OFFLU SWINE INFLUENZA REPORT

JULY 2023 TO DECEMBER 2023

SCOPE

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

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 July 1, 2023 – December 31, 2023) 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 flutile (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

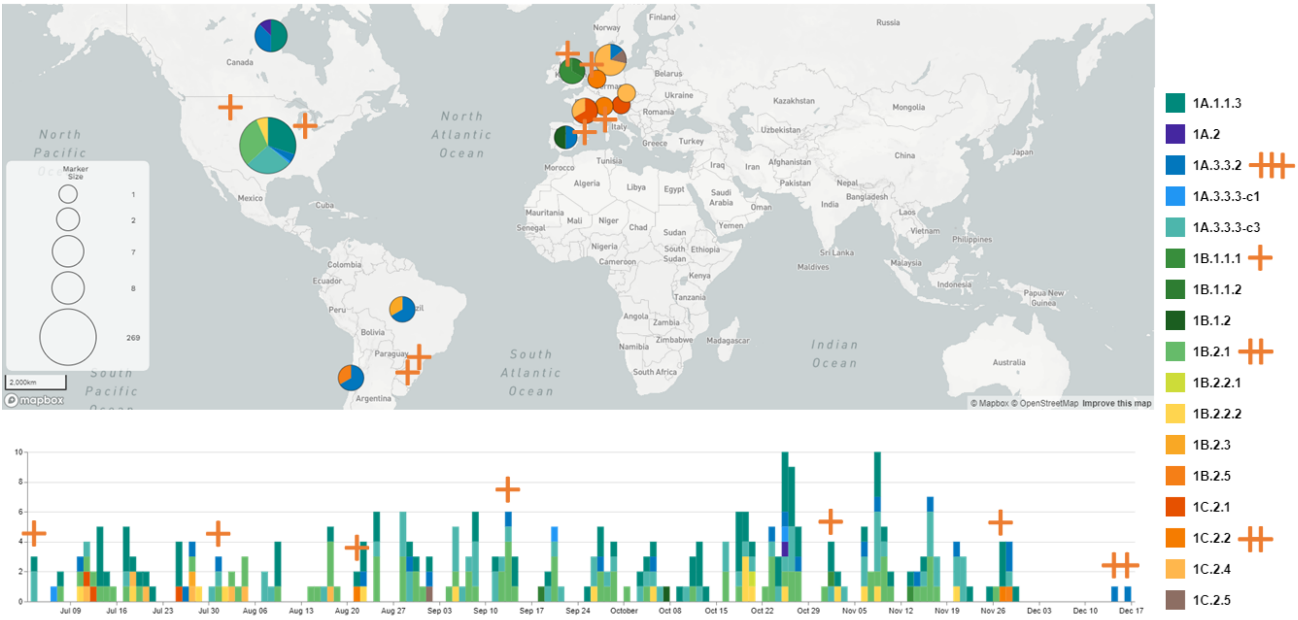


Figure 1. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between July 1, 2023 and December 31, 2023 (n=302); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions. Clades containing variants, location, and date of detection are marked with orange crosses.

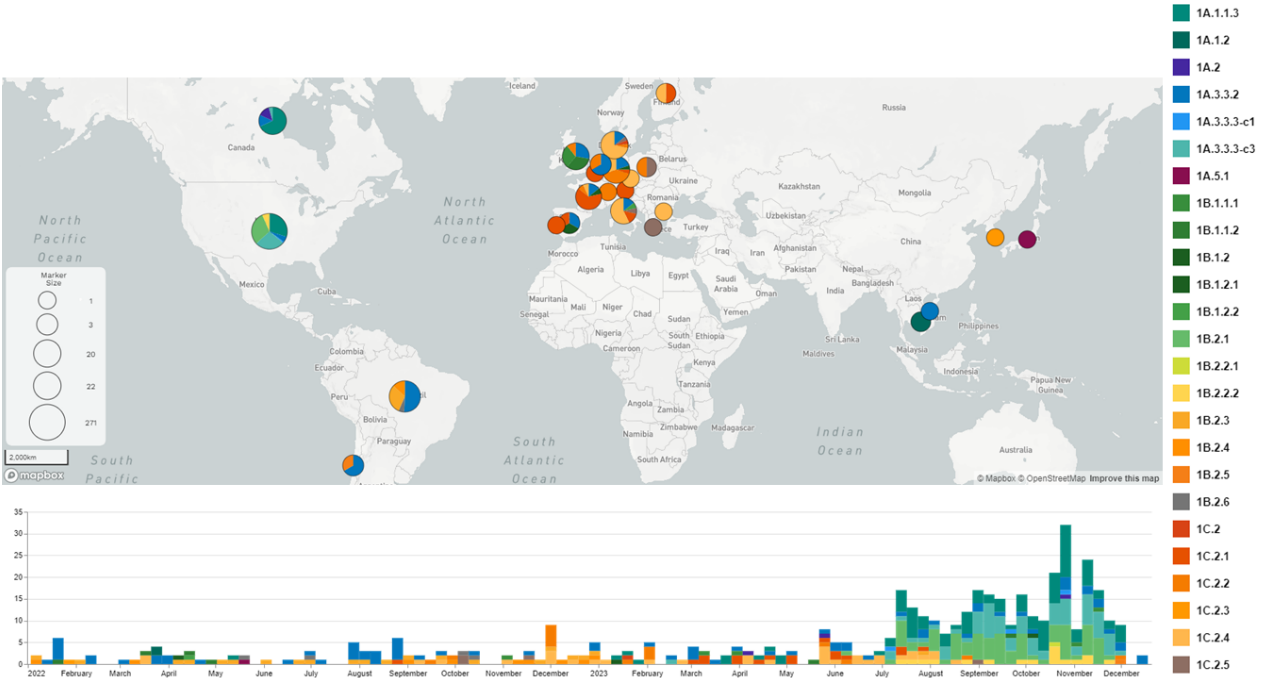


Figure 2. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between December 31, 2021 and December 31, 2023 (n=474); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions.

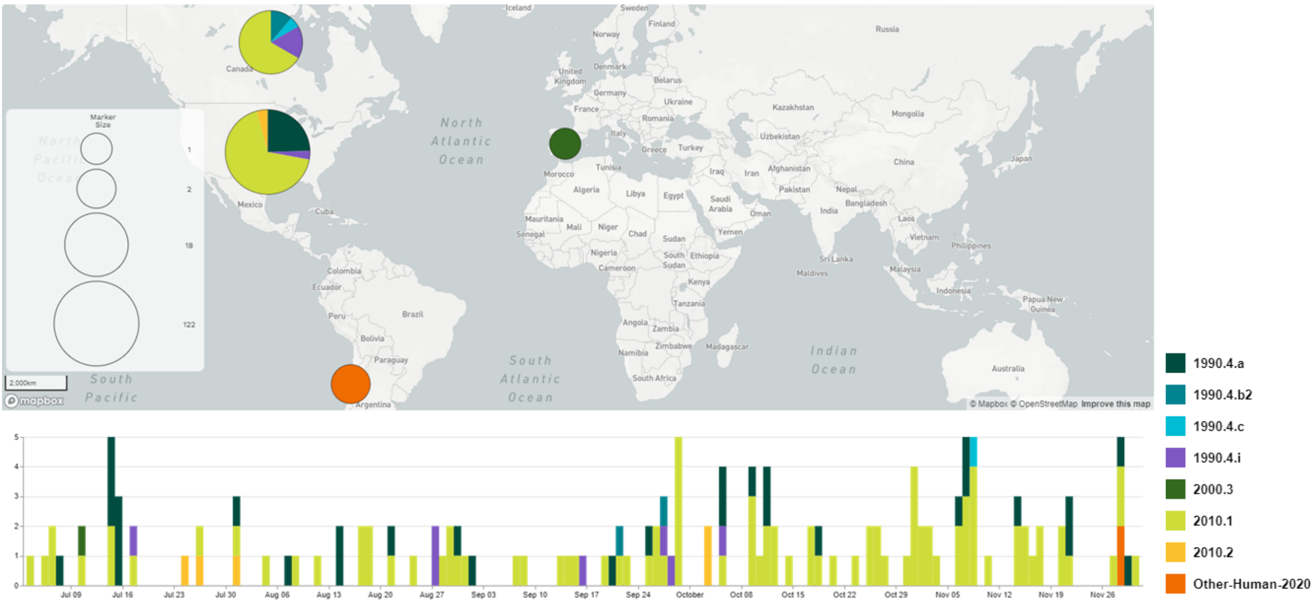


Figure 3. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between July 1, 2023 and December 31, 2023 (n=143). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions. Clades containing variants, location, and date of detection are marked with orange crosses.

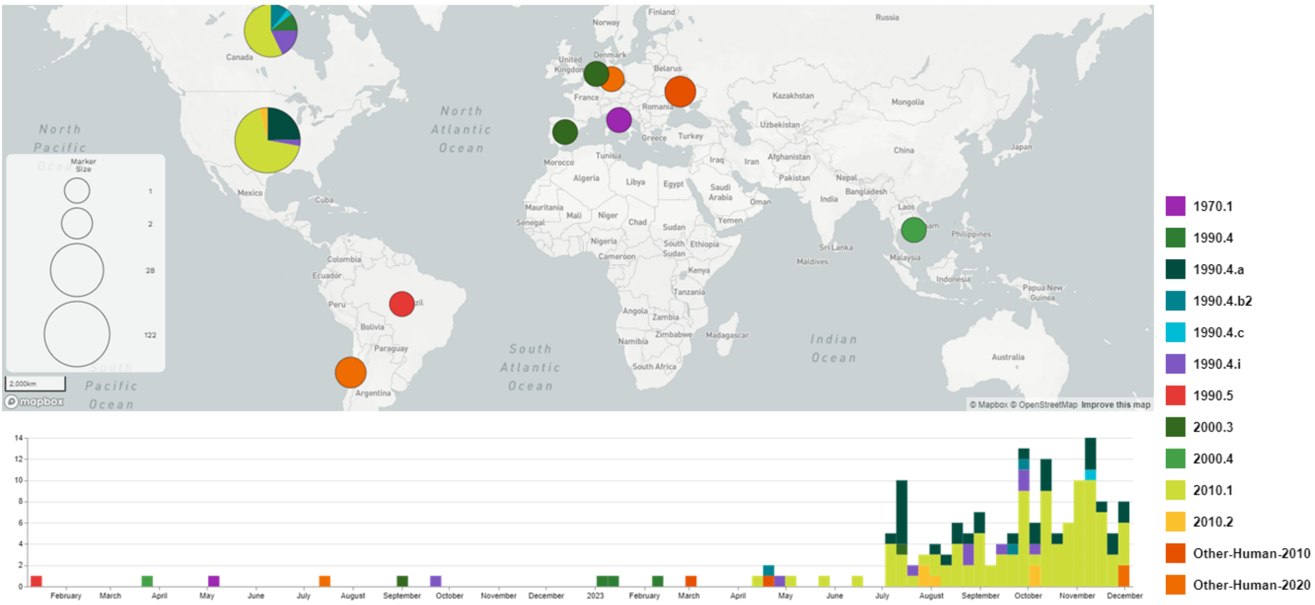
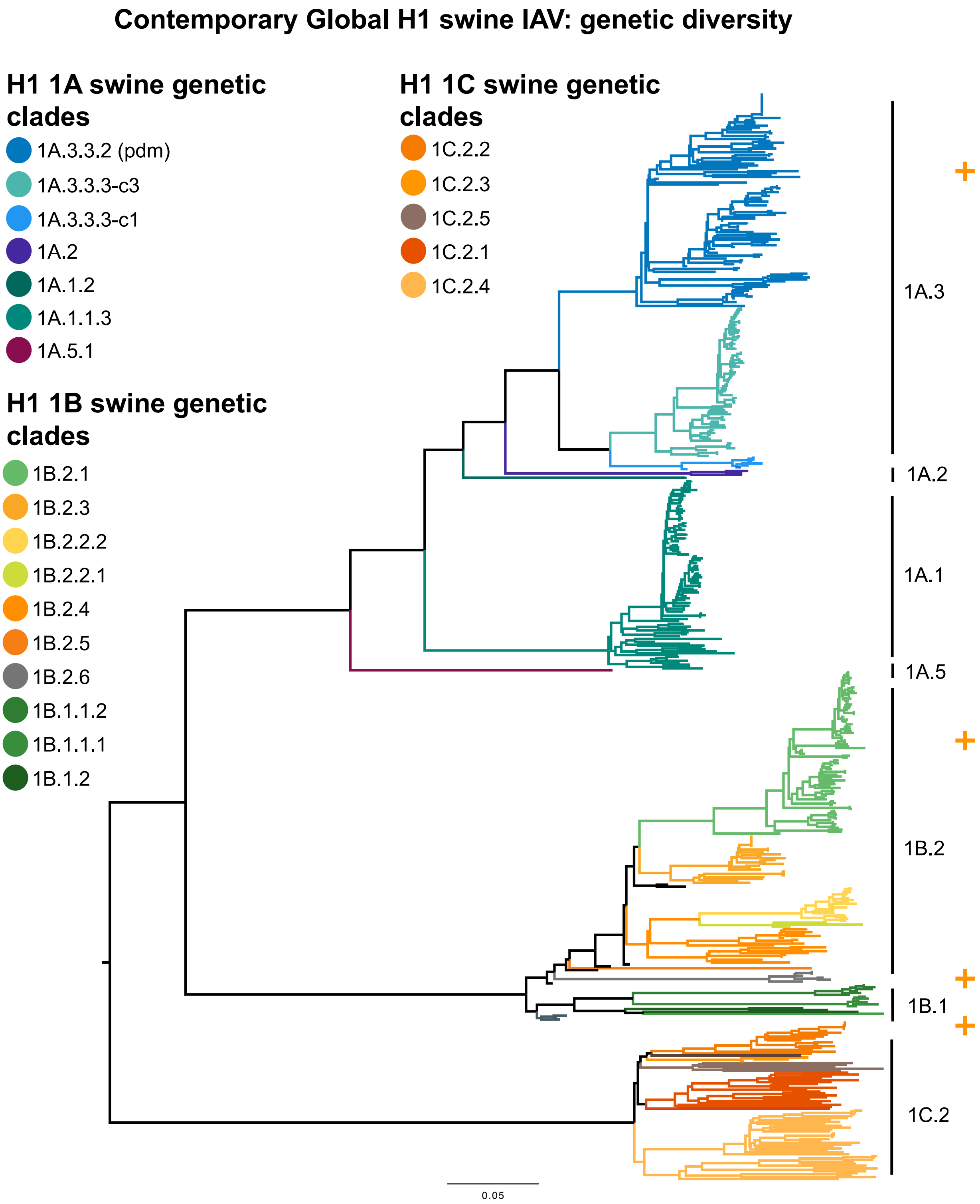


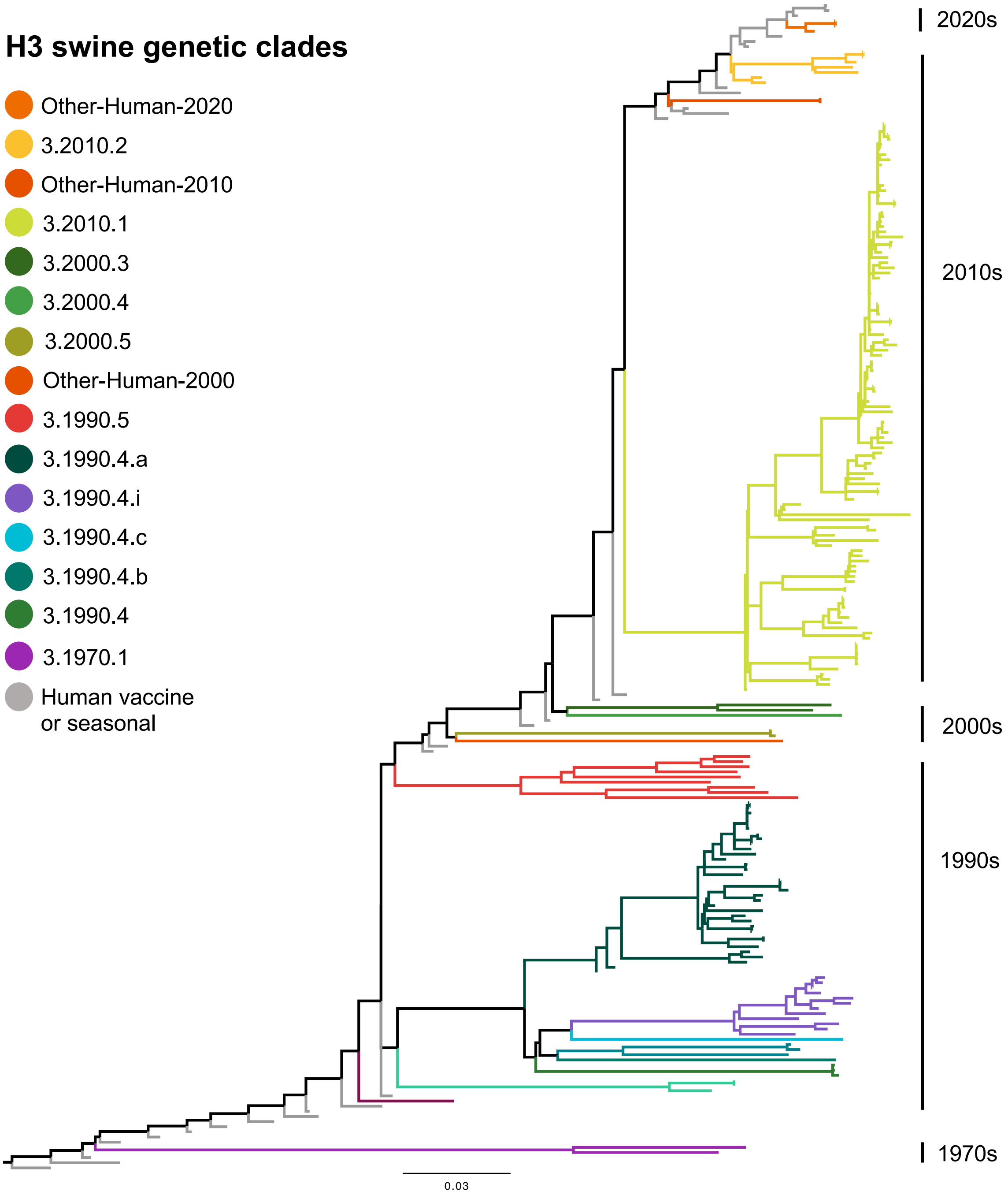
Figure 4. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between December 31, 2021 and December 31, 2023 (n=160). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions.



n=510, data deposited within last 6 months.

