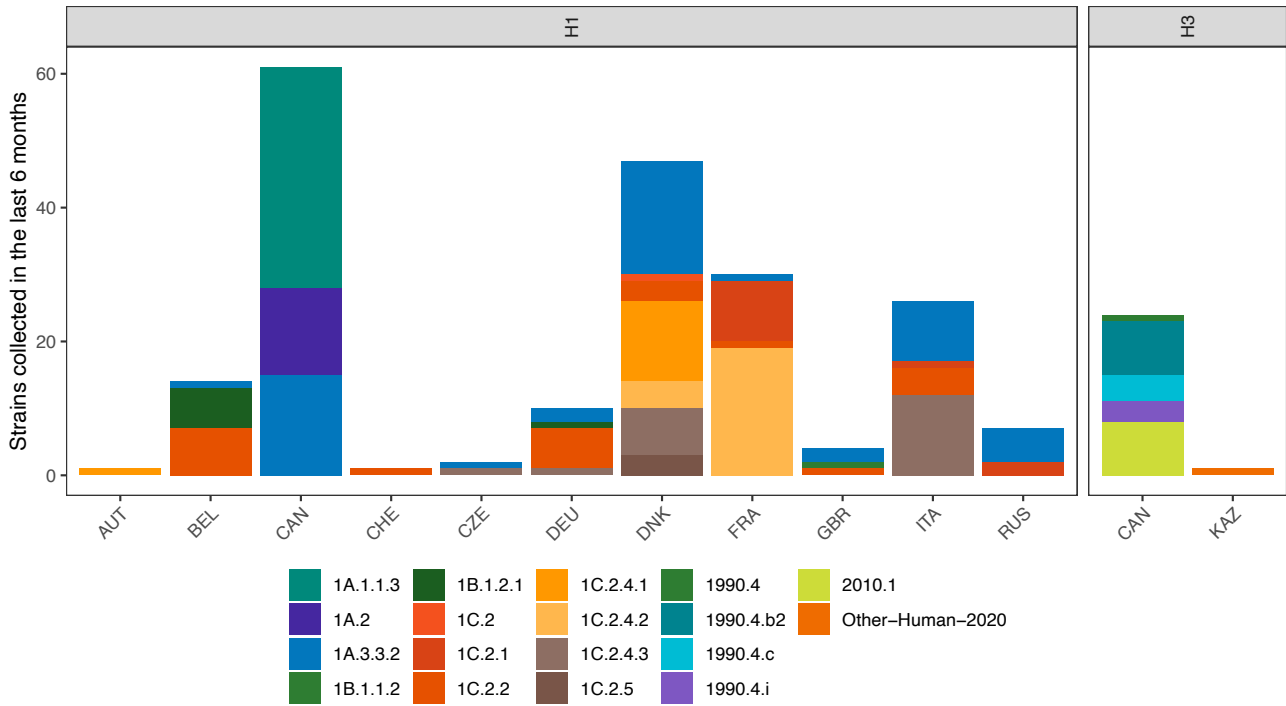


Figure A3. Summary of swine HA genes colored by phylogenetic clade for sequences deposited on or after January 2024 and truncated to those collected within the last 6 months (n = 228) in any country other than the United States of America.