Figure 5. Global swine H1 phylogenetic tree (n=510) 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 July 1, 2023 – December 31, 2023) from GISAID or shared via the OFFLU swine IAV working group.

Contemporary Global H3 swine IAV: genetic diversity



n=170, data deposited within last 6 months, and n=56 reference genes.

Figure 6. Global swine H3 phylogenetic tree (n=170) with reference strains (n=56) colored by clade and annotated by decade of introduction from human seasonal H3. Analyses were conducted with reference sequences and data (all data deposited July 1, 2023 – December 31, 2023) from GISAID or shared via the OFFLU swine IAV working group.

Regional geographic summary

Phylogenetic analysis identified 34 genetic clades from H1 and H3 IAV in swine in sequence data deposited between July 1 - December 31, 2023.

22 clades were H1 subtype, with detections from: 1A classical swine lineage (7 clades); 1B human-seasonal lineage (10 clades); and 1C Eurasian avian lineage (5 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 and Canada; 1A.5.1 in Japan. The 1A.3.3.2 (pdm09) circulated in all countries that deposited sequence data during this 6-month period (5 countries).
- 1B.1 human seasonal lineage was only in Europe, and the 1B.2 human seasonal lineage was only in the Americas.
- The 1C.2 Eurasian avian lineage was detected in Europe. Additional detections of 1C.2 were reported in Asia outside the reporting period of Jan 1 – June 30, 2023.
- Diversity, detection frequency in swine, and an H1N2v within the 1B.1.1.x clade in the United Kingdom warranted new nomenclature: 1B.1.1.1, 1B.1.1.2, and 1B.1.1.3.
- In the 2-yr window, undersurveilled regions reported novel clades including: 1A.1.2 (Cambodia), 1B.2.3 (Brazil), 1B.2.4 (Brazil), 1B.2.5 (Chile), and 1B.2.6 (Brazil).

12 H3 clades were detected in swine within 8 distinct lineages derived from human seasonal H3 virus spillovers grouped by the decade of introduction (1970.1; 1990.4; 1990.5; 2000.3; 2000.4; 2000.5; 2010.1; 2010.2) and 3 novel human seasonal spillovers without evidence for onward circulation (Other-Human-2000, Other-Human-2010, Other-Human-2020). The 1990.4 lineage diversified into 4 co-circulating genetic clades.

- Most lineages exhibited regional circulation: the 1970.1 lineage in Italy; the 1990.5 lineage in Brazil; the 2000.4 in Cambodia; the 2000.5 lineage was detected in Japan; and the 2010.2 lineage was detected in the USA.
- The 1990.4 and the 2010.1 lineages were detected in the USA and Canada.
- Regionally restricted lineages had new detections outside of prior geography: 2010.1 (Canada); 2000.3 (Netherlands).
- In the 2-yr window, an uncharacterized lineage, 2000.4 was reported (Cambodia).
- Novel human-to-swine spillovers were detected in Brazil (Other-Human-2000), the Ukraine (Other-Human-2010), and Germany and Chile (Other-Human-2020).

Global Variant Cases:

During the reporting period (July 1 – December 31, 2023), 6 variant cases were reported and included with the swine analyses when sequence data were available:

- **United Kingdom: H1N2v (1B.1.1.1)***
- **Netherlands: H1N1v (1C.2.2)**
- **Switzerland: H1N1v (1C.2.2)**
- **USA: H1N2v (2x 1B.2.1), H3v (no sequence data)**

**(1B.1.1.1 characterization: https://doi.org/10.2807/1560-7917.ES.2024.29.3.2400002)*

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

- **Brazil: H1N1v (2x 1A.3.3.2)**
- **Spain: H1N1v (1A.3.3.2)**

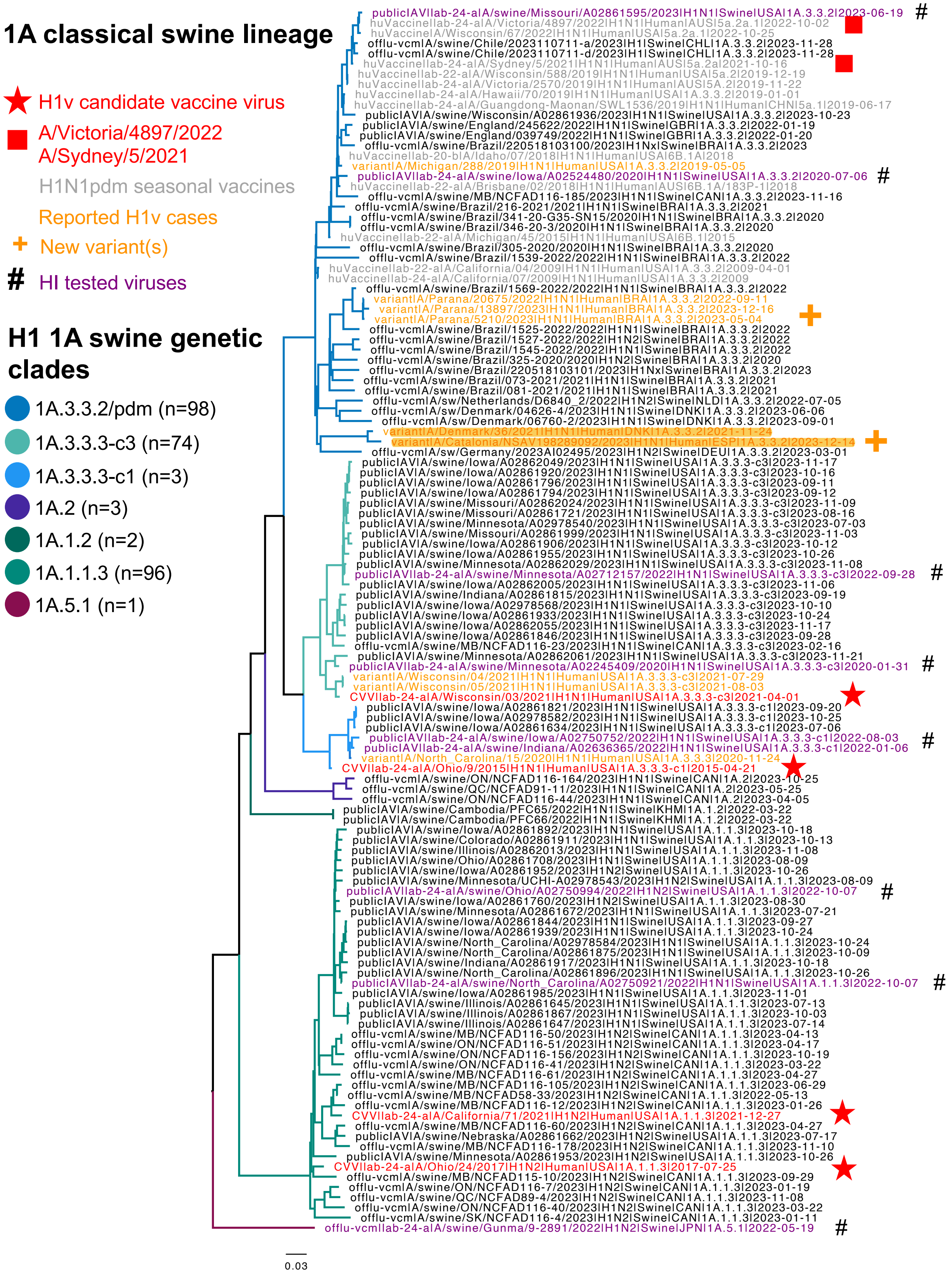
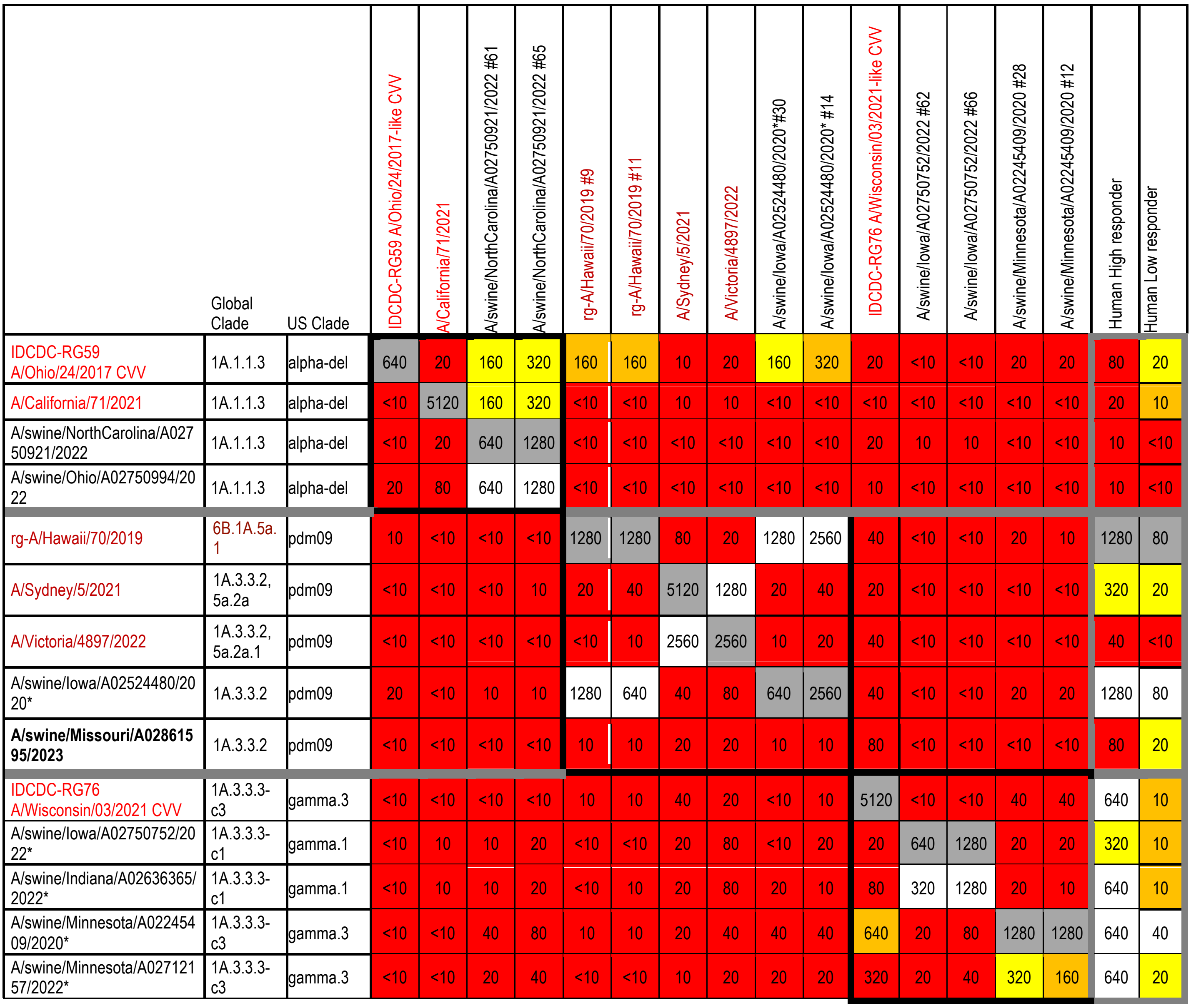


Figure 7. Swine H1 HA genes of the 1A lineage (tree was proportionally downsampled to 95 swine HA genes, excluding references). Number of detections of each clade from data deposited between July 1 – December 31, 2023 are presented adjacent to the clade name in the figure key.

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/NorthCarolina/A02750921/2022 #61	A/swine/NorthCarolina/A02750921/2022 #65	rg-A/Hawaii/70/2019 #9	rg-A/Hawaii/70/2019 #11	A/Sydney/5/2021	A/Victoria/4897/2022	A/swine/Iowa/A02524480/2020*#30	A/swine/Iowa/A02524480/2020* #14	IDCDC-RG76 A/Wisconsin/03/2021-like CVV	A/swine/Iowa/A02750752/2022 #62	A/swine/Iowa/A02750752/2022 #66	A/swine/Minnesota/A02245409/2020 #28	A/swine/Minnesota/A02245409/2020 #12	Human High responder	Human Low responder
IDCDC-RG59 A/Ohio/24/2017 CVV	1A.1.1.3	alpha-del	640	20	160	320	160	160	10	20	160	320	20	<10	<10	20	20	80	20
A/California/71/2021	1A.1.1.3	alpha-del	<10	5120	160	320	<10	<10	10	10	<10	<10	<10	<10	<10	<10	<10	20	10
A/swine/NorthCarolina/A02750921/2022	1A.1.1.3	alpha-del	<10	20	640	1280	<10	<10	<10	<10	<10	<10	20	10	10	<10	<10	10	<10
A/swine/Ohio/A02750994/2022	1A.1.1.3	alpha-del	20	80	640	1280	<10	<10	<10	<10	<10	<10	10	<10	<10	<10	<10	10	<10
rg-A/Hawaii/70/2019	6B.1A.5a.1	pdm09	10	<10	<10	<10	1280	1280	80	20	1280	2560	40	<10	<10	20	10	1280	80
A/Sydney/5/2021	1A.3.3.2, 5a.2a	pdm09	<10	<10	<10	10	20	40	5120	1280	20	40	20	<10	<10	<10	<10	320	20
A/Victoria/4897/2022	1A.3.3.2, 5a.2a.1	pdm09	<10	<10	<10	<10	<10	10	2560	2560	10	20	40	<10	<10	<10	<10	40	<10
A/swine/Iowa/A02524480/2020*	1A.3.3.2	pdm09	20	<10	10	10	1280	640	40	80	640	2560	40	<10	<10	20	20	1280	80
A/swine/Missouri/A02861595/2023	1A.3.3.2	pdm09	<10	<10	<10	<10	10	10	20	20	10	10	80	<10	<10	<10	<10	80	20
IDCDC-RG76 A/Wisconsin/03/2021 CVV	1A.3.3.3-c3	gamma.3	<10	<10	<10	<10	10	10	40	20	<10	10	5120	<10	<10	40	40	640	10
A/swine/Iowa/A02750752/2022*	1A.3.3.3-c1	gamma.1	<10	10	10	20	<10	<10	20	80	<10	20	20	640	1280	20	20	320	10
A/swine/Indiana/A02636365/2022*	1A.3.3.3-c1	gamma.1	<10	10	10	20	<10	10	20	80	20	10	80	320	1280	20	10	640	10
A/swine/Minnesota/A02245409/2020*	1A.3.3.3-c3	gamma.3	<10	<10	40	80	10	10	20	40	40	40	640	20	80	1280	1280	640	40
A/swine/Minnesota/A02712157/2022*	1A.3.3.3-c3	gamma.3	<10	<10	20	40	<10	<10	10	20	20	20	320	20	40	320	160	640	20

Reference CVV in red, seasonal vaccines in dark red, new swine in bold. Homologous titers highlighted in gray. *Previously tested; rg=synthetic HA/NA on PR8 backbone. Human sera were collected in USA with high or low responder relative to A/Hawaii/70/2019, pools of 2 male and 2 female adults in each.

- 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 and had significant decreases in titer in adult human sera.
- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine had >8-fold decrease to the A/Sydney/5/2021 5a.2a and A/Victoria/4897/2022 5a.2a.1 vaccine strains and adult human sera.
- The contemporary swine 1A.3.3.3-c1 (gamma.1) virus had >8-fold decrease to the 1A.3.3.3-c3 CVV A/Wisconsin/2021 but reacted with adult human high responder sera. The contemporary swine 1A.3.3.3-c3 (gamma.3) virus had >8-fold decrease from the within-clade 1A.3.3.3-c3 CVV A/Wisconsin/2021 but retained reactivity with adult human 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/Ohio/9/2015 CVV	A/California/71/21	A/California/7/2009	A/Guangdong-Maonan/SWL1536/2019	A/Victoria/2570/2019 (IVR-215)
A/Ohio/9/2015 CVV	H1N1 (1A.3.3.3)	320	40	80	320	320
A/California/71/21	H1N1 (1A.1.1.3)	160	2560	80	320	320
A/California/7/2009	H1N1 (1A.3.3.2)	80	40	320	320	160
A/Guangdong-Maonan/SWL1536/2019	1A.5a.1	80	160	320	10240	2560
A/Victoria/2570/2019 (IVR-215)	1A.5a.2	1280	640	160	640	10240
A/swine/Gunma/9-2891/2022 H1N2	1A.5.1	80	80	0	160	320

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

- The contemporary swine 1A5.1 from Japan has no within clade CVV and showed 4-fold loss in reactivity with the 1A.3.3.3 A/Ohio/9/2015 CVV.

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).

site	A/California/71/2021	A/Ohio/24/2017	A/swine/Ohio/A02750994/2022	A/swine/North_Carolina/A02750921/2022	annotations
48	A		S	S	
69	P	S	S	S	
71	P	L	L	L	Cb
103	V	E	E	E	
119	T	I	I	I	
124	S	P	P		Sa
126	H		Y	Y	
127	E	K	K	K	
132	A	V	E	E	RBS
138	Y	D			
140	G			E	Ca2
141	K	A			
149	M	I		V	
155	G		A	A	Sa
156	D	N			Sb
157	S			L	Sa
160	M	R	R	K	Sa
166	A	V	V	V	Ca1
168	D	N	N		
170	G	R			Ca1
185	D	G	G		
186	S	N	N	N	
189	R	Q	Q	Q	Sb, RBS
195	T	A	A	A	Sb, RBS
209	K	E			
222	N	G	G	G	Ca2, RBS
224	A	T			RBS
232	A	T	T	T	
252	R	K			
272	I			T	
308	R	K	K	K	
310	T		R	R	
311	R	K			
aadiff		26	21	22	

Reference CVVs in red, HI swine strains in purple.