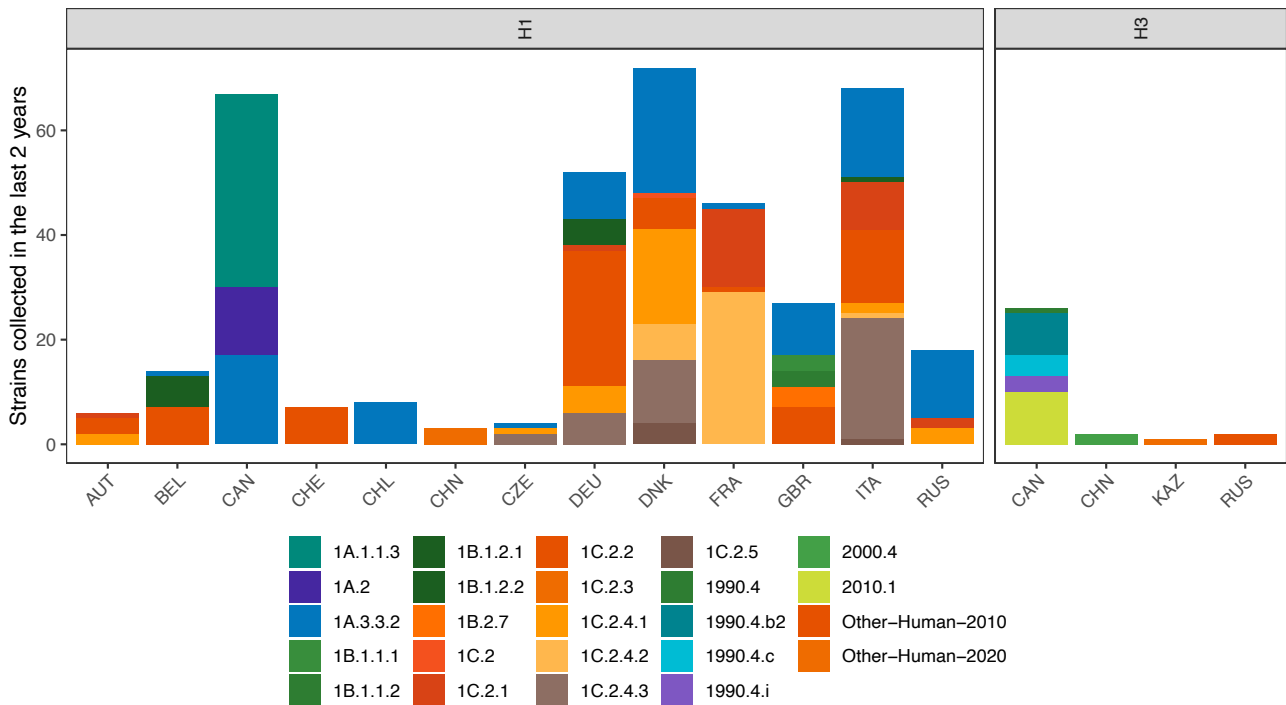


Figure A4. Summary of swine HA genes colored by phylogenetic clade. Expanded collection date criteria for non-USA sequences to a 24-month window, post June 2022 (n = 423). Note that USA data has been excluded.

Table A1a. Summary of swine H1 clades by country collected in the past 6 months and deposited on or after January 2024.

| clade | Country | count |
|--------------|----------------|--------------|
| 1C.2.4.1 | AUT | 1 |
| 1A.3.3.2 | BEL | 1 |
| 1B.1.2.1 | BEL | 6 |
| 1C.2.2 | BEL | 7 |
| 1A.1.1.3 | CAN | 33 |
| 1A.2 | CAN | 13 |
| 1A.3.3.2 | CAN | 15 |
| 1C.2.2 | CHE | 1 |
| 1A.3.3.2 | CZE | 1 |
| 1C.2.4.3 | CZE | 1 |
| 1A.3.3.2 | DEU | 2 |
| 1B.1.2.1 | DEU | 1 |
| 1C.2.2 | DEU | 6 |
| 1C.2.4.3 | DEU | 1 |
| 1A.3.3.2 | DNK | 17 |
| 1C.2 | DNK | 1 |
| 1C.2.2 | DNK | 3 |
| 1C.2.4.1 | DNK | 12 |
| 1C.2.4.2 | DNK | 4 |
| 1C.2.4.3 | DNK | 7 |
| 1C.2.5 | DNK | 3 |
| 1A.3.3.2 | FRA | 1 |
| 1C.2.1 | FRA | 9 |
| 1C.2.2 | FRA | 1 |
| 1C.2.4.2 | FRA | 19 |
| 1A.3.3.2 | GBR | 2 |
| 1B.1.1.2 | GBR | 1 |
| 1C.2.2 | GBR | 1 |
| 1A.3.3.2 | ITA | 9 |
| 1C.2.1 | ITA | 1 |
| 1C.2.2 | ITA | 4 |
| 1C.2.4.3 | ITA | 12 |
| 1A.3.3.2 | RUS | 5 |
| 1C.2.1 | RUS | 2 |
| 1A.1.1.3 | USA | 111 |
| 1A.3.3.2 | USA | 31 |
| 1A.3.3.3-c1 | USA | 3 |
| 1A.3.3.3-c3 | USA | 89 |
| 1A.4 | USA | 1 |
| 1B.2.1 | USA | 120 |
| 1B.2.2.1 | USA | 1 |
| 1B.2.2.2 | USA | 16 |

Table A1b. Summary of swine H1 clades by country collected in the past 24 months and deposited on or after July 2022.

cont.

| clade | country | count |
|----------|---------|-------|
| 1C.2.1 | AUT | 1 |
| 1C.2.2 | AUT | 3 |
| 1C.2.4.1 | AUT | 2 |
| 1A.3.3.2 | BEL | 1 |
| 1B.1.2.1 | BEL | 6 |
| 1C.2.2 | BEL | 7 |
| 1A.1.1.3 | CAN | 37 |
| 1A.2 | CAN | 13 |
| 1A.3.3.2 | CAN | 17 |
| 1C.2.2 | CHE | 7 |
| 1A.3.3.2 | CHL | 8 |
| 1C.2.3 | CHN | 3 |
| 1A.3.3.2 | CZE | 1 |
| 1C.2.4.1 | CZE | 1 |
| 1C.2.4.3 | CZE | 2 |
| 1A.3.3.2 | DEU | 9 |
| 1B.1.2.1 | DEU | 5 |
| 1C.2.1 | DEU | 1 |
| 1C.2.2 | DEU | 26 |
| 1C.2.4.1 | DEU | 5 |
| 1C.2.4.3 | DEU | 6 |
| 1A.3.3.2 | DNK | 24 |
| 1C.2 | DNK | 1 |
| 1C.2.2 | DNK | 6 |
| 1C.2.4.1 | DNK | 18 |
| 1C.2.4.2 | DNK | 7 |
| 1C.2.4.3 | DNK | 12 |
| 1C.2.5 | DNK | 4 |

| clade | country | count |
|-------------|---------|-------|
| 1A.3.3.2 | FRA | 1 |
| 1C.2.1 | FRA | 15 |
| 1C.2.2 | FRA | 1 |
| 1C.2.4.2 | FRA | 29 |
| 1A.3.3.2 | GBR | 10 |
| 1B.1.1.1 | GBR | 3 |
| 1B.1.1.2 | GBR | 3 |
| 1B.2.7 | GBR | 4 |
| 1C.2.2 | GBR | 7 |
| 1A.3.3.2 | ITA | 17 |
| 1B.1.2.2 | ITA | 1 |
| 1C.2.1 | ITA | 9 |
| 1C.2.2 | ITA | 14 |
| 1C.2.4.1 | ITA | 2 |
| 1C.2.4.2 | ITA | 1 |
| 1C.2.4.3 | ITA | 23 |
| 1C.2.5 | ITA | 1 |
| 1A.3.3.2 | RUS | 13 |
| 1C.2.1 | RUS | 2 |
| 1C.2.4.1 | RUS | 3 |
| 1A.1.1.3 | USA | 111 |
| 1A.3.3.2 | USA | 31 |
| 1A.3.3.3-c1 | USA | 3 |
| 1A.3.3.3-c3 | USA | 89 |
| 1A.4 | USA | 1 |
| 1B.2.1 | USA | 120 |
| 1B.2.2.1 | USA | 1 |
| 1B.2.2.2 | USA | 16 |