Table 4. 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, CVV, and recent 1A.3.3.2 variants.

site	A/Wisconsin/67/2022	A/Victoria/4897/2022	A/California/07/2009	A/Victoria/2570/2019	A/Guangdong-Maonan/SWL1536/2019	A/Hawaii/70/2019	A/Sydney/5/2021	A/swine/Iowa/A02524480/2020	A/swine/Missouri/A02861595/2023	A/Parana/5210/2023	A/Catalonia/NSAV198289092/2023	A/Parana/13897/2023	annotations
19	V									I	I	I	
26	V										I		
43	K										Q		
54	Q		K	K	K	K		K		K	K	K	
69	S										T		
70	L										I		Cb
71	S										P		Cb
74	R		S								G		Cb
82	T										A		
83	S		P							P		P	
84	N		S							S	S	S	
85	S										P		
94	D						N			X			
97	N		D							X	D	E	
104	Q										H		
112	E								D	X			
113	R							K					
120	T									S		S	
125	N									X		D	Sa
127	D								N	X			
128	S									X		A	
129	D		N					N		N	S	N	
130	N		K		K	K		K			K		
135	A									S		S	RBS
137	S		P	P	P	P	P			P	P	P	Ca2
139	A										N		
141	A									T		T	
142	R		K	K	K	K	K	K		S	N	S	Ca2
152	V									I	I	I	
155	G										E		Sa
156	K		N		N	N		N	Q	N	N	N	Sb
161	I		L		L	L		L		L	L	L	

162	N		S							S		S	Sa
163	Q		K							K	I	K	Sa
164	T		S							S	S	S	Sa
166	I										V		Ca1
173	V							I					
176	L										V		
183	P		S										
185	I		S					T		N	S	N	
186	T		A	A	A	A		A			D		
187	D				A	A				S	S	S	RBS
189	E		Q	Q				Q		Y	Q	Y	Sb, RBS
190	S										W		Sb, RBS
195	A									G		G	Sb, RBS
196	D										N		
202	G										S		
203	T		S										
205	R										K		
210	F										Y		
211	K										N		
216	T	A	I				A		A	I	R	I	
217	R								X				
222	D									N		N	Ca2, RBS
223	Q	R		R									RBS
224	A		E	E	E	E		E		E	E	E	RBS
249	V										M		
250	A		V		V	V		V		V		V	
256	T		A							A	A	A	
259	K		R	R	R	R		R		R	R	R	
260	E		N	D	D	D	D	N		N	N	N	
261	A										T		
270	T										A		
272	V										I		
274	D										N		
277	A		T	T	T	T	T	T		T	T	T	
283	E		K							K	K	K	
287	N											K	
295	V		I							I	I	I	
302	K									X		Q	
308	R		K	K	K	K		K		X		K	
311	K									X	R		
321	V		I							X	T		
324	I									X		A	
aadiff		2	30	11	14	14	6	17	5	41	51	37	

Reference vaccine strains in gray, CVV in red, HI strains in purple, variants in orange.

Table 5a. Amino acid substitutions between representative swine 1A.3.3.3-c1 strains compared to the 1A.3.3.3-c3 CVV (A/Ohio/9/2015) used in the HI assay.

site	A/Ohio/9/2015	A/swine/Indiana/A02636365/2022	A/swine/Iowa/A02750752/2022	annotations
22	K	R	R	
48	X	A	A	
68	E	D	D	
72	T	R	R	
82	T	N	N	
83	S	P	P	
120	T		N	
125	N	K	K	Sa
169	R	K	K	
194	N		H	RBS
204	S	P	P	Ca1
205	R		K	
222	G	D	D	Ca2, RBS
228	N		S	
235	E		A	
269	E	G	G	
278	T	I	I	
283	N	S	S	
295	I	V	V	
aadiff		14	19	

Reference CVV in red, HI swine strains in purple.

Table 5b. Amino acid substitutions between representative 1A.3.3.3-c3 swine strains compared to the most similar within-clade CVV (A/Wisconsin/03/2021).

site	A/Wisconsin/03/2021	A/swine/Minnesota/A02245409/2020	A/swine/Minnesota/A02712157/2022	annotations
35	T	N	N	
45	K	R	R	
56	N		S	
74	S		R	Cb
83	S	P		
84	N		K	
120	T	A		
127	E	D	D	
129	D	N	N	
130	K	R		
137	P	S		Ca2
142	R	N	N	Ca2
161	V		I	
163	T	K	K	Sa
168	N		D	
169	K	R		
171	K		R	
189	R	Q	Q	Sb, RBS
195	E	A	A	Sb, RBS
205	R	K	K	
224	S	A	A	RBS
252	R		I	
262	G		E	
270	A	T	T	
278	T		A	
295	I		V	
302	E		K	
326	S		T	
aadiff		16	23	

Reference CVV in red, HI swine strains in purple.

Table 6. Amino acid substitutions between representative 1A.5.1 swine strain compared to most similar HI-tested vaccine strain (A/California/07/2009).

cont.

site	A/California/07/2009	A/swine/Gunma/9-2891/2022	annotations
2	T	I	
5	I	V	
19	V	I	
36	K	T	
39	G	K	
54	K	R	
61	I	L	
72	T	N	
73	A	V	Cb
83	P	S	
84	S	K	
96	I	S	
97	D	N	
119	K	N	
120	T	A	
127	D	K	
128	S	T	
129	N	D	
130	K	R	
137	P	S	Ca2
138	H	K	
139	A	S	
141	A	K	
142	K	S	Ca2
152	V	L	
156	N	D	Sb
157	S	-	Sa
161	L	I	
162	S	N	Sa
166	I	V	Ca1
173	V	I	
183	S	P	
185	S	R	
186	A	N	
187	D	S	RBS
190	S	E	Sb, RBS
193	Q	N	Sb, RBS
195	A	E	Sb, RBS
196	D	N	
197	A	T	
203	S	T	
205	R	T	
207	S	N	
208	K	Q	
211	K	M	
216	I	E	
224	E	A	RBS
239	K	T	
250	V	A	
257	M	V	
258	E	D	
260	N	G	
261	A	S	
270	T	V	
272	V	L	
274	D	N	
276	N	T	
278	T	E	
295	I	V	
298	I	V	
302	K	E	
aadiff		61	

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

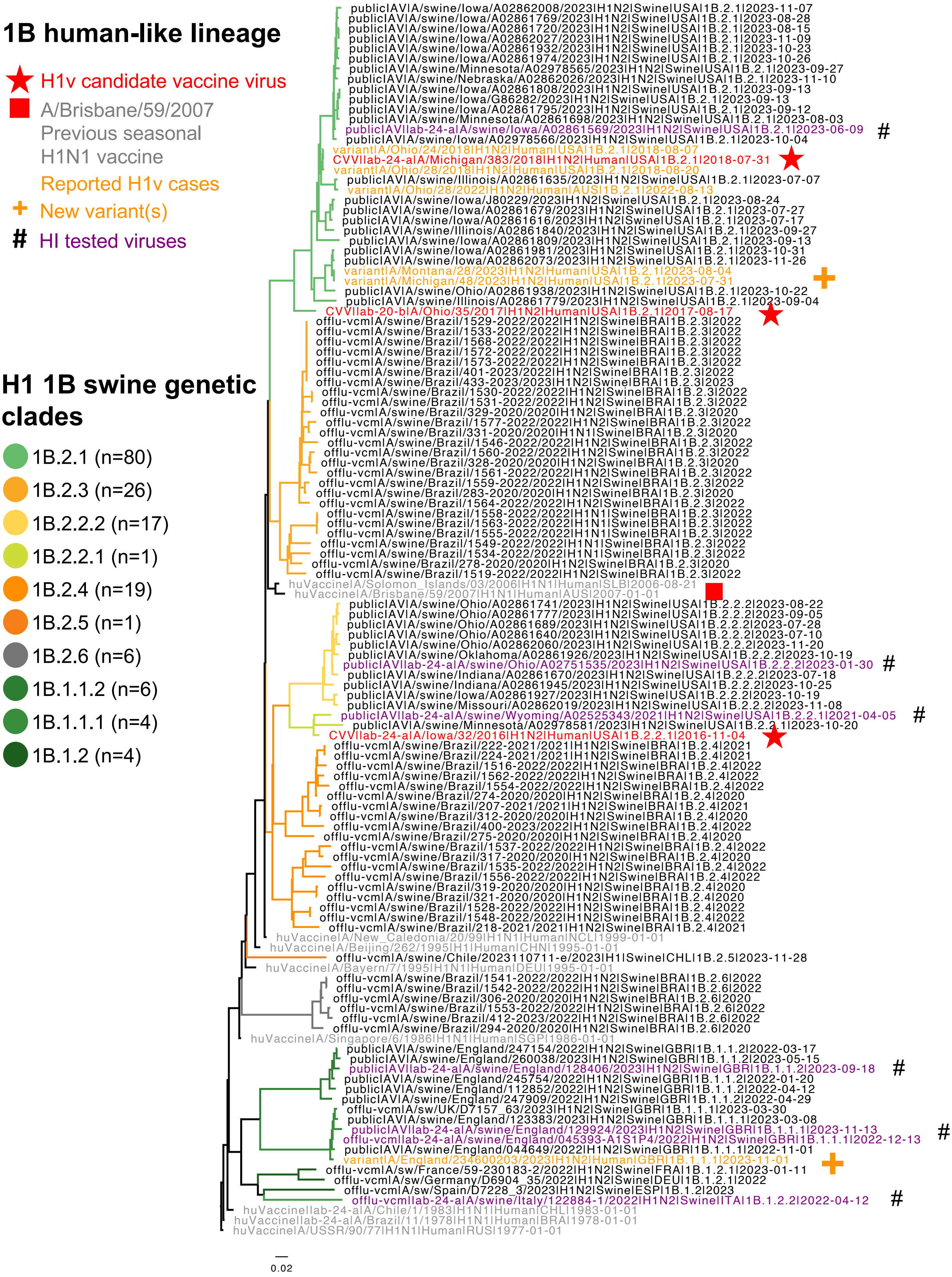


Figure 8. Swine H1 HA genes of the 1B lineage (tree was proportionally down sampled to 104 swine HA genes, excluding references). Number of detections of each clade from data deposited between July 1 - December 31, 2023 are presented adjacent to the clade name in the figure key.

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 High responder	Human Low responder
A/Michigan/383/2018 RG58A	1B.2.1	delta-2	160	<10	<10	<10	10	20
A/swine/Iowa/A02861569/2023	1B.2.1	Delta-2	160	40	10	10	10	10
A/Iowa/32/2016	1B.2.2.1	delta-1a	<10	160	40	20	80	40
A/swine/Wyoming/A02525343/2021	1B.2.2.1	delta-1a	<10	20	160	80	40	20
A/swine/Ohio/A02751535/2023*	1B.2.2.2	delta-1b	<10	10	<10	<10	<10	<10
rg-A/Hawaii/70/2019	6B.1A.5a.1	pdm09	nt	nt	nt	nt	1280	80

Reference CVV in red, new swine strains in bold. Homologous titers highlighted gray. *Previously tested in report 2023a; Human sera were collected in USA with high or low responder relative to A/Hawaii/70/2019, pools of 2 male and 2 female adults in each. nt=not tested.

- The 1B.2.1 (delta-2) clade swine representative had no loss of titer the within clade CVV but displayed limited titers in adult human sera.
- The 1B.2.2.1 A. had 8-fold reduction from the within clade CVV A/Iowa/32/2016 and significant loss in titers in adult human high responder 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 limited titers in adult human 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/Michigan/383/2018 CVV	A/Chile/1/1983	A/Brazil/11/1978
A/Michigan/383/2018 CVV	H1N2 1B.2.1	2560	160	640
A/Chile/1/1983	H1N1 1B.1.1	20	640	640
A/Brazil/11/1978	H1N1 1B1.1	40	320	2560
A/swine/England/045393-A1S1P4/2022 H1N2	1B.1.1.1	40	10	80
A/Swine/England/129924/2023	**1B.1.1.1**	80	20	320
A/Swine/England/128406/2023	**1B.1.1.2**	160	320	5120
A/swine/Italy/122884-1/2022 H1N2	**1B.1.2.2**	40	80	320

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

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

- There are no within-clade CVVs for 1B lineages from Europe and the 1B.2.1 CVV has significant loss in reactivity with 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.

Table 9. Amino acid substitutions between representative 1B.2.1 swine strains 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/A02635863/2021	A/swine/Michigan/A02780683/2023	A/Michigan/48/2023	annotations
5	V		I	I	
16	V		I	I	
69	L		M	M	
71	N	T	I	I	Cb
82	T		K	K	
83	S		P	P	
89	T		A	A	
113	K		R	R	
119	K		E	E	
149	L	V			
168	K		T	T	
169	E		K	K	
170	G	E	E	E	Ca1
173	V	I	I	I	
185	M		I	I	
193	H		N	N	Sb, RBS
224	E		G	G	RBS
237	G		K	K	Ca1
260	S	G	G	G	
261	F	S			
277	T		A	A	
310	A		T	T	
aadiff		6	20	20	

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

Table 10. Amino acid substitutions between representative swine 1B.2.2.1 strain compared to the within-clade CVV (A/Iowa/32/2016).

site	A/Iowa/32/2016	A/swine/Wyoming/A02525343/2021	annotations
19	L	V	
72	S	P	
74	K	E	Cb
96	T	A	
106	S	N	
130	T	D	
132	K	T	RBS
157	L	R	Sa
168	E	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	

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

Table 11. 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

site	A/Iowa/32/2016	A/swine/Ohio/A02751535/2023	annotations
19	L	V	
36	S	N	
50	L	I	
82	A	T	
85	S	P	
86	D	E	
94	Q	D	
96	T	A	
119	R	K	
129	V	T	
142	G	S	Ca2
146	R	K	
149	I	V	
153	V	E	Sb
168	E	D	
170	G	E	Ca1
175	I	V	
176	L	I	
183	S	P	
184	N	D	
186	G	E	
187	D	N	RBS
193	H	R	Sb, RBS
194	T	K	RBS
202	M	A	
208	R	E	
222	D	N	Ca2, RBS
228	N	K	
236	P	A	
244	T	S	
249	I	V	
256	A	T	
269	K	N	
271	P	S	
273	D	N	
274	E	Q	
277	A	T	
283	K	Q	
289	N	S	
310	T	A	
aadiff		40	

Relative to most similar vaccine

site	A/Michigan/2/2003	A/swine/Ohio/A02751535/2023	annotations
36	S	N	
50	L	I	
69	L	S	
74	E	K	Cb
94	Y	D	
129	V	T	
132	V	K	RBS
141	K	E	
146	R	K	
149	L	V	
153	G	E	Sb
162	I	S	Sa
166	A	K	Ca1
168	N	D	
175	I	V	
176	L	I	
184	N	D	
186	G	E	
187	D	N	RBS
193	H	R	Sb, RBS
194	T	K	RBS
202	V	A	
208	R	E	
222	D	N	Ca2, RBS
228	N	K	
236	P	A	
244	A	S	
249	I	V	
256	A	T	
261	F	L	
271	P	S	
273	D	N	
274	E	Q	
277	A	T	
aadiff		34	

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

Table 12. Amino acid substitutions between representative swine 1B.1.1.x strains compared to HI-tested vaccine strain (A/Chile/1/1983) and a recent 1B.1.1.1 variant.

cont. →

site	A/Chile/1/1983	A/swine/England/045393-A1S1P4/2022	A/swine/England/128406/2023	A/swine/England/129924/2023	A/England/234600203/2023	annotations
14	D	E		E	E	
19	V		I			
35	D		N			
36	N	S	S	S	S	
44	L		M			
48	A	P		P	P	
54	K	T		T	T	
66	E	K		K	K	
70	L		S			Cb
73	K		R			Cb
74	K	N		N	N	Cb
82	T	L		S	L	
85	S	P	P	P	P	
89	T	I	I	I	I	
94	Y	H		H	H	
96	A	S	S	S	S	
111	F		I			
116	I		M		M	
120	E	D		D	D	
125	K	G	N	G	G	Sa
130	K		R			
132	V		K	L		RBS
134	A		V			RBS
135	A	S	S	S	S	RBS
137	S	P		P	P	Ca2
139	K	D	N	D	D	
146	R		K			
149	L	V		V	V	
153	E	G		G	G	Sb
155	N		D			Sa
156	G	N		N	N	Sb
157	S	L	L	L	L	Sa
161	L	V	I	V	V	
162	S	N		N	N	Sa
163	K		N			Sa
164	S		R			Sa
170	E			G		Ca1
176	L		I			

site	A/Chile/1/1983	A/swine/England/045393-A1S1P4/2022	A/swine/England/128406/2023	A/swine/England/129924/2023	A/England/234600203/2023	annotations
181	H		N			
185	I		M			
189	K		R			Sb, RBS
190	T	A	A	A	A	Sb, RBS
193	R	H		H	H	Sb, RBS
194	K	N		N	N	RBS
196	N		T			
197	A	T		T	T	
208	R			K		
209	R	K		K	K	
215	A		E			
216	K	R		R	R	
220	V	I	I	I	I	RBS
237	G	D	S	D	D	Ca1
238	D	E	E	E	E	
239	T	E	I	E	E	
248	L		F			
252	W	Q		L	Q	
260	G	S		S	S	
261	F		L		Y	
262	G		R			
267	T	I	I	I	I	
270	A	T		T	T	
272	M		L		I	
273	D		G			
276	D	N		N	N	
277	A	T	T	T	T	
278	K		E			
287	N			T		
288	S		N			
299	T		A			
305	K		R			
307	V		I			
308	R	K	K	K	K	
310	T			K		
311	K		R			
324	I		V			
aadiff		39	49	44	42	

Reference human seasonal vaccine in gray, HI swine strains in purple, recent variant in orange.