Table A2a. Summary of swine H3 clades by country collected in the past 6 months and deposited on or after January 2024.

| clade | country | count |
|------------------|----------------|--------------|
| 1990.4 | CAN | 1 |
| 1990.4.b2 | CAN | 8 |
| 1990.4.c | CAN | 4 |
| 1990.4.i | CAN | 3 |
| 2010.1 | CAN | 8 |
| Other-Human-2020 | KAZ | 1 |
| 1990.4 | USA | 1 |
| 1990.4.a | USA | 81 |
| 1990.4.b1 | USA | 7 |
| 1990.4.i | USA | 11 |
| 2010.1 | USA | 103 |
| 2010.2 | USA | 3 |
| Other-Human-2020 | USA | 7 |

Table A2b. Summary of swine H3 clades by country collected in the past 24 months and deposited on or after July 2022.

| clade | country | count |
|------------------|----------------|--------------|
| 1990.4 | CAN | 1 |
| 1990.4.b2 | CAN | 8 |
| 1990.4.c | CAN | 4 |
| 1990.4.i | CAN | 3 |
| 2010.1 | CAN | 10 |
| 2000.4 | CHN | 2 |
| Other-Human-2020 | KAZ | 1 |
| Other-Human-2010 | RUS | 2 |
| 1990.4 | USA | 1 |
| 1990.4.a | USA | 81 |
| 1990.4.b1 | USA | 8 |
| 1990.4.i | USA | 11 |
| 2010.1 | USA | 103 |
| 2010.2 | USA | 3 |
| Other-Human-2020 | USA | 7 |

Table A3a. Recent swine and variant A(H1) activity shared with international agencies and collected from sequence repositories.

| Country, area or territory | Host [#] | Genetic clade |
|--|-------------------|---|
| Austria | Swine | 1C.2.4.1 |
| Belgium | Swine | 1A.3.3.2; 1B.1.2.1; 1C.2.2 |
| Canada | Swine | 1A.1.1.3; 1A.2; 1A.3.3.2 |
| Czech Republic | Swine | 1A.3.3.2; 1C.2.4.3 |
| Denmark | Swine | 1A.3.3.2; 1C.2; 1C.2.2; 1C.2.4.1; 1C.2.4.2; 1C.2.4.3; 1C.2.5 |
| France | Swine | 1A.3.3.2; 1C.2.1; 1C.2.2; 1C.2.4.2 |
| Germany | Swine | 1A.3.3.2; 1B.1.2.1; 1C.2.2; 1C.2.4.3 |
| Italy | Swine | 1A.3.3.2; 1C.2.1; 1C.2.2; 1C.2.4.3 |
| Russia | Swine | 1A.3.3.2; 1C.2.1 |
| Switzerland | Swine | 1C.2.2 |
| United Kingdom of Great Britain and Northern Ireland | Swine | 1A.3.3.2; 1B.1.1.2; 1C.2.2 |
| United States of America | Human (5) | 1A.1.1.3; 1B.2.1 |
| United States of America | Swine | 1A.1.1.3; 1A.3.3.2; 1A.3.3.3-c1; 1A.3.3.3-c3; 1A.4; 1B.2.1; 1B.2.2.1; 1B.2.2.2 |
| Vietnam | Human (1) | 1C.2.3 |

[#]Swine H1 clades by country collected in the past 6 months and deposited on or after January 2024. *Number of cases and/or detections.

Table A3b. Recent swine and variant A(H3) activity shared with international agencies and collected from sequence repositories.

| Country, area or territory | Host [#] | Genetic clade |
|----------------------------|-------------------|--|
| Canada | Human (1) | 1990.4 |
| Canada | Swine | 1990.4; 1990.4.b2; 1990.4.c; 1990.4.i; 2010.1 |
| Kazakhstan | Swine | 2020.1 |
| United States of America | Human (2) | 1990.4.a; 2010.1 |
| United States of America | Swine | 1990.4; 1990.4.a; 1990.4.b1; 1990.4.i; 2010.1; 2010.2; 2020.1 |

[#]Swine H3 clades by country collected in the past 6 months and deposited on or after January 1, 2024. *Number of cases and/or detections.