Table 13. Amino acid substitutions between representative swine 1B.1.2.2 strain compared to the most similar human seasonal vaccine strain (A/Chile/1/1983) used in HI assays.

site	A/Chile/1/1983	A/swine/Italy/122884-1/2022	annotations
47	I	V	
54	K	R	
71	F	L	Cb
82	T	A	
85	S	A	
89	T	L	
94	Y	H	
96	A	S	
111	F	I	
127	N	S	
128	V	I	
129	T	-	
130	K	-	
132	V	A	RBS
135	A	S	RBS
137	S	P	Ca2
138	H	K	
139	K	Q	
141	K	R	
146	R	T	
161	L	M	
162	S	T	Sa
163	K	M	Sa
166	V	M	Ca1
168	N	D	
170	E	R	Ca1
171	K	R	
189	K	R	Sb, RBS
190	T	A	Sb, RBS
196	N	T	
202	V	M	
207	N	S	
208	R	K	
209	R	K	
220	V	I	RBS
222	N	D	Ca2, RBS
237	G	S	Ca1
258	S	N	
262	G	E	
267	T	V	
270	A	V	
276	D	N	
278	K	T	
283	Q	R	
288	S	N	
295	V	I	
298	V	I	
308	R	K	
313	R	K	
315	V	A	
aadiff		50	

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

Figure 9. Swine H1 HA genes of the 1C lineage (n=70, excluding references). Number of detections of each clade from data deposited between July 1 - December 31, 2023 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.

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

	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	H1N1 1C.2.1	1280	80	160	<10	<10
A/Netherlands/10370-1b/2020 CVV	H1N1 1C.2.1	40	320	320	40	<10
A/Hessen/47/2020-like CVV	H1 1C.2.2	40	160	5120	640	40
A/swine/England/048998/2023	**1C.2.2**	20	80	640	40	<10
A/Hunan/42443/2015 CNIC-1601 CVV	H1N1 1C.2.3	80	160	1280	640	<10
A/Bretagne/24241/2021 CVV	H1N1 1C.2.4	20	40	40	<10	1280
A/swine/Italy/359928-10/2022	**1C.2.4**	80	40	80	<10	<10

Reference CVV in red, new swine strains in bold. Homologous titers highlighted in gray. *Previously tested in report 2023a.

- The 1C.2.2 swine strain from England had 8-fold loss to the within-clade CVV A/Hessen/47/2020.
- The 1C.2.4 swine strain from Italy had >8-fold loss to the within-clade CVV A/Bretagne/24241/2021.

Table 15. Amino acid substitutions between representative swine 1C.2.2 strain compared to the within-clade CVV (A/Hessen/47/2020), similar 1C.2.3 CVV (A/Hunan/42443/2015), and recent 1C.2.2 variants.

cont. →

site	A/Hessen/47/2020	A/Hunan/42443/2015	A/swine/England/048998/2023	A/Netherlands/10534/2023	A/Switzerland/114/2023	annotations
3	I		L			
22	K				R	
35	T	N				
36	S		N		N	
43	S		N			
48	A	I				
51	Q				H	
53	K	G	R	R	G	
54	N				S	
56	N	S				
57	L	V			V	
66	E	K				
74	N				D	Cb
80	V	I			I	
82	T			A		
83	P	S				
84	D	N	N			
86	K				E	
89	T	A				
96	A				T	
97	D		H	N		
102	R	K				
125	N		D			Sa
127	D			E		
130	R			K		
134	I	V				RBS
141	A				T	
142	N				K	Ca2
152	V				I	
155	G				X	Sa
157	S				A	Sa
161	L		I		I	
162	R	S	K			Sa
163	N	K	K	K	K	Sa
175	V			I		
176	I				V	
185	D		E			
186	S			N		
199	I	V				
205	K			E		
208	Q	K				
211	T		K			
214	T	I	I		I	
216	A				T	
220	I	V				RBS
227	I	M				
235	D		N	K		
242	F				I	
253	Y	H	H			
258	N	K	D			
261	S		P			
262	S				N	
265	I				V	
266	I	M				
267	M	R	I	R		
269	N	D	D		D	
271	R	Q	H		H	
276	T				S	
278	M	K	V		K	
287	K				E	
288	S	G				
289	N			D		
298	I	V				
302	E		K	K	K	
308	K			R		
310	T		N			
311	H	Q	Y		Q	
aadiff		29	24	14	30	

Reference CVVs in red, HI swine strain in purple, new variant detections in orange.

Table 16. Amino acid substitutions between representative swine 1C.2.4 strain compared to the within-clade CVV (A/Bretagne/24241/2021).

site	A/Bretagne/24241/2021	A/swine/Italy/359928-10/2022	annotations
5	I	V	
15	T	I	
20	M	L	
36	N	S	
46	G	E	
54	N	S	
56	D	N	
71	V	L	Cb
83	S	P	
86	K	E	
104	L	Q	
120	-	A	
121	N	T	
125	S	D	Sa
128	A	T	
129	-	T	
130	-	K	
134	V	A	RBS
138	K	H	
141	A	D	
142	N	K	Ca2
149	L	I	
152	T	V	
155	G	K	Sa
156	K	N	Sb
157	P	S	Sa
159	S	P	Sa
161	L	I	
166	K	T	Ca1
175	V	I	
184	T	N	
186	S	G	
199	V	I	
202	V	E	
208	Q	R	
216	P	T	
218	P	Q	RBS
219	E	K	RBS
222	E	D	Ca2, RBS
224	R	A	RBS
237	E	G	Ca1
252	R	W	
258	E	D	
261	S	I	
266	M	V	
267	K	M	
269	D	E	
283	H	L	
288	S	G	
289	D	N	
290	R	L	
311	Q	R	
aadiff		52	

Reference CVV in red, HI swine strain in purple.

Table 17. Amino acid substitutions between representative swine 1C.2.5 strain compared to most similar CVV (A/Netherlands/3315/2016) and other 1C.2 CVVs.

cont. →

site	A/Hessen/47/2020	A/Netherlands/3315/2016	A/Netherlands/10370-1b/2020	A/Hunan/42443/2015	A/Bretagne/24241/2021	A/swine/Italy/381004-06/2022	annotations
5	V				I		
20	L	M	M		M		
31	N		D				
35	T	N	N	N		N	
36	S				N		
44	L		M			M	
47	K					N	
48	A			I			
53	K	G	G	G	G	R	
56	N			S	D	D	
57	L	V	V	V	V	V	
66	E			K			
69	L					F	
71	L	I			V		Cb
74	N		K				Cb
80	V	I	I	I	I	I	
82	T					A	
83	P	S		S	S		
84	D	N	N	N	N	N	
85	S					P	
89	T	A	A	A		A	
97	D		N				
102	R			K			
104	Q				L	L	
111	F					L	
120	A	E			-	R	
121	T				N		
125	N				S	D	Sa
127	D	E	E		E	E	
128	T				A		
129	T				-		
130	R	K	K		-	K	
132	T	S					RBS
134	I	V	V	V	V	S	RBS
135	A				S	S	RBS
137	S		P				Ca2
138	H				K		
141	A	I					
142	N	K					Ca2
152	V				T		
153	K				S		Sb
155	G	E					Sa
156	N				K		Sb
157	S				P		Sa
159	P				S		Sa
161	L	I					
162	R	S	S	S	S	N	Sa
163	N	T	K	K	K		Sa
166	T				K	I	Ca1
169	K					R	
170	G					E	Ca1
175	V	I					
185	D				Y		
190	T					A	Sb, RBS
199	I	V		V	V	V	
202	G		E		V		
208	Q	K		K		R	
214	T	I	I	I	I	I	
215	V		A			I	
216	A				P		
219	K				E		RBS
220	I	V	V	V		V	RBS
222	E					N	Ca2, RBS
224	A				R		RBS
227	I	M	M	M	M		
230	Y					H	
237	G				E		Ca1
245	T					N	
252	W				R		
253	Y		H	H			
258	N		D	K	E	K	
262	S	N	N		N		
266	I	M	M	M	M	M	
267	M		I	R	K	I	
269	N	D	D	D	D	D	
271	R	N	H	Q	H	H	
278	M	K	K	K	K	K	
287	K					N	
288	S			G		N	
289	N				D		
290	L				R		
298	I			V			
310	T					K	
311	H	Q	R	Q	Q	Q	
321	I	T					
324	I	V					
aadiff		34	31	29	49	43	

Reference CVVs in red, HI swine strain in purple.

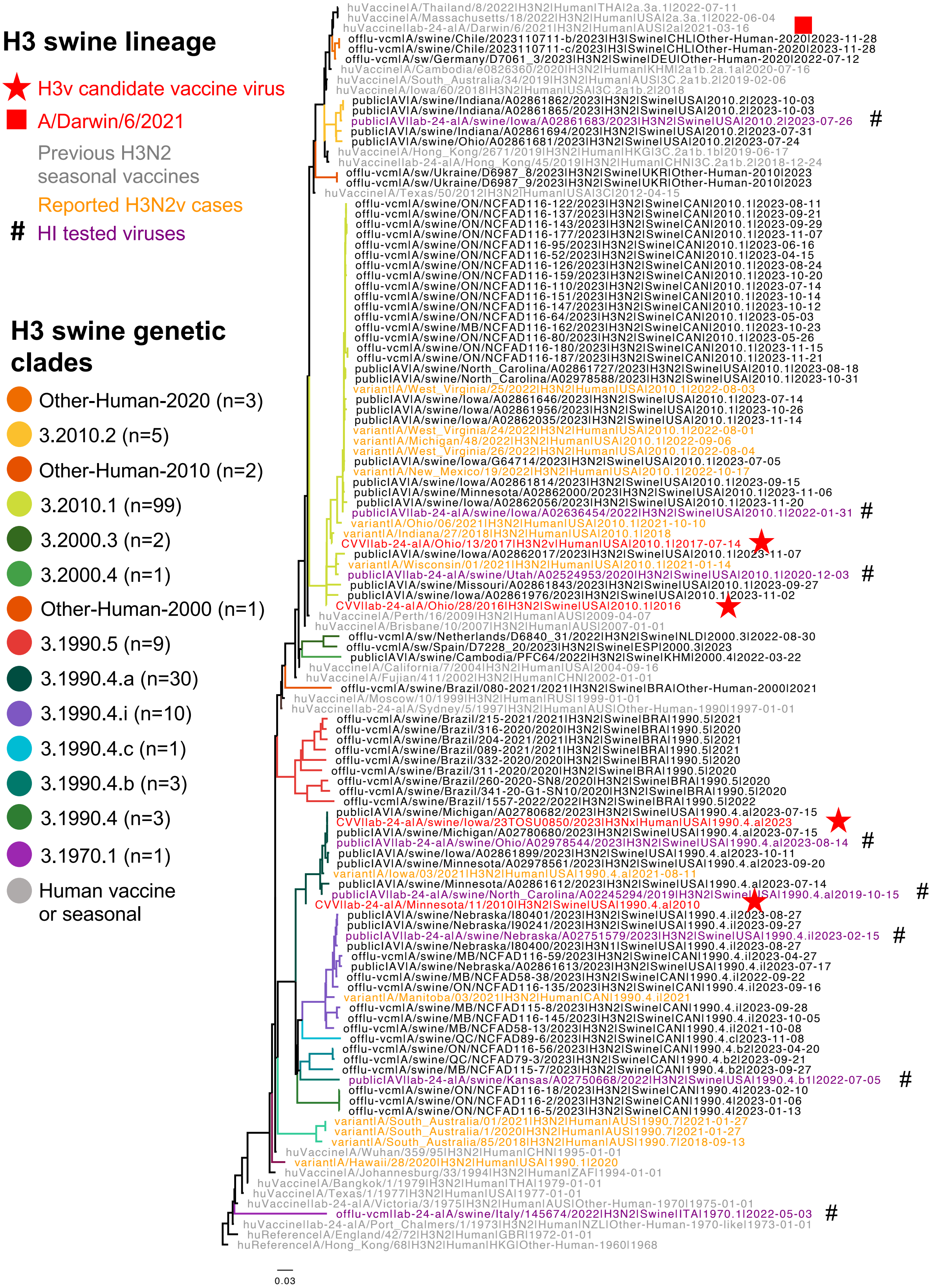


Figure 10. Swine H3 HA genes (tree was proportionally down sampled to 80 swine HA genes, excluding references). Number of detections of each clade from data deposited between July 1 - December 31, 2023 are presented adjacent to the clade name in the figure key.

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/North Carolina/A02245294/2019 (H3N2) #32	A/swine/North Carolina/A02245294/2019 (H3N2) #18	A/swine/Iowa/23TOSU0850/2023	IDCDC-RG60A A/Ohio/13/2017 CVV	A/swine/Utah/A02524953/2020 (H3N2) #19	A/swine/Utah/A02524953/2020 (H3N2) #20	rg-A/Darwin/6/2021 (H3N2)-like #5	rg-A/Darwin/6/2021 (H3N2)-like #6	Human High H3 responder	Human Low H3 responder
A/Minnesota/11/2010 x 203	1990.4.a	1280	80	160	40	20	40	80	80	10	80	20
A/swine/NorthCarolina/A02245294/2019*	1990.4.a	80	640	640	320	20	20	40	40	10	160	80
A/swine/Ohio/A02978544/2023	1990.4.a	40	320	320	640	10	10	20	<10	<10	160	160
A/swine/Iowa/23TOSU0850/2023	1990.4.a	80	640	320	1280	nt	nt	nt	nt	nt	nt	nt
A/swine/Kansas/A02750668/2022*	1990.4.b1	<10	<10	10	nt	<10	<10	10	10	<10	320	160
A/swine/Nebraska/A02751579/2023 **	1990.4.i	<10	10	10	nt	<10	10	10	<10	<10	160	40
IDCDC-RG60A A/Ohio/13/2017 CVV	2010.1	<10	20	80	nt	1280	160	160	20	10	320	80
A/swine/Utah/A02524953/2020*	2010.1	10	40	40	nt	160	640	640	20	10	320	80
A/swine/Iowa/A02636454/2022*	2010.1	10	20	40	nt	160	320	320	40	<10	320	80
A/swine/Iowa/A02861683/2023	2010.2	10	10	10	nt	<10	10	20	<10	<10	320	320
A/HongKong/45/2019*	3C.2a1b.1b	<10	nt	nt	nt	<10	nt	nt	nt	nt	1280	160
rg-A/Darwin/6/2021-like*	3C.2a1b.2a.2	<10	<10	10	nt	<10	20	20	320	640	40	20

Reference CVV in red, seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted in gray. *Previously tested in report 2021a; **Previously tested in report 2023a; rg is a synthetic HA/NA on PR8 backbone. Human sera were collected in USA with high or low responder relative to A/Hong Kong/45/2019, pools of 2 male and 2 female adults in each. nt=not tested.

- The contemporary swine 1990.4.a demonstrated a >8-fold decrease from CVV A/Minnesota/11/2010 and had a 4- to 8-fold reduction in reactivity in adult human high responder sera.
- The contemporary swine 1990.4.b1 does not have a CVV contained within clade and demonstrated a >8-fold decrease from CVV A/Minnesota/11/2010 but retained some reactivity with adult human 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 a 4- to 8-fold reduction in reactivity in adult human sera.
- The contemporary swine 2010.1 representative strain had 8-fold loss to A/Ohio/13/2017 but retained reactivity with adult human sera.
- The contemporary swine 2010.2 representative strain does not contain a CVV and demonstrated had >8-fold loss to human vaccine strains but retained reactivity with adult human sera.

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

Table 19. EU Study Design: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine H3 strains selected to represent clade consensus.

	Global Clade	A/Port Chalmers/1/1973	A/Ohio/28/2016_CVV	A/Victoria/3/1975	A/Sydney/5/97
A/Port Chalmers/1/1973	Human Seasonal	5120	160	160	80
A/Ohio/28/2016 CVV	H3N2 2010.1	20	2560	<10	40
A/Victoria/3/1975	Human Seasonal	2560	160	5120	80
A/Sydney/5/97	Human Seasonal	<10	40	<10	640
A/swine/Italy/145674/2022 H3N2	**1970.1**	80	320	160	80

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

- The 1970.1 representative swine strain from Italy demonstrated 8-fold or greater loss in reactivity with human seasonal vaccine anti-sera.

Table 20. Amino acid substitutions between representative swine 1990.4.x strains compared to the within-lineage CVVs (A/Minnesota/11/2010 x 203 and A/swine/Iowa/23TOSU0850/2023).

cont. →

site	A/Minnesota/11/2010	A/swine/Iowa/23TOSU0850/2023	A/swine/Ohio/A02978544/2023	A/swine/North_Carolina/A02245294/2019	A/swine/Kansas/A02750668/2022	A/swine/Nebraska/A02751579/2023	annotations
1	Q					K	
2	K				N		
6	S	N	N				
8	N					K	
10	M					T	
46	S				T		
48	T				K		
49	G					D	
50	R				E		
53	N				S		Site C
57	Q				K	K	
58	I					V	
62	K					G	
80	Q					E	
81	N				D		
82	K				R		
83	E				T		
96	N	S	S	S	D		
104	D				E		
107	T				S	S	
117	N				T	T	
119	E			K			
121	T					N	
122	Q					P	Site A
124	S	I	I	I		N	
131	A			T	N	G	
133	D					N	Site A
135	S				A		
137	Y				F	F	Site A
138	A	S	S	S	S	S	
140	R					K	
142	G					N	
143	S				A		Site A
144	V				E		Site A
145	N				K	K	Site A

site	A/Minnesota/11/2010	A/swine/Iowa/23TOSU0850/2023	A/swine/Ohio/A02978544/2023	A/swine/North_Carolina/A02245294/2019	A/swine/Kansas/A02750668/2022	A/swine/Nebraska/A02751579/2023	annotations
155	Y				H	H	Site B
156	N	H	H	H	S	H	
158	N				K		
159	Y					H	
163	E				A	A	
164	Q				L		
172	D	E	E		G		
174	F				S		
189	K				A	M	Site B
192	T				I		
193	N				S	S	Site B
196	V	I	I		T		
198	A					E	
201	R				G		Site D
202	V				I		
203	I				T	T	
207	K	R	R	R			Site D
210	Q					L	
216	N				T		
217	I				V		Site D
228	S				G		
260	I					M	
261	Q					R	
262	S					I	
269	R					K	
273	H					L	
275	D				G	G	Site C
276	E				N	N	
278	N				E		Site C
289	P			S			
299	K				R	R	
304	A				P		
312	N				S	G	
323	V			I			
aadiff		8	8	9	43	38	

Reference CVVs in red, HI swine strains in purple.