Annex 2. Phylogenies annotated by amino acid changes

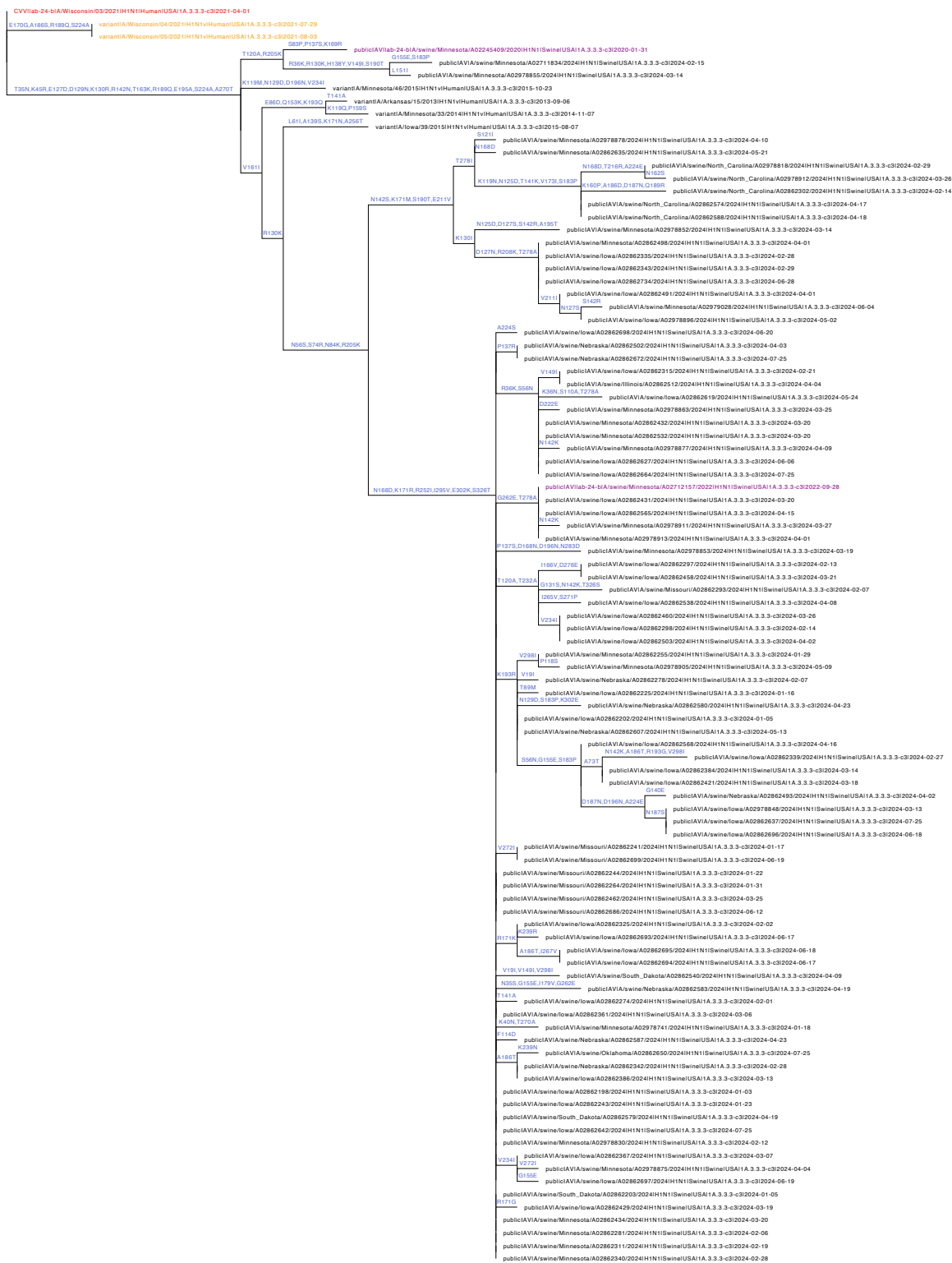


Figure A9. A phylogeny of the **1A.3.3.3-c3** clade displaying sequences deposited on or after January 1, 2024 and collected in 2024 ($n=91$), and 7 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Wisconsin/03/2021) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