Table 21. Amino acid substitutions between representative swine 2010.1 strains compared to the within-clade CVVs (A/Ohio/28/2016 and DCDC-RG60A A/Ohio/13/2017).

Site	A/Ohio/28/2016	A/Ohio/13/2017	A/swine/Utah/A02524953/2020	A/swine/Iowa/A02636454/2022	annotations
31	N			D	
45	S			N	
56	H	Y		Y	
62	E			G	
88	V		I		
92	K		R		
96	N			D	
106	A			V	
112	V		A		
122	N			K	Site A
138	A	S	S	S	
144	S		T		Site A
145	K	N	N	N	Site A
156	H		Q		
167	T		A		
202	I			T	
209	S	N	G	N	
212	A			T	
223	V			I	
238	R			K	
264	K			R	
312	N		K		
aadiff		4	10	15	

Reference CVVs in red, HI swine strains in purple.

Table 22. Amino acid substitutions between a representative 2010.2 strain compared to four current WHO recommended human seasonal vaccines used in HI assays (A/Darwin/6/2021, A/Darwin/9/2021, A/Thailand/8/2022 and A/Massachusetts/18/2022), and additional HI-tested human seasonal vaccine and CVV.

cont. →

site	A/Darwin/6/2021	A/Darwin/9/2021	A/Thailand/8/2022	A/Massachusetts/18/2022	A/Hong_Kong/45/2019	A/Ohio/28/2016	A/swine/Iowa/A02861683/2023	annot.
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	E		K	K				
53	G	D	N	N	D	N	N	Site C
58	I						V	
62	G					E	E	
67	I						V	
82	K					N		
83	E				K	K	Q	
92	R					K		
94	N				Y	H	Y	
96	N		S	S				
106	A						V	
121	K					N		
128	T				A			
131	K				T	T	T	
133	N					D		Site A
135	T				K	A	I	
137	S				F			Site A
138	A				S		S	
140	I		K	K		K	K	
142	G					R		
144	S						K	Site A
145	S					K		Site A
156	S				H	H	Q	
158	N						D	
159	N				Y	F	Y	
160	I				T	K	K	
164	Q				L	L	L	
165	N					E		
167	T						I	
171	K					N	N	
174	F						X	
186	D	N			G	A	G	Site B
189	K						S	Site B
190	N				D	D	D	
192	I		F	F			T	
195	F				Y	Y	Y	
197	Q						R	
198	S					A		
199	S					A		
203	T					I	I	
223	I		V	V		V		
225	D	G						
238	K					R		
242	I						T	
278	K					N		Site C
280	E					A		
291	D						N	
312	S				N	N		
323	V					I		
aadiff		3	6	6	16	38	31	

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

Table 23. Amino acid substitutions between a representative swine 1970.1 strain compared to HI-tested human seasonal vaccines (A/Port_Chalmers/1/1973, A/Sydney/5/1997, and A/Victoria/3/1975).

cont. →

site	A/Port_Chalmers/1/1973	A/Sydney/5/1997	A/Victoria/3/1975	A/swine/Italy/145674/2022	annotations
2	D	K			
3	F	I	L		
6	N			K	
7	D			G	
7+1	-		N		
9	S			N	
31	N			D	
45	S			N	
46	S			F	
48	T			M	
50	K	R			
53	N	D		R	Site C
54	N	S			Site C
62	I	E		A	
67	I			V	
69	A			S	
80	Q			R	
82	E	K			
83	T	E	K	K	
88	V			I	
92	K			R	
94	F	Y			
104	D			E	
106	A			T	
117	T			S	
121	I	N		T	
124	G	S		N	
126	T	N	N	N	
131	T	A			
135	G	T			
137	N	Y		S	Site A
142	G	S			
143	P	S			Site A
144	D	I		N	Site A
145	S	K		N	Site A
146	G	S		S	Site A
155	Y	H			Site B
156	K	Q			
157	S	L			
158	G	K			
159	S	Y		N	
160	A	K	T	T	
163	V	A		M	
164	L		Q		
171	N			S	
173	N	K		D	
174	F		S		
189	Q	S	K	R	Site B
190	E	D			
193	N	S			Site B
194	L	I			
196	V	A		I	
198	A			T	
201	R		K	K	Site D
202	V			I	
203	T			I	
213	I	V			
217	I		V		Site D
226	L	I			
233	Y	H			
244	V	L		I	
248	N	T			
260	M	I		I	
261	R			Q	
262	T	S			
267	I			V	
269	R			K	
276	T	K			
278	I	N	S	D	Site C
299	K	R		R	
307	K	R		H	
309	V			I	
323	V			I	
aadiff		44	11	46	

Reference vaccines in gray, HI swine strain in purple.

Summary and Risk Assessment
Global Variant Cases:
During the reporting period (July 1 – December 31, 2023), 6 variant cases were reported:

- United Kingdom: H1N2v (1B.1.1.1)
- Netherlands: H1N1v (1C.2.2)
- Switzerland: H1N1v (1C.2.2)
- USA: H1N2v (2x 1B.2.1), H3v (no sequence data)

Additional variant cases were detected after this period:

- Brazil: H1N1v (2x 1A.3.3.2)
- Spain: H1N1v (1A.3.3.2)

Europe Swine:

- H1 1A: The contemporary swine 1A.5.1 from Japan has no within clade CVV and showed 4-fold loss in reactivity with the 1A.3.3.3 A/Ohio/9/2015 CVV.
- H1 1B: There are no within-clade CVVs 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.
- H1 1C: The 1C.2.2 swine strain from England had 8-fold loss to the within-clade CVV A/Hessen/47/2020. The 1C.2.4 swine strain from Italy had >8-fold loss to the within-clade CVV A/Bretagne/24241/2021.
- H3N2: The 1970.1 representative swine strain from Italy demonstrated 8-fold or greater loss in reactivity with human seasonal vaccine anti-sera.

North America Swine:

- H1 1A: The contemporary swine 1A.1.1.3 had >8-fold decrease from 1A.1.1.3 CVV A/Ohio/24/2017 and A/California/71/2021 ferret anti-sera and had significant decreases in titer in adult human sera. The contemporary swine 1A.3.3.2 with onward transmission in swine had >8-fold decrease to the A/Sydney/5/2021 5a.2a and A/Victoria/4897/2022 5a.2a.1 vaccine strains and adult human sera. The contemporary swine 1A.3.3.3-c1 virus had >8-fold decrease to the 1A.3.3.3-c3 CVV A/Wisconsin/2021 but reacted with adult human high responder sera. The contemporary swine 1A.3.3.3-c3 virus had >8-fold decrease from the within-clade 1A.3.3.3-c3 CVV A/Wisconsin/2021 but retained reactivity with adult human sera.
- H1 1B: The 1B.2.1 clade swine representative had no loss of titer the within clade CVV but displayed limited titers in adult human sera. The 1B.2.2.1 A. had 8-fold reduction from the within clade CVV A/Iowa/32/2016 and significant loss in titers in adult human high responder sera. The contemporary swine 1B.2.2.2 does not have a within-clade CVV and had a >8-fold decrease from CVV A/Iowa/32/2016 and limited titers in adult human sera.
- H3N2: The contemporary swine 1990.4.a demonstrated a >8-fold decrease from CVV A/Minnesota/11/2010 and had 4- to 8-fold reduction in reactivity in adult human high responder sera. The contemporary swine 1990.4.b1 does not have a CVV within clade and demonstrated a >8-fold decrease from CVV A/Minnesota/11/2010 but retained some reactivity with adult human sera. The contemporary swine 1990.4.i does not contain a within clade CVV and demonstrated >8-fold decrease from CVV A/Minnesota/11/2010 and a 4- to 8-fold reduction in reactivity in adult human sera. The contemporary swine 2010.1 representative strain had 8-fold loss to A/Ohio/13/2017 but retained reactivity with adult human sera. The contemporary swine 2010.2 representative strain does not have a within clade CVV and demonstrated >8-fold loss to human vaccine strains but retained reactivity with adult human sera.

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

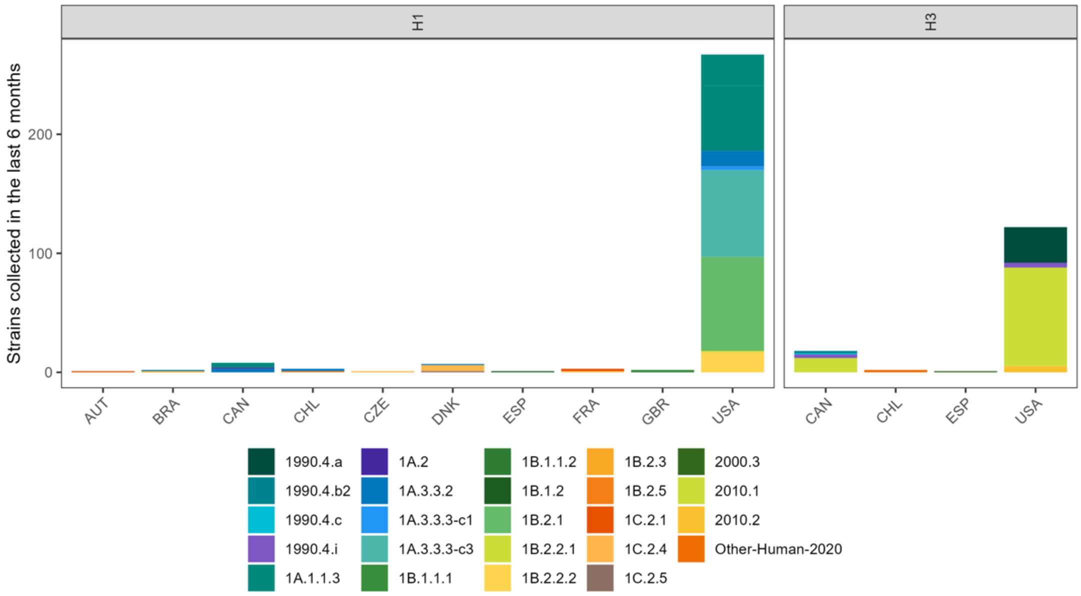


Figure A1. Summary of swine HA genes colored by phylogenetic clade for sequences deposited July 2023 – December 2023 and truncated to those collected within the last 6 months (n = 445).

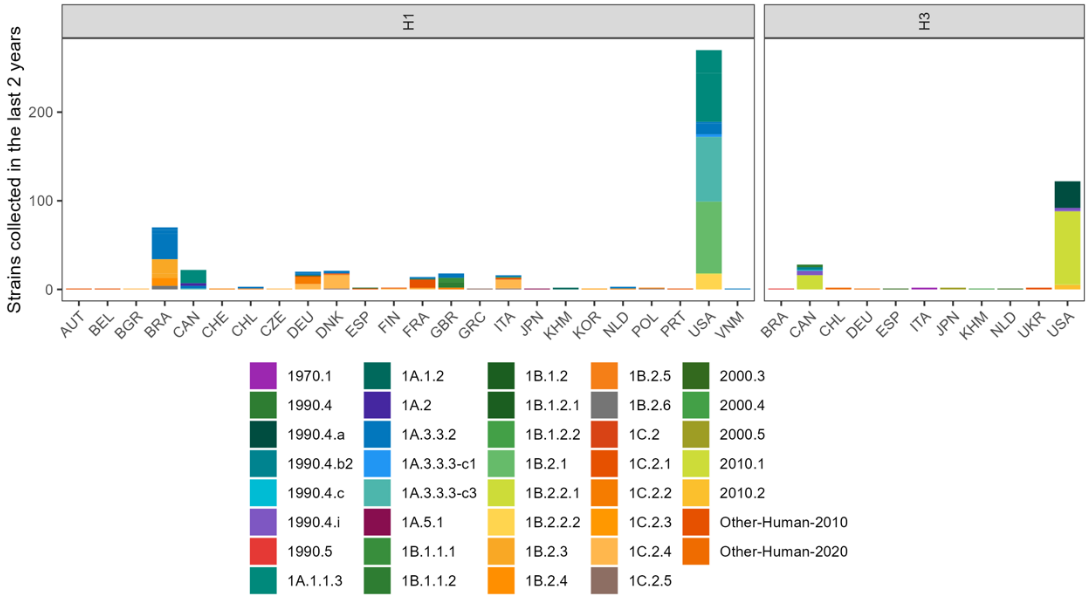


Figure A2. Summary of swine HA genes colored by phylogenetic clade for sequences deposited December 2021 – December 2023 and truncated to those collected within the last 24 months (n = 687).

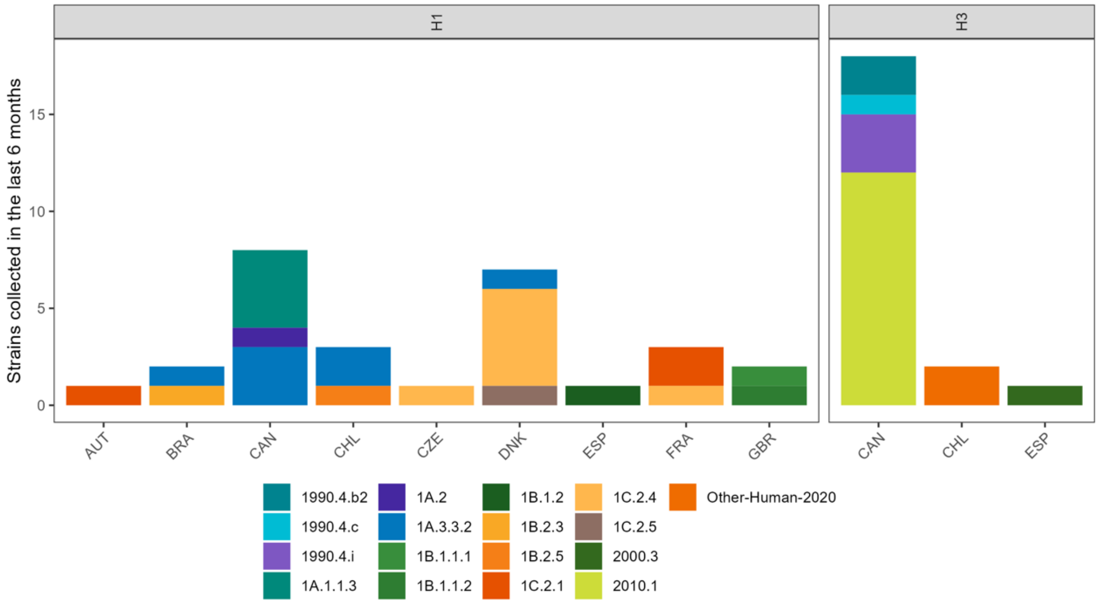


Figure A3. Summary of swine HA genes colored by phylogenetic clade for sequences deposited July 2023 – December 2023 and truncated to those collected within the last 6 months (n = 54) in any country other than the United States of America.

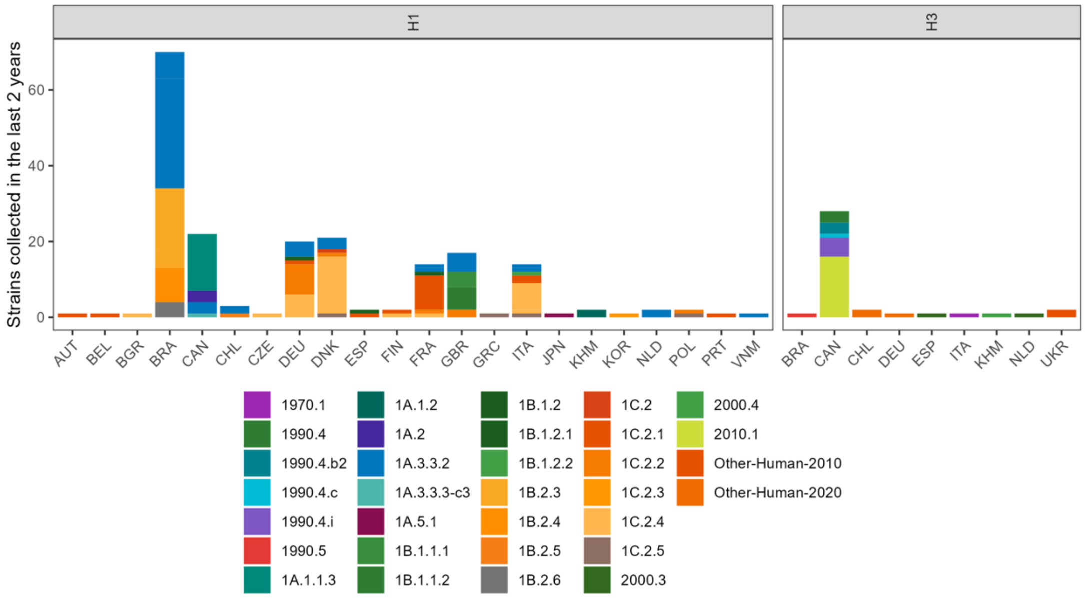


Figure A4. Summary of swine HA genes colored by phylogenetic clade for sequences deposited December 2021 – December 2023 and truncated to those collected within the last 24 months (n = 294) in any country other than the United States of America.

Table A1a. Summary of swine H1 clades by country collected in the past 6 months and sequences deposited July 2023 – December 2023.

clade	**country**	**count**
1C.2.1	AUT	1
1A.3.3.2	BRA	1
1B.2.3	BRA	1
1A.1.1.3	CAN	4
1A.2	CAN	1
1A.3.3.2	CAN	3
1A.3.3.2	CHL	2
1B.2.5	CHL	1
1C.2.4	CZE	1
1A.3.3.2	DNK	1
1C.2.4	DNK	5
1C.2.5	DNK	1
1B.1.2	ESP	1
1C.2.1	FRA	2
1C.2.4	FRA	1
1B.1.1.1	GBR	1
1B.1.1.2	GBR	1
1A.1.1.3	USA	81
1A.3.3.2	USA	13
1A.3.3.3-c1	USA	3
1A.3.3.3-c3	USA	73
1B.2.1	USA	79
1B.2.2.1	USA	1
1B.2.2.2	USA	17

Table A1b. Summary of swine H1 clades by country collected in the past 24 months and sequences deposited January 2022 – December 2023.

cont.

clade	country	count
1C.2.1	AUT	1
1C.2.1	BEL	1
1C.2.4	BGR	1
1A.3.3.2	BRA	34
1B.2.3	BRA	21
1B.2.4	BRA	9
1B.2.6	BRA	4
1A.1.1.3	CAN	15
1A.2	CAN	3
1A.3.3.2	CAN	3
1A.3.3.3-c3	CAN	1
1A.3.3.2	CHL	2
1B.2.5	CHL	1
1C.2.4	CZE	1
1A.3.3.2	DEU	4
1B.1.2.1	DEU	1
1C.2.1	DEU	1
1C.2.2	DEU	8
1C.2.4	DEU	6
1A.3.3.2	DNK	3
1C.2	DNK	1
1C.2.2	DNK	1
1C.2.4	DNK	15
1C.2.5	DNK	1
1B.1.2	ESP	1
1C.2.1	ESP	1
1C.2.1	FIN	1
1C.2.4	FIN	1
1A.3.3.2	FRA	2
1B.1.2.1	FRA	1
1C.2.1	FRA	9
1C.2.2	FRA	1
1C.2.4	FRA	1
1A.3.3.2	GBR	5
1B.1.1.1	GBR	4
1B.1.1.2	GBR	6
1C.2.2	GBR	2
1C.2.5	GRC	1
1A.3.3.2	ITA	2
1B.1.2.2	ITA	1
1C.2.1	ITA	2
1C.2.4	ITA	8
1C.2.5	ITA	1
1A.5.1	JPN	1
1A.1.2	KHM	2
1C.2.3	KOR	1
1A.3.3.2	NLD	2
1C.2.2	POL	1
1C.2.5	POL	1
1C.2.1	PRT	1
1A.1.1.3	USA	81
1A.3.3.2	USA	14
1A.3.3.3-c1	USA	3
1A.3.3.3-c3	USA	73
1B.2.1	USA	80
1B.2.2.1	USA	1
1B.2.2.2	USA	17
1A.3.3.2	VNM	1

Table A2a. Summary of swine H3 clades by country collected in the past 6 months and sequences deposited July 2023 – December 2023.

clade	country	count
1990.4.b2	CAN	2
1990.4.c	CAN	1
1990.4.i	CAN	3
2010.1	CAN	12
Other-Human-2020	CHL	2
2000.3	ESP	1
1990.4.a	USA	30
1990.4.i	USA	4
2010.1	USA	83
2010.2	USA	5

Table A2b. Summary of swine H3 clades by country collected in the past 24 months and sequences deposited January 2022 – December 2023.

clade	country	count
1990.5	BRA	1
1990.4	CAN	3
1990.4.b2	CAN	3
1990.4.c	CAN	1
1990.4.i	CAN	6
2010.1	CAN	16
Other-Human-2020	CHL	2
Other-Human-2020	DEU	1
2000.3	ESP	1
1970.1	ITA	1
2000.4	KHM	1
2000.3	NLD	1
Other-Human-2010	UKR	2
1990.4.a	USA	30
1990.4.i	USA	4
2010.1	USA	83
2010.2	USA	5

Annex 2. Phylogenies of swine HA lineages annotated by antigenic analyses or by inferred amino acid mutations.

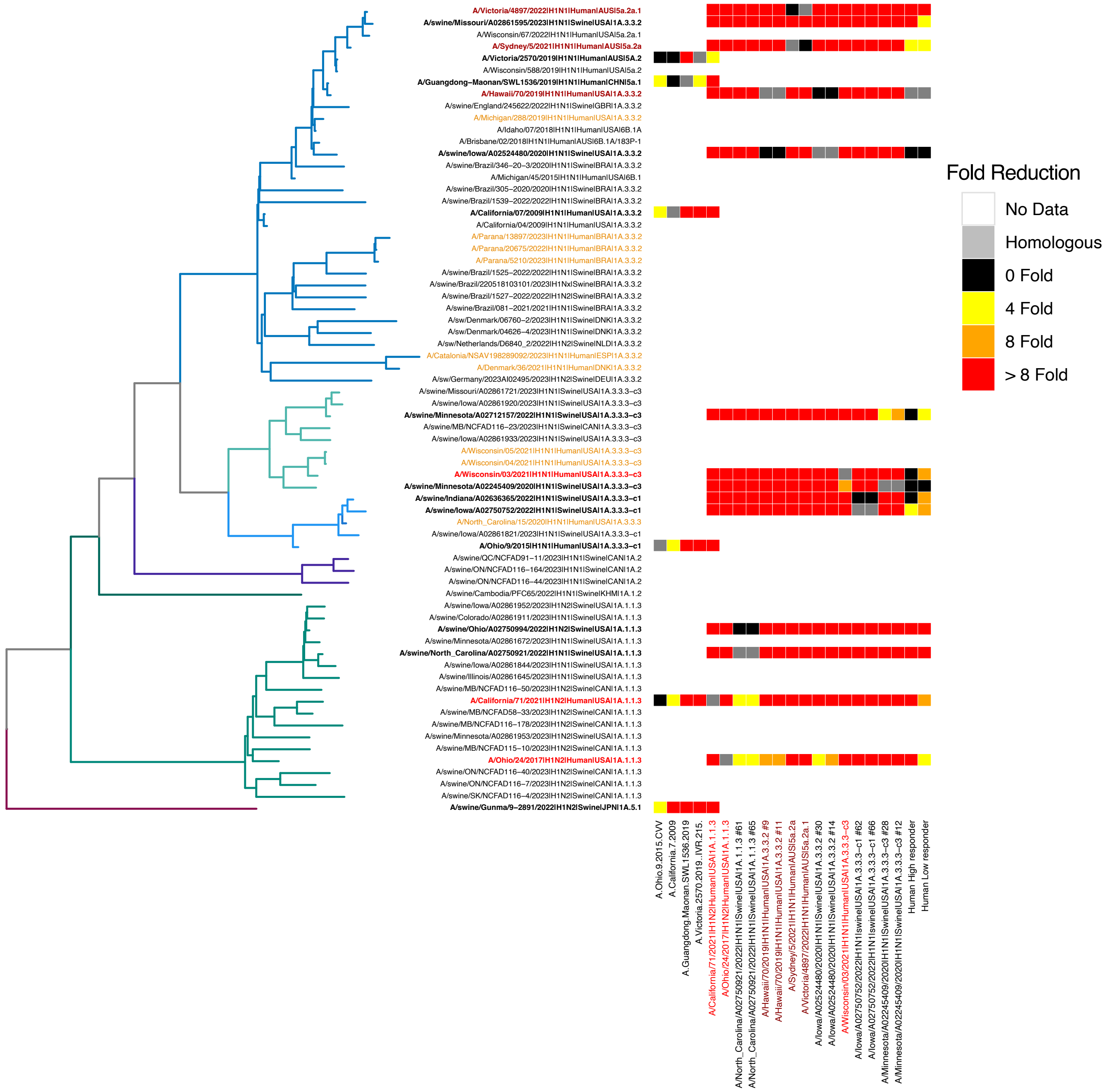


Figure A5. A phylogeny of the **1A lineage** sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023 (n=76) and 3 reference genes. All branches are colored to be consistent with Figure 7, with HI assay data presented as fold-change to within clade vaccine strain beside strain name.

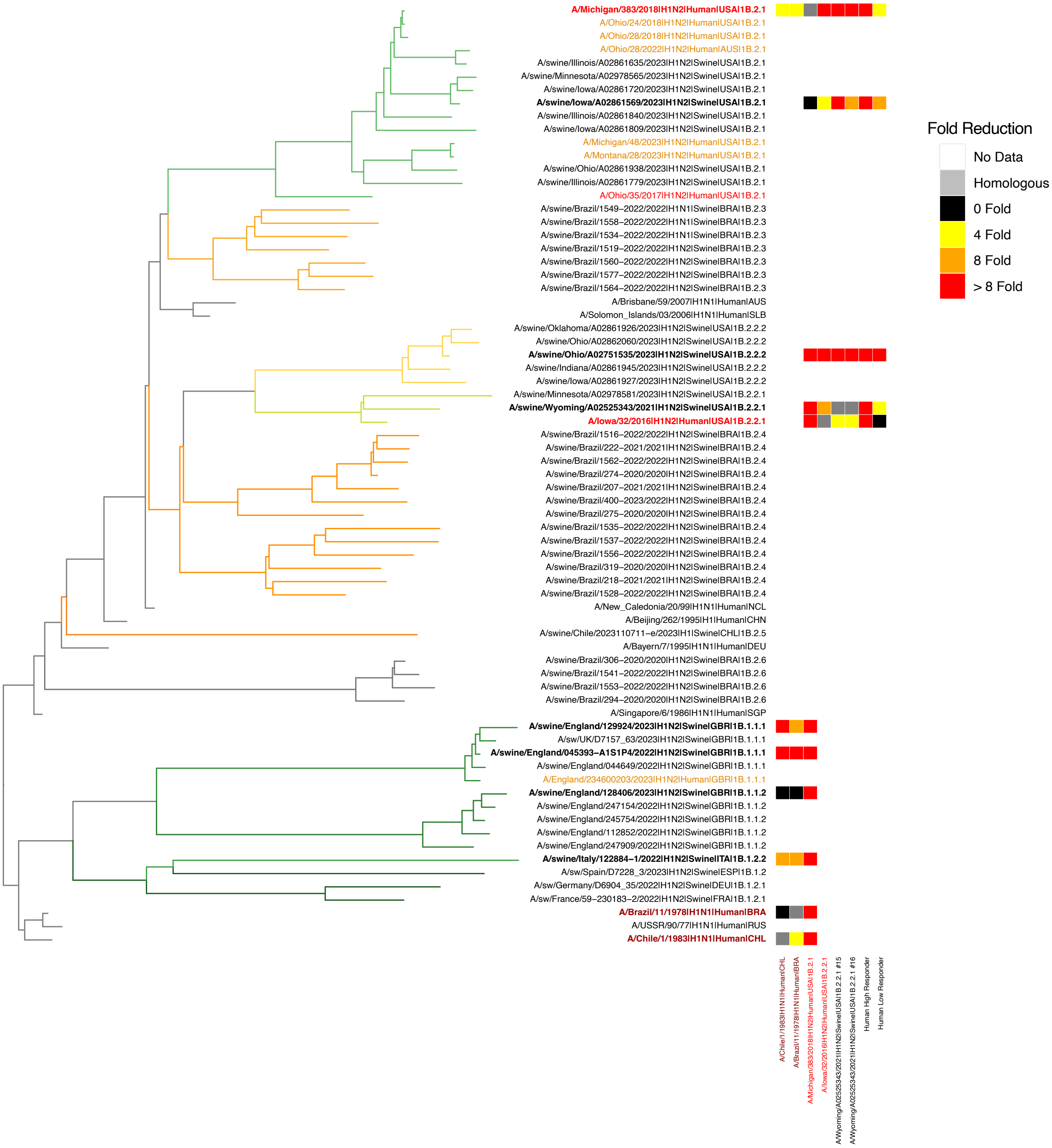


Figure A6. A phylogeny of the **1B lineage** sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023 (n=76) and 3 reference genes. All branches are colored to be consistent with Figure 7, with HI assay data presented as fold-change to within clade vaccine strain beside strain name.

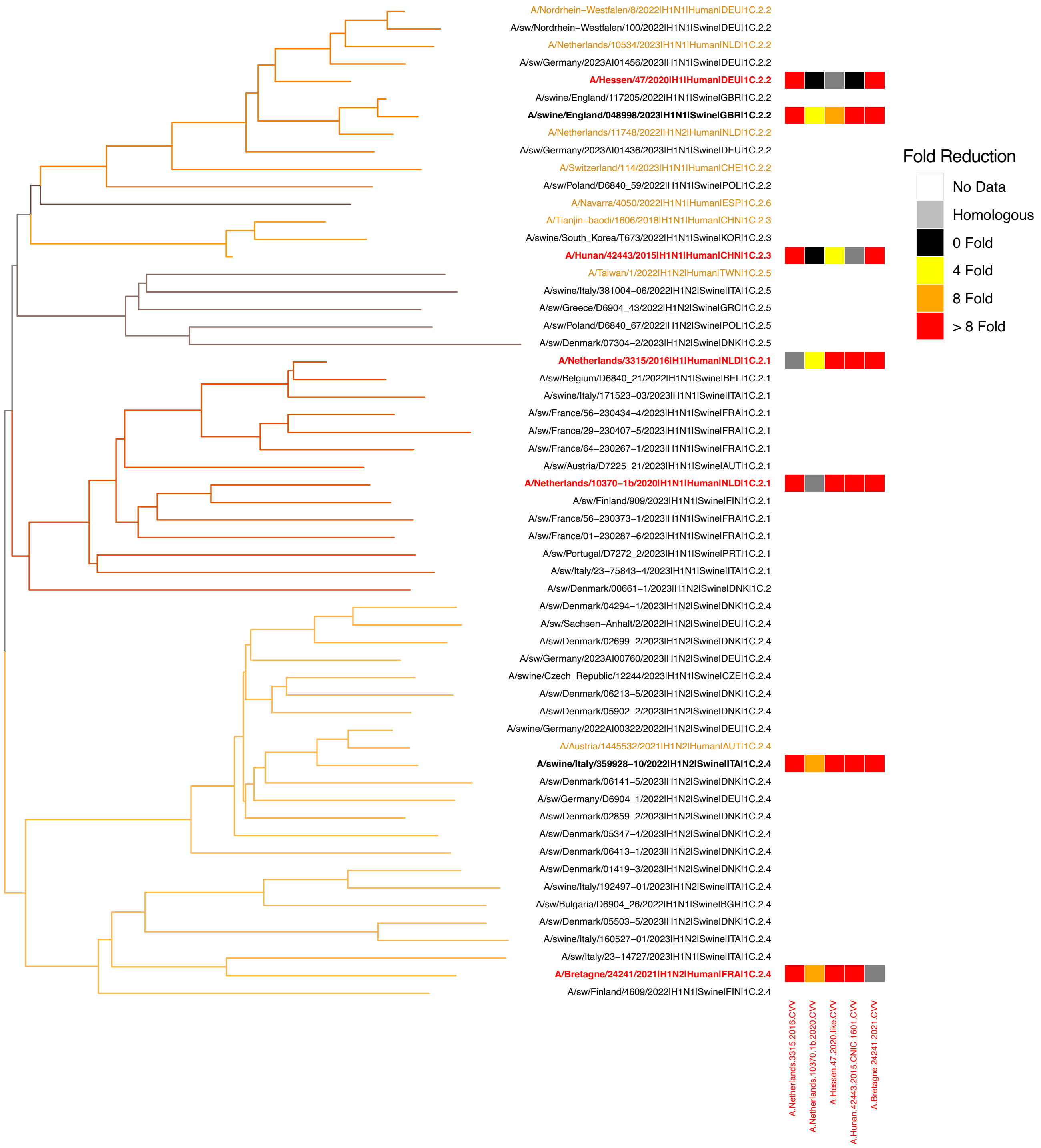


Figure A7. A phylogeny of the **1C lineage** sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023. All branches are colored to be consistent with Figure 7, with HI assay data presented as fold-change to within clade vaccine strain beside strain name.

Figure A8. A phylogeny of the **H3 lineage** sequences deposited July 1, 2023 – December 31, 2023, and collected in 2023 (n=76) and 3 reference genes. All branches are colored to be consistent with Figure 7, with HI assay data presented as fold-change to within clade vaccine strain beside strain name.