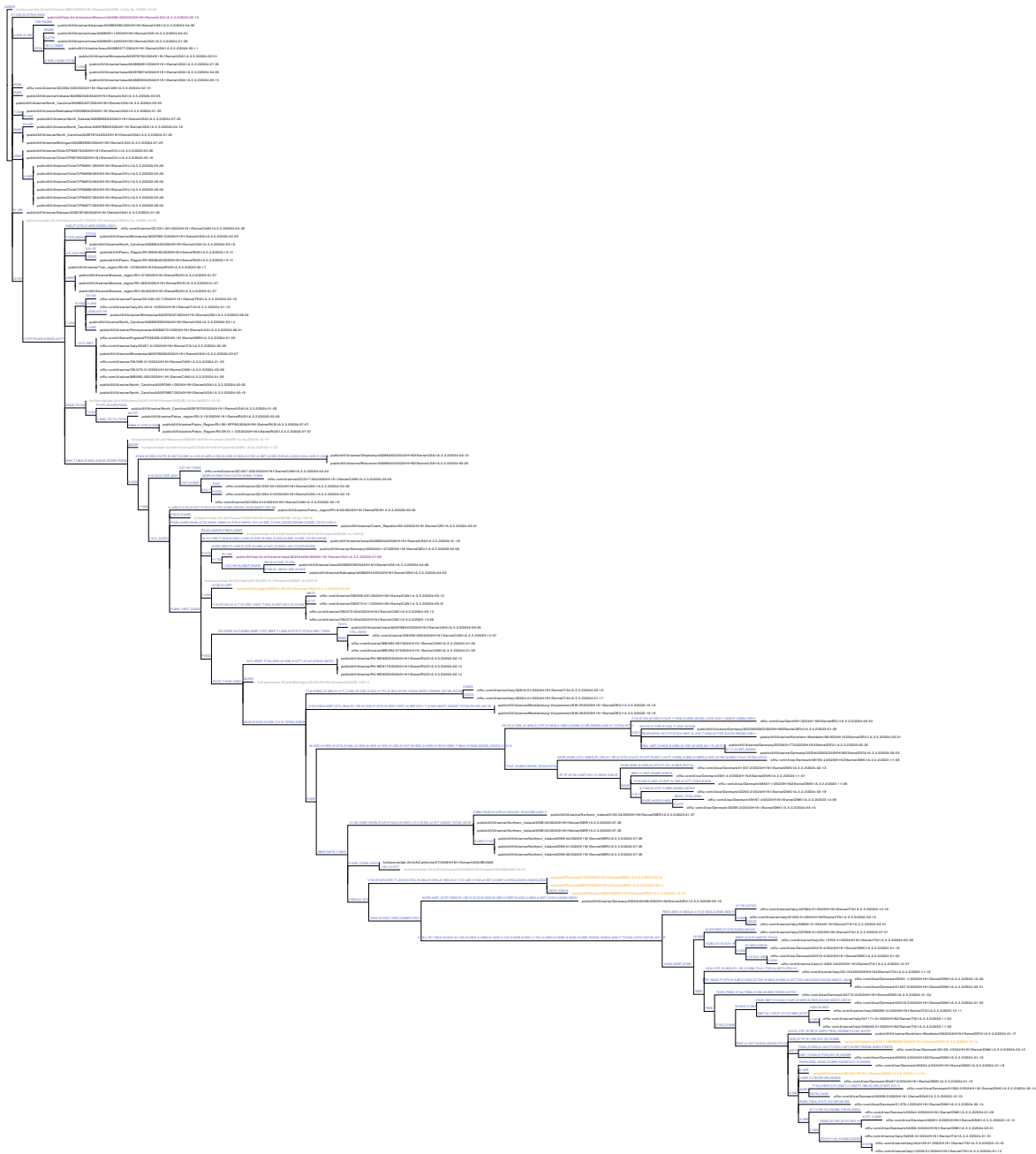


Figure A10. A phylogeny of the **1A.3.3.2 (pdm)** clade displaying n=130 sequences deposited on or after January 1, 2024 and collected in 2022-2024, and n=17 reference genes. The clade is rooted relative to the most recent human vaccine strain (A/Victoria/4897/2022) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

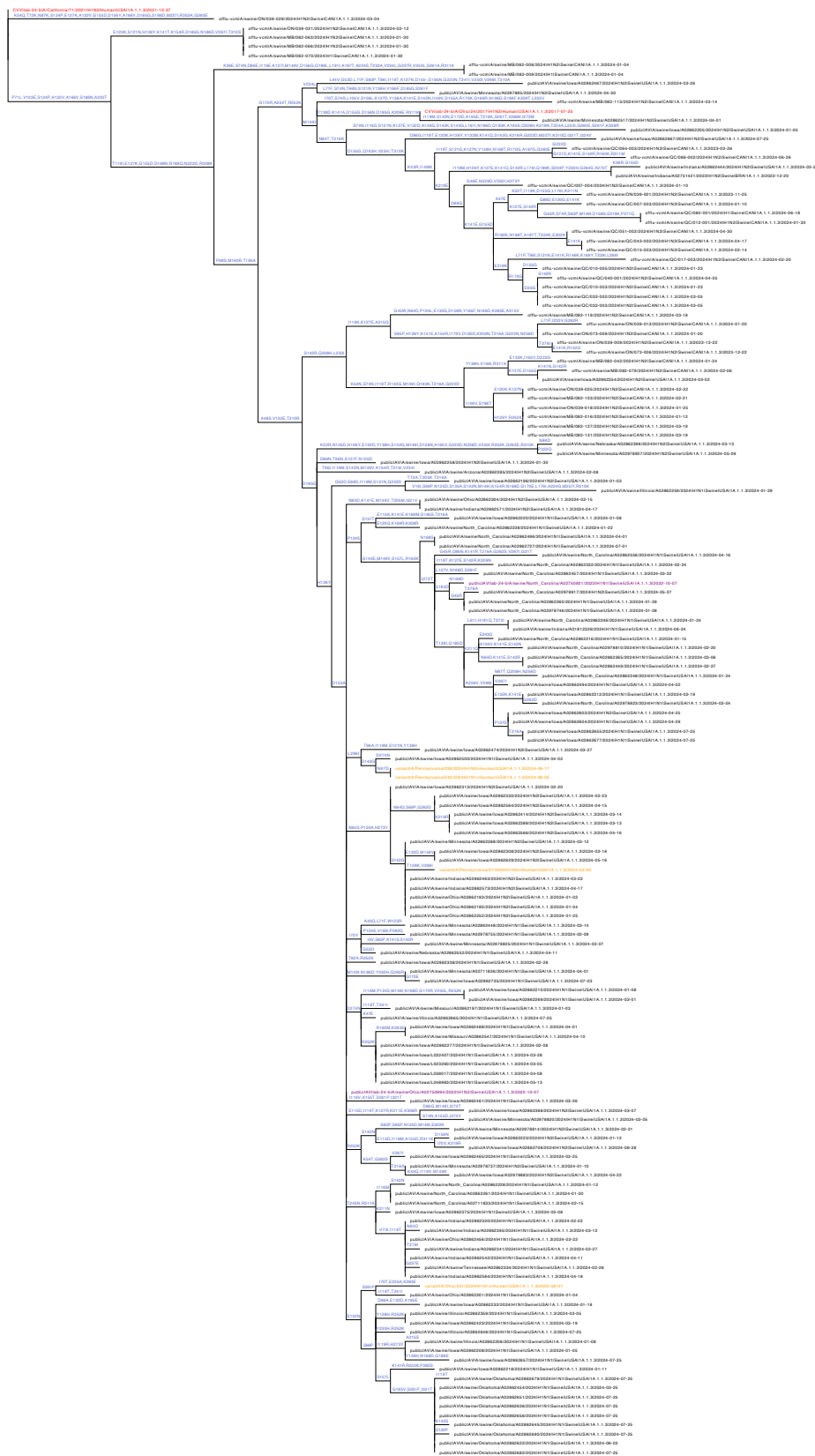


Figure A11. A phylogeny of the **1A.1.1.3** clade displaying n=151 sequences deposited on or after January 1, 2024 and collected in 2022-2024, and n=6 reference genes. The clade is rooted relative to the most recent in-clade CVV (*A/California/71/2021*) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