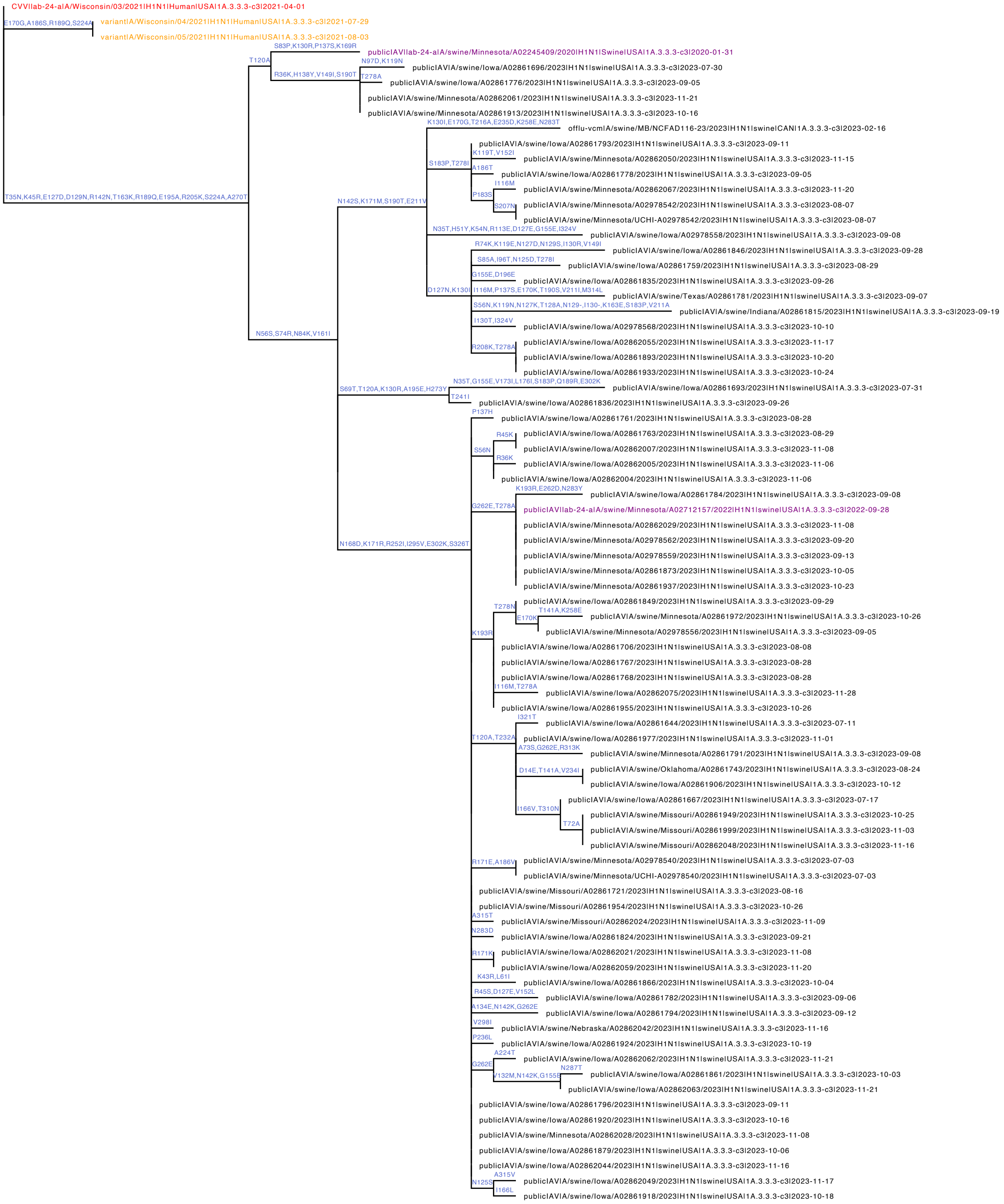


Figure A9. A phylogeny of the **1A.3.3.3-c3** clade displaying sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023 (n=76) and 3 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Wisconsin/03/2021) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

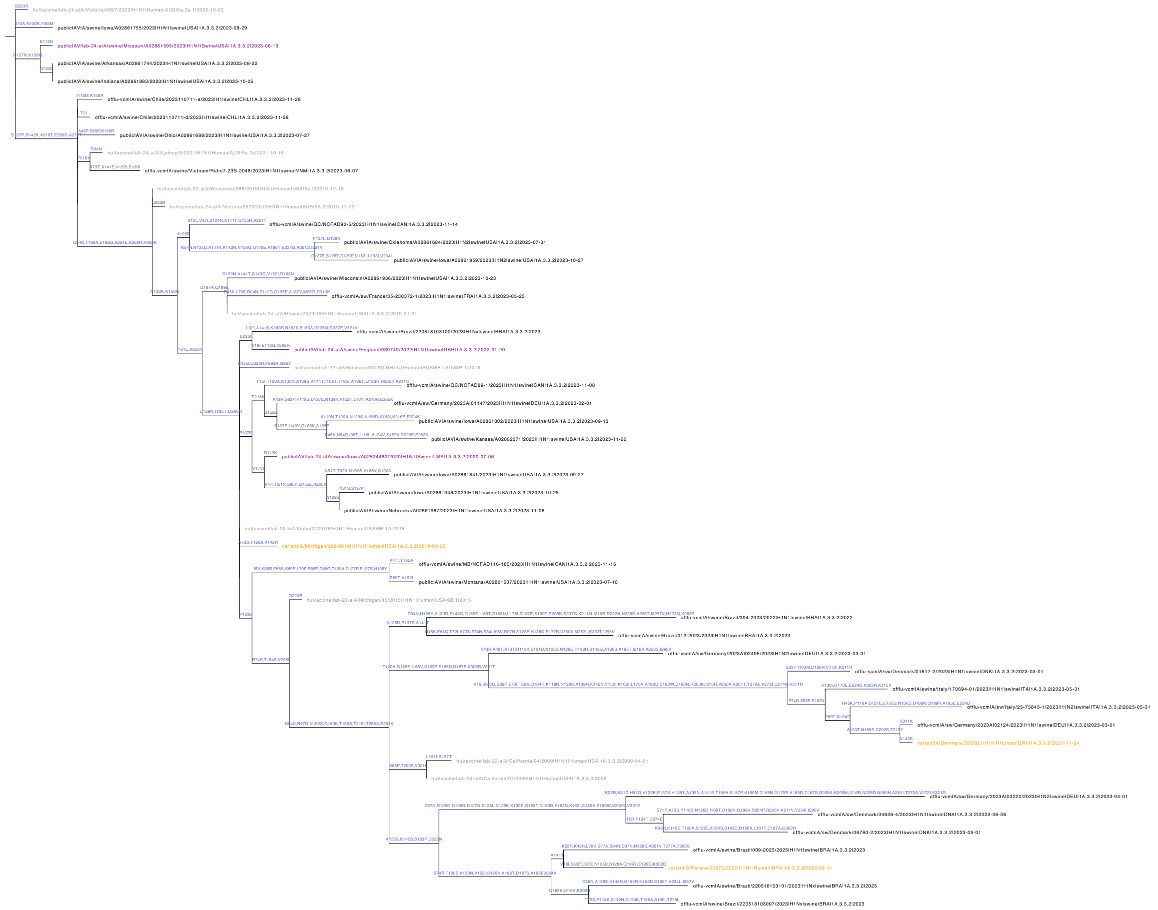


Figure A10. A phylogeny of the **1A.3.3.2 (pdm)** clade displaying n=38 sequences deposited July 1, 2023 - December 31, 2023 and collected in 2023, and n=13 reference genes. The clade is rooted relative to the most recent human vaccine strain (A/Victoria/4897/2022) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

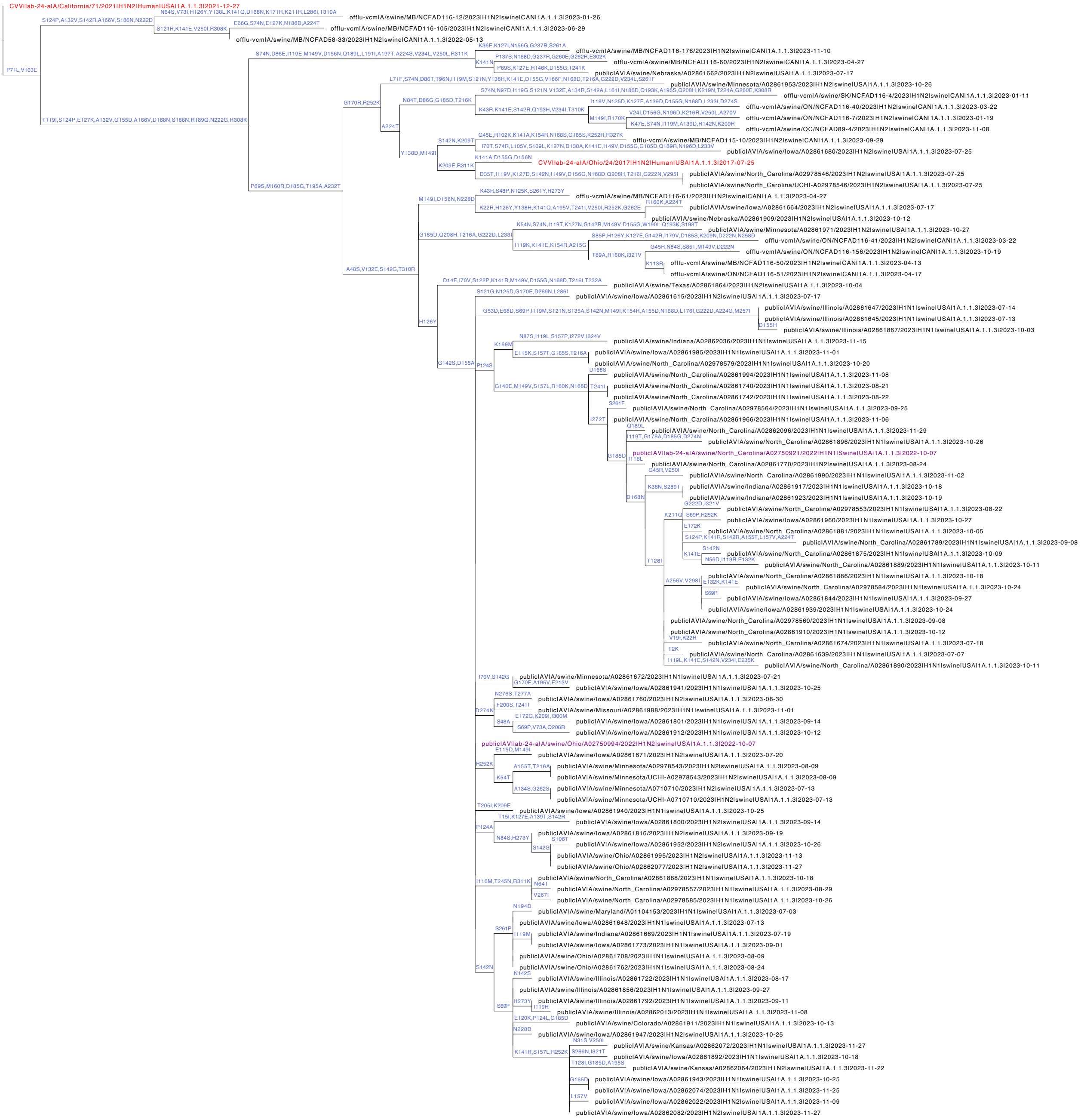


Figure A11. A phylogeny of the **1A.1.1.3** clade displaying n=98 sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023, and n=2 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Ohio/24/2017) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

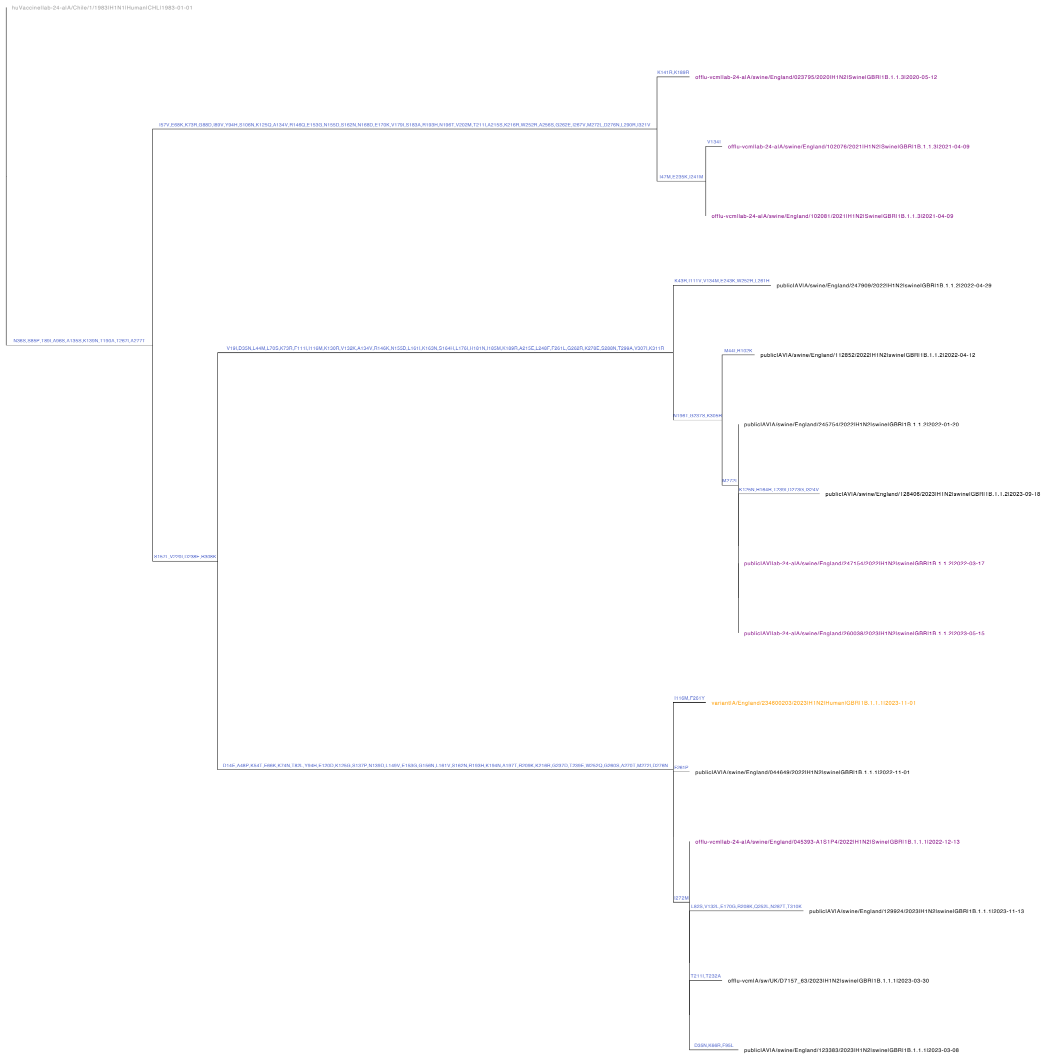


Figure A12. A phylogeny of the **1B.1.1.x** clade displaying n=14 sequences deposited July 1, 2023 – December 31, 2023 and collected in 2022-2023, and n=2 reference genes. The clade is rooted relative to the most similar human vaccine (A/Chile/1/1983) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

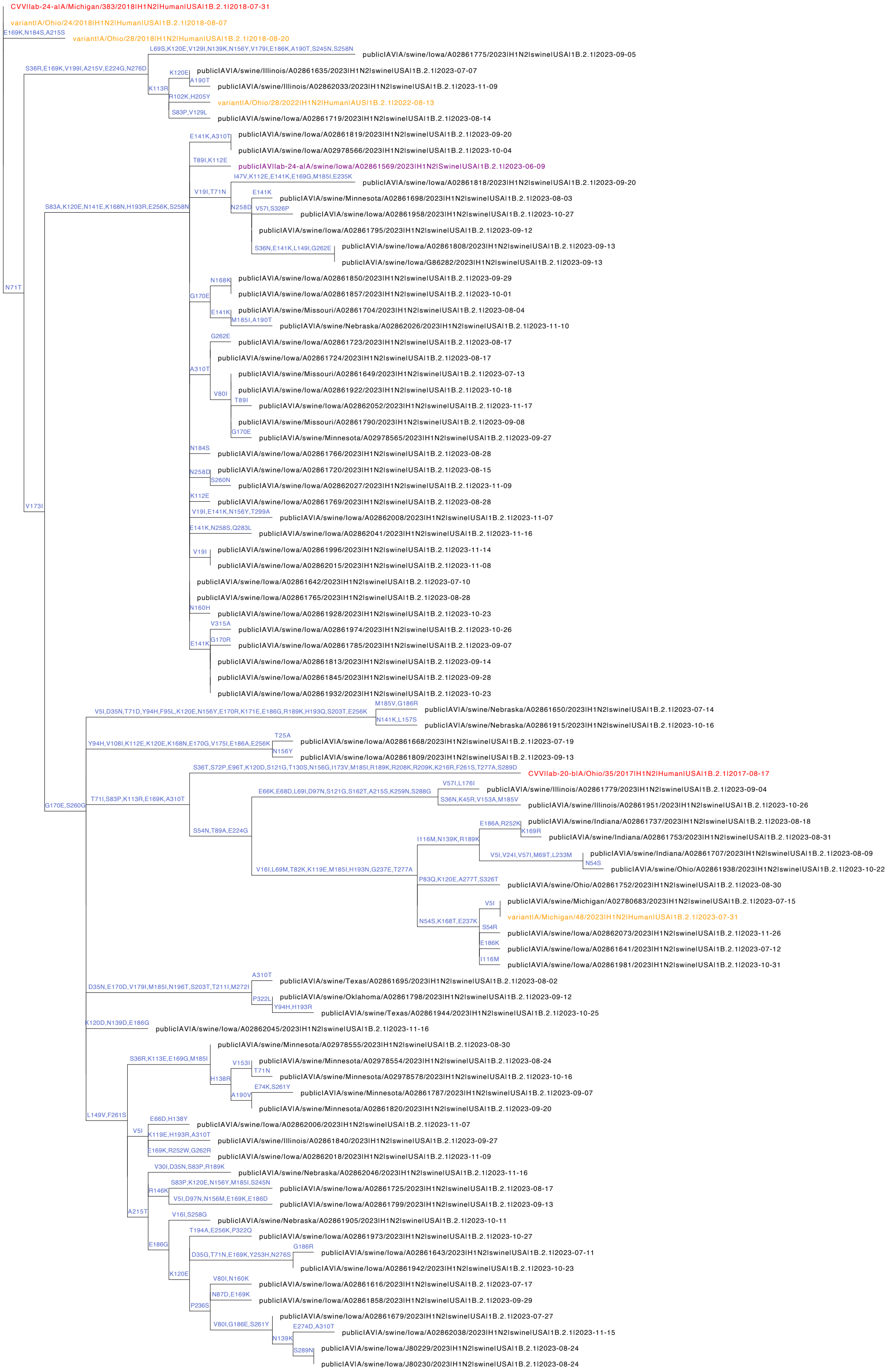


Figure A13. A phylogeny of the **1B.2.1** clade displaying n=80 sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023, and n=6 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Michigan/383/2018) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 8.