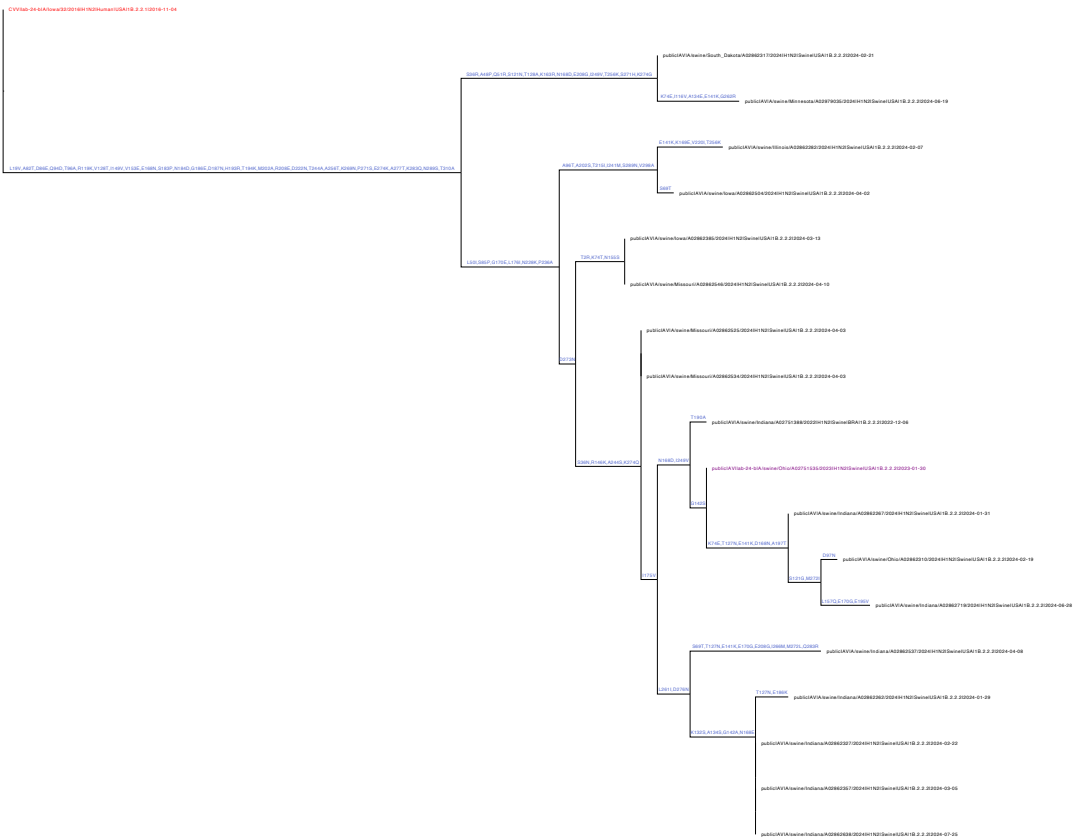


Figure A13. A phylogeny of the **1B.2.2.2** clade displaying n=18 sequences deposited on or after January 1, 2024 and collected in 2022-2024, and n=1 reference genes. The clade is rooted relative to the closest CVV (*A/Iowa/32/2016*) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 8.

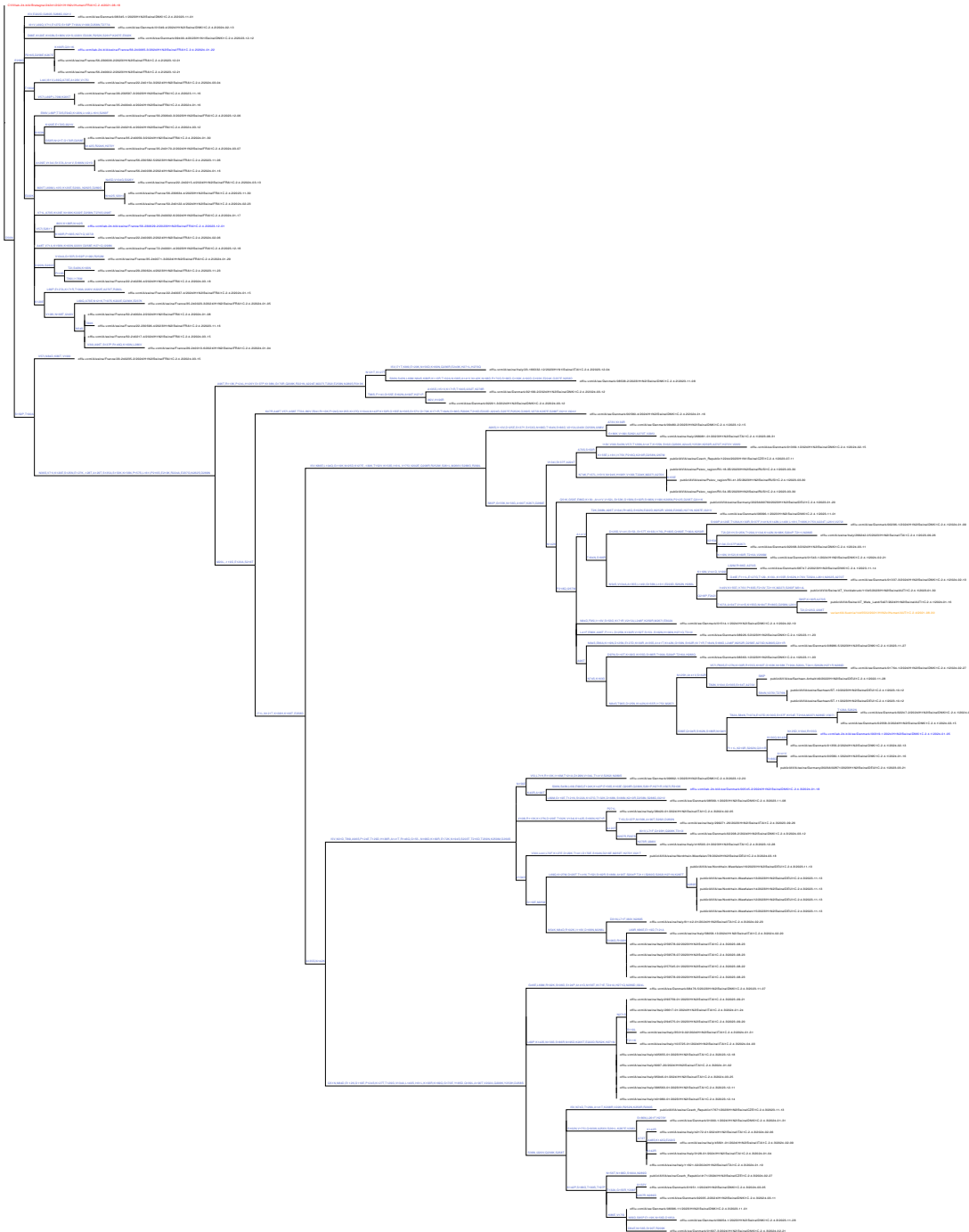


Figure A14. A phylogeny of the **1C.2.4** clade displaying n=110 sequences deposited on or after January 1, 2024 and collected in 2022-2024, and n=2 reference genes. The clade is rooted relative to the closest CVV (A/Bretagne/24241/2021) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.