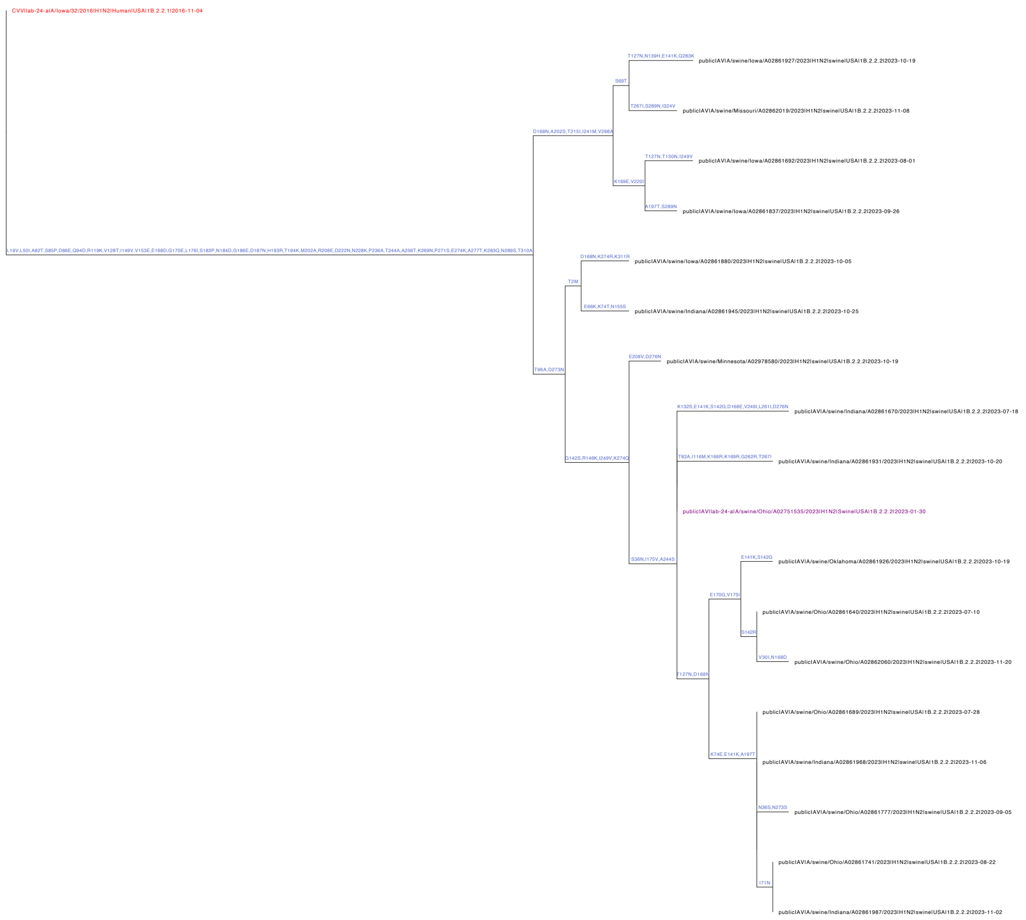


Figure A14. A phylogeny of the **1B.2.2.2** clade displaying n=18 sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023, and n=1 reference genes. The clade is rooted relative to the closest CVV (A/Iowa/32/2016) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 8.

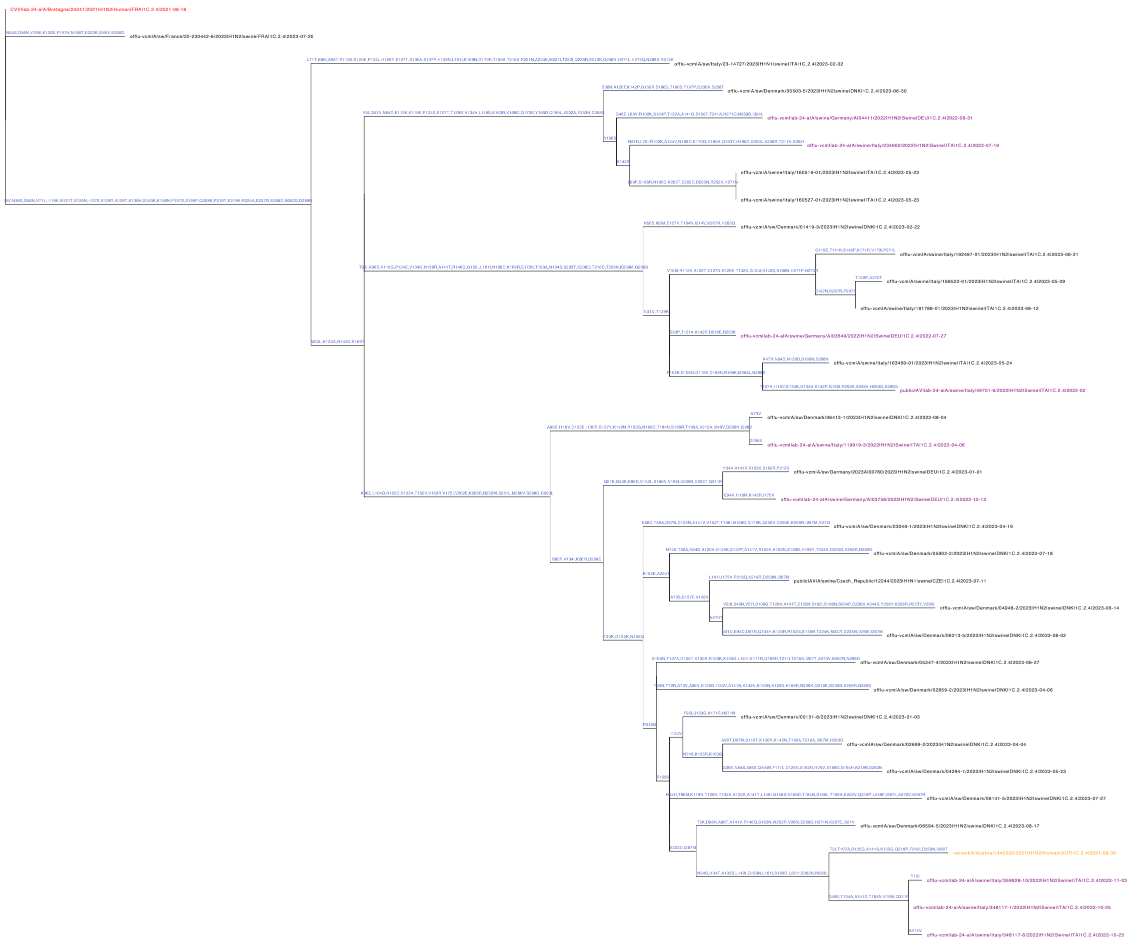


Figure A15. A phylogeny of the **1C.2.4** clade displaying n=33 sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023, and n=2 reference genes. The clade is rooted relative to the closest CVV (A/Bretagne/24241/2021) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.

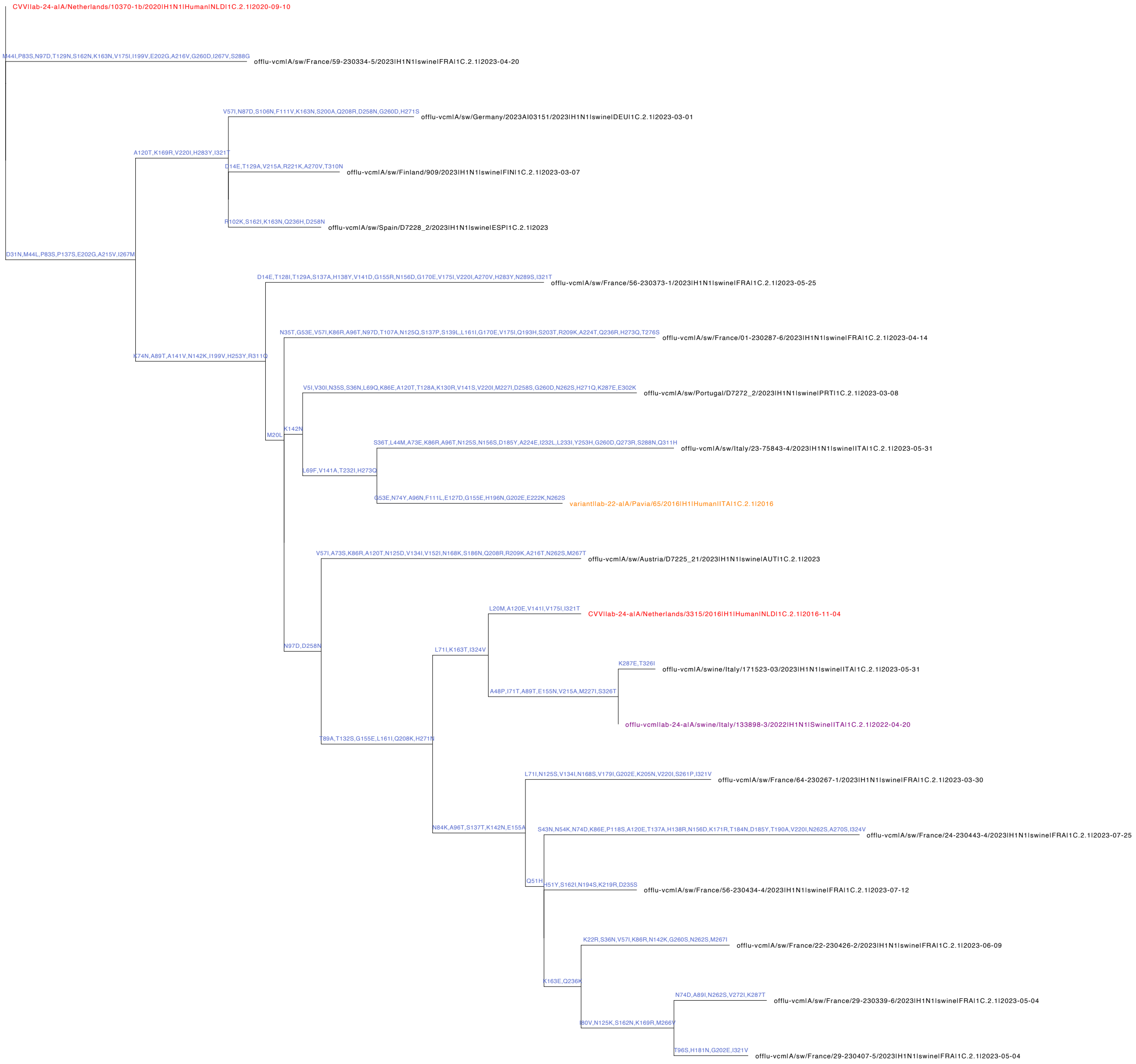


Figure A16. A phylogeny of the **1C.2.1** clade displaying n=17 sequences deposited July 1, 2023 - December 31, 2023 and collected in 2023, and n=3 reference genes. The clade is rooted relative to the closest CVV (A/Netherlands/10370-1b/2020) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.

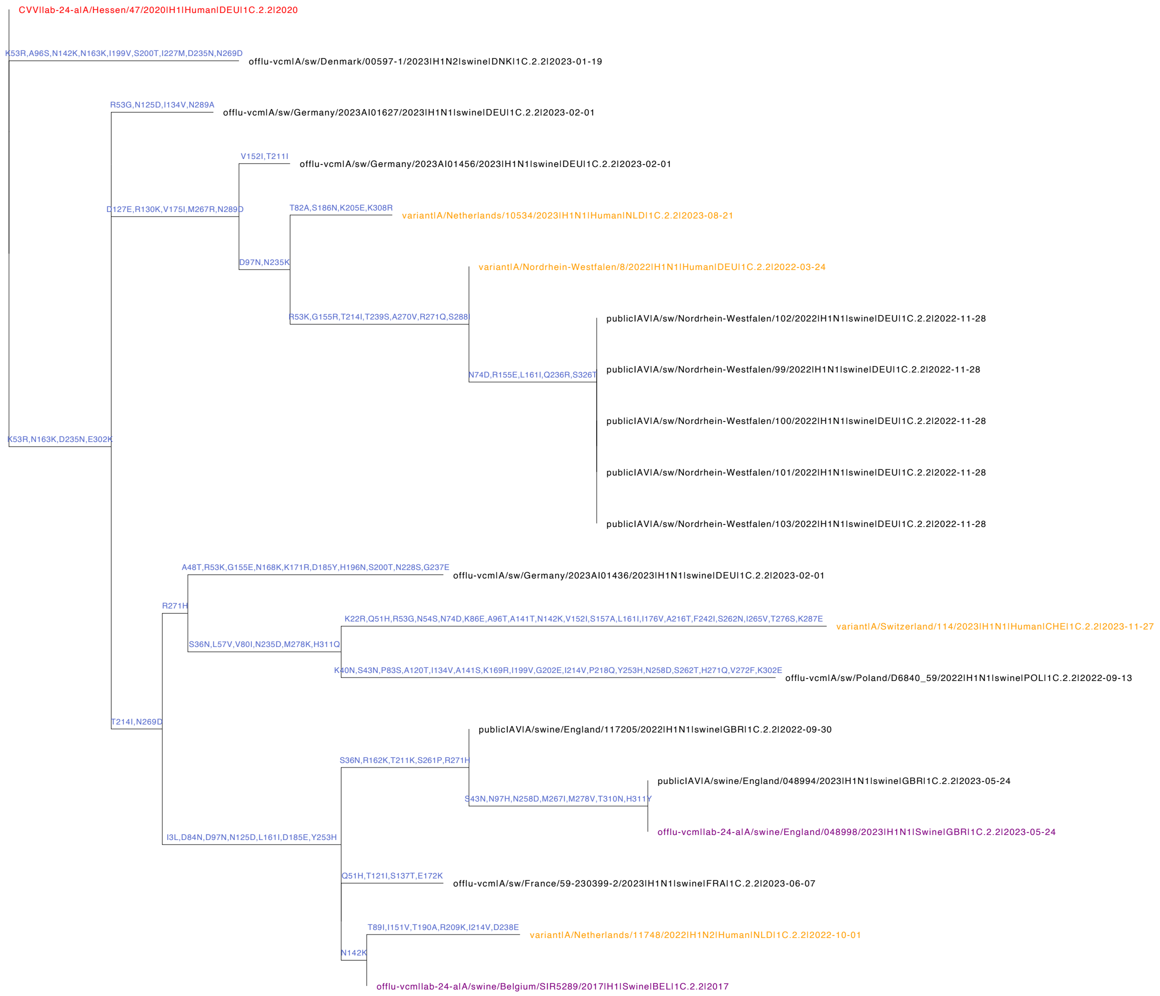


Figure A17. A phylogeny of the **1C.2.2** clade displaying n=15 sequences deposited July 1, 2023 – December 31, 2023 and collected in 2022-2023, and n=5 reference genes. The clade is rooted relative to the in-clade CVV (A/Hessen/47/2020) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.

Figure A18. A phylogeny of the **3.2010.1** clade displaying n=101 sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023, and n=11 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Ohio/28/2016) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 10.

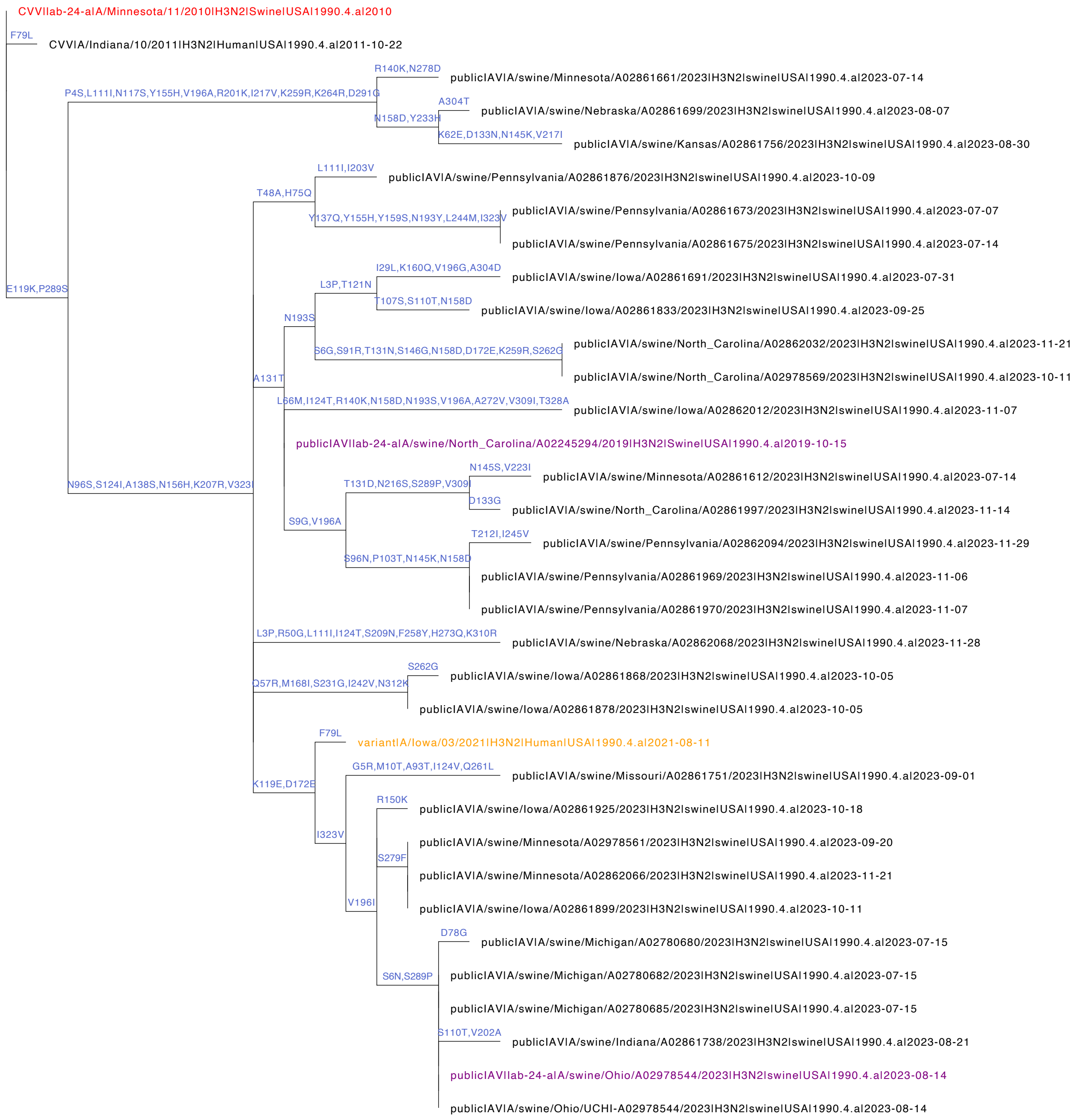


Figure A19. A phylogeny of the **3.1990.4.a** clade displaying n=31 swine sequences deposited July 1, 2023 – December 31, 2023 and collected in 2023, and n=3 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Minnesota/11/2010) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 10.