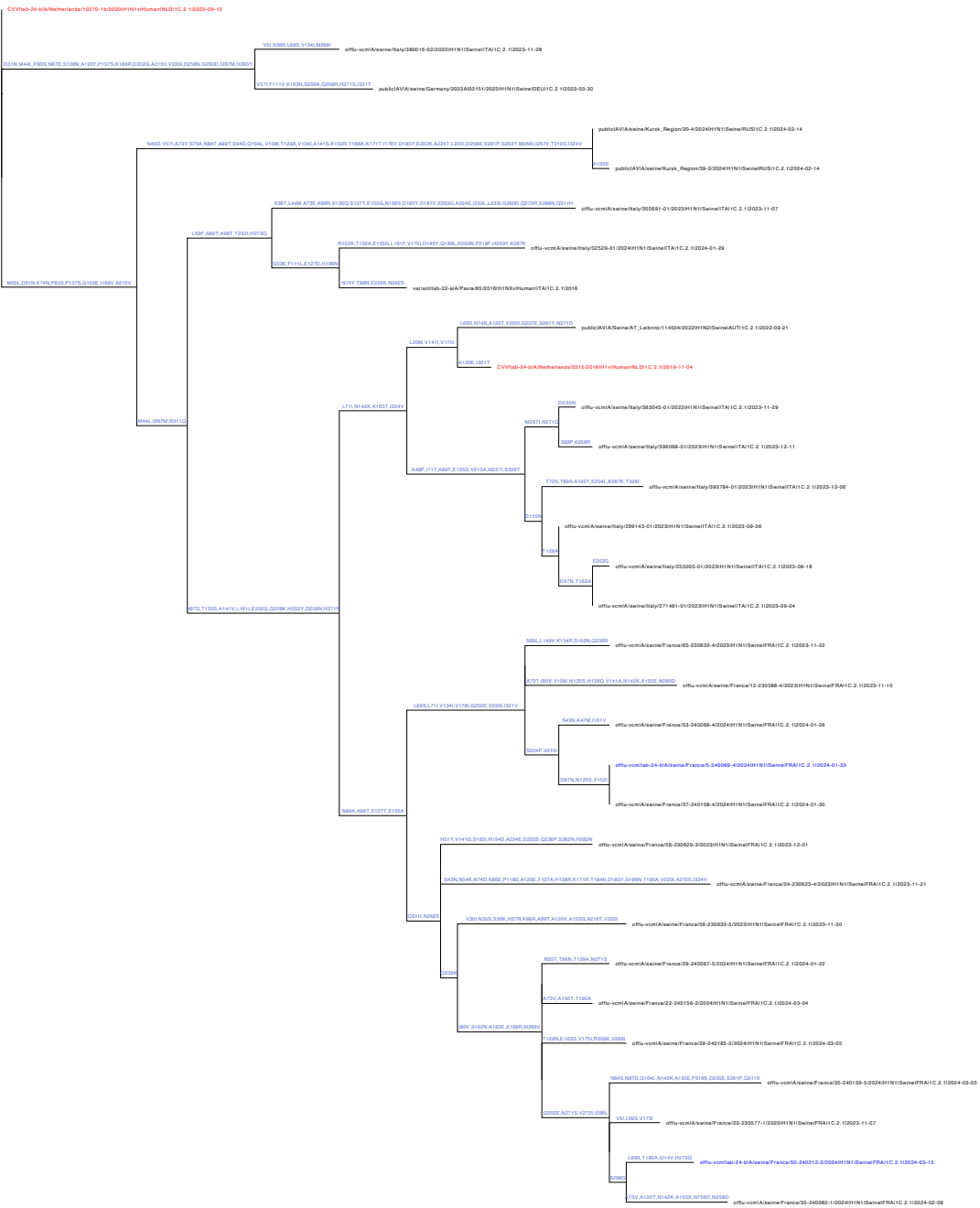


Figure A15. A phylogeny of the **1C.2.1** clade displaying n=28 sequences deposited on or after January 1, 2024 and collected in 2022-2024, and n=3 reference genes. The clade is rooted relative to the closest CVV (A/Netherlands/10370-1b/2020) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.

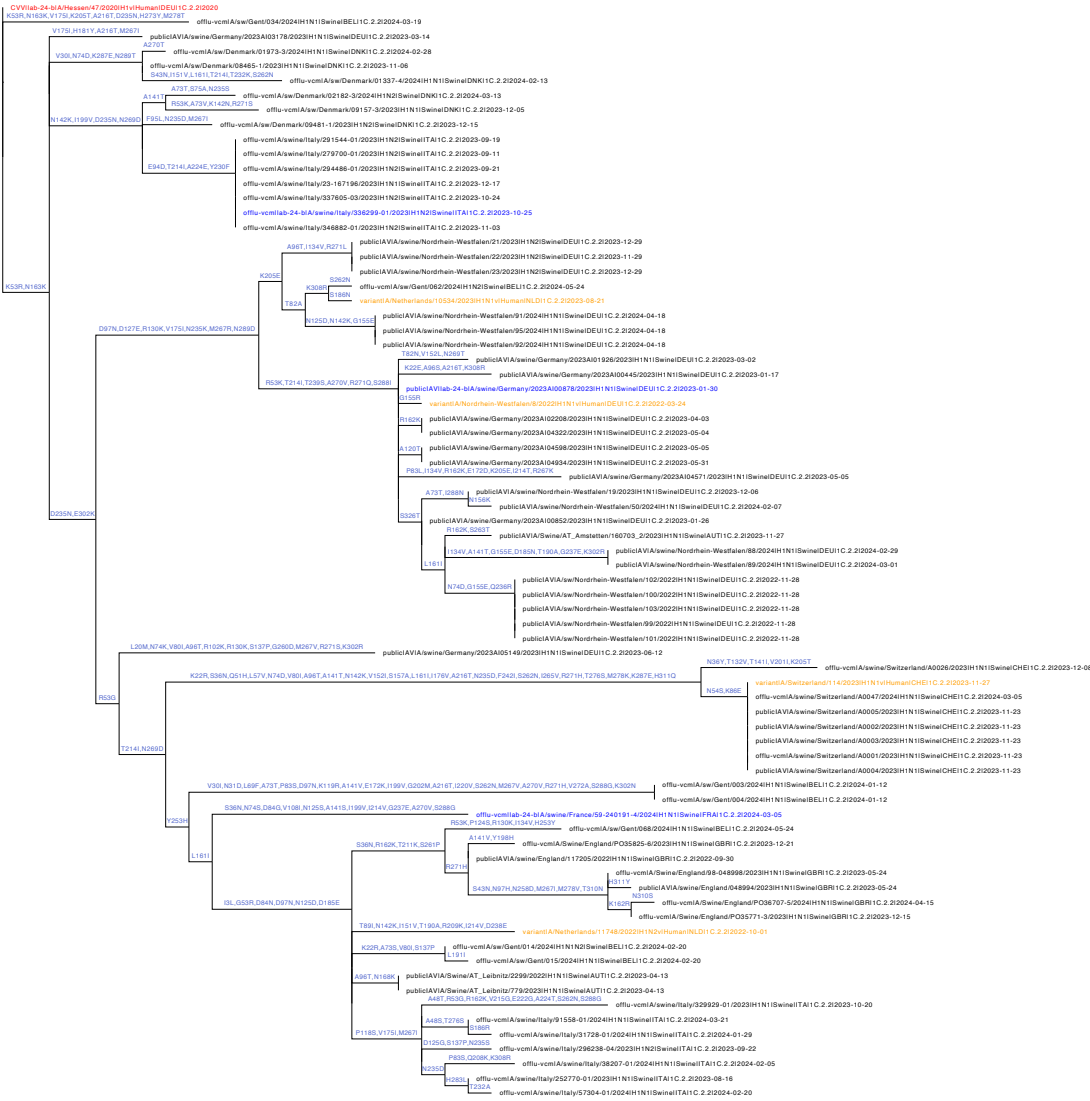


Figure A16. A phylogeny of the **1C.2.2** clade displaying n=70 sequences deposited on or after January 1, 2024 and collected in 2022-2024, and n=5 reference genes. The clade is rooted relative to the in-clade CVV (A/Hessen/47/2020) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.



Figure A18. A phylogeny of the **3.1990.4.a** clade displaying n=84 swine sequences deposited on or after January 1, 2024 and collected in 2022-2024, and n=5 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Minnesota/11/2010) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 10.