



# OFFLU SWINE INFLUENZA REPORT

JANUARY 2023 TO JUNE 2023

## 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 January 1, 2023 – June 30, 2023) downloaded from GISAID or GenBank were aligned with MAFFT (Kato and Standley, 2013) using default settings. Alignments for each segment were inspected manually and trimmed to the start and stop codon. Exploratory trees were run using FastTree (Price et al., 2009). Tabular comparisons between current CVVs or human seasonal vaccine strains and new swine data were generated using the NADC IAV bioinformatic toolkit (<https://github.com/flu-crew>). A clade representative was selected using PARNAS (<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.

# Global influenza A events in swine Epidemiology

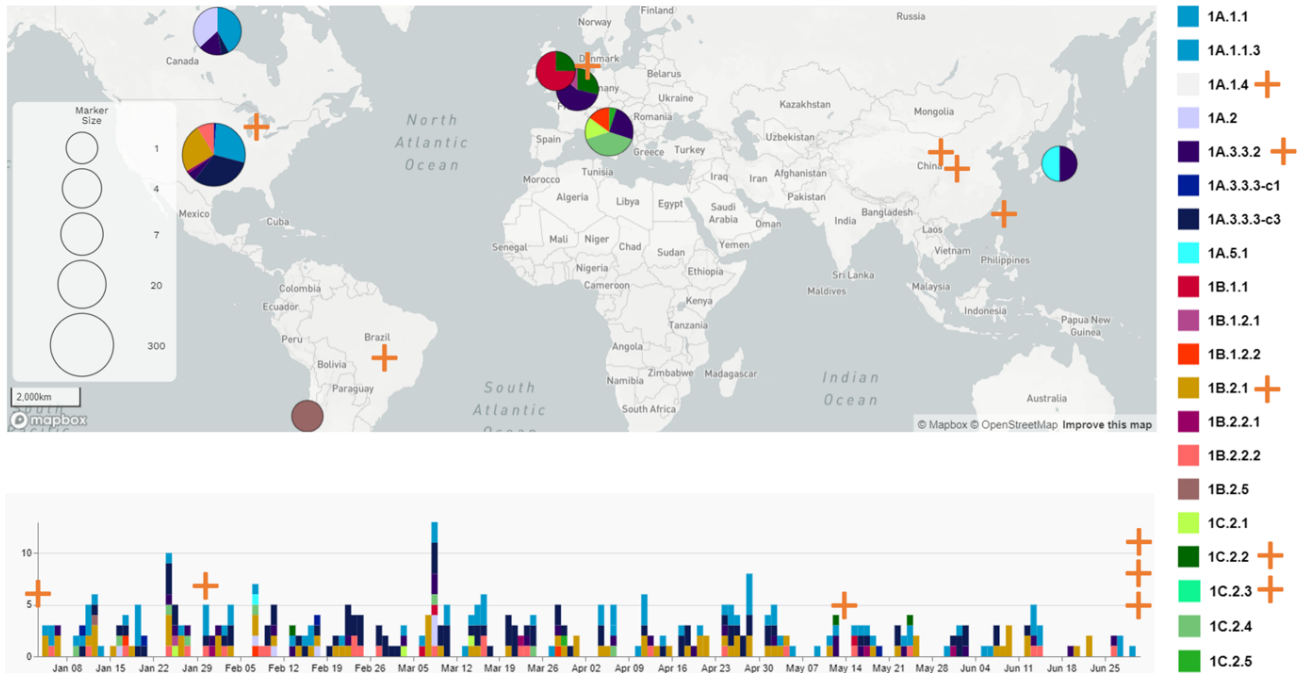


Figure 1. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between January 1, 2023 – June 30, 2023 (n=353); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions. Clades containing variants, location, and date of detection are marked with orange crosses.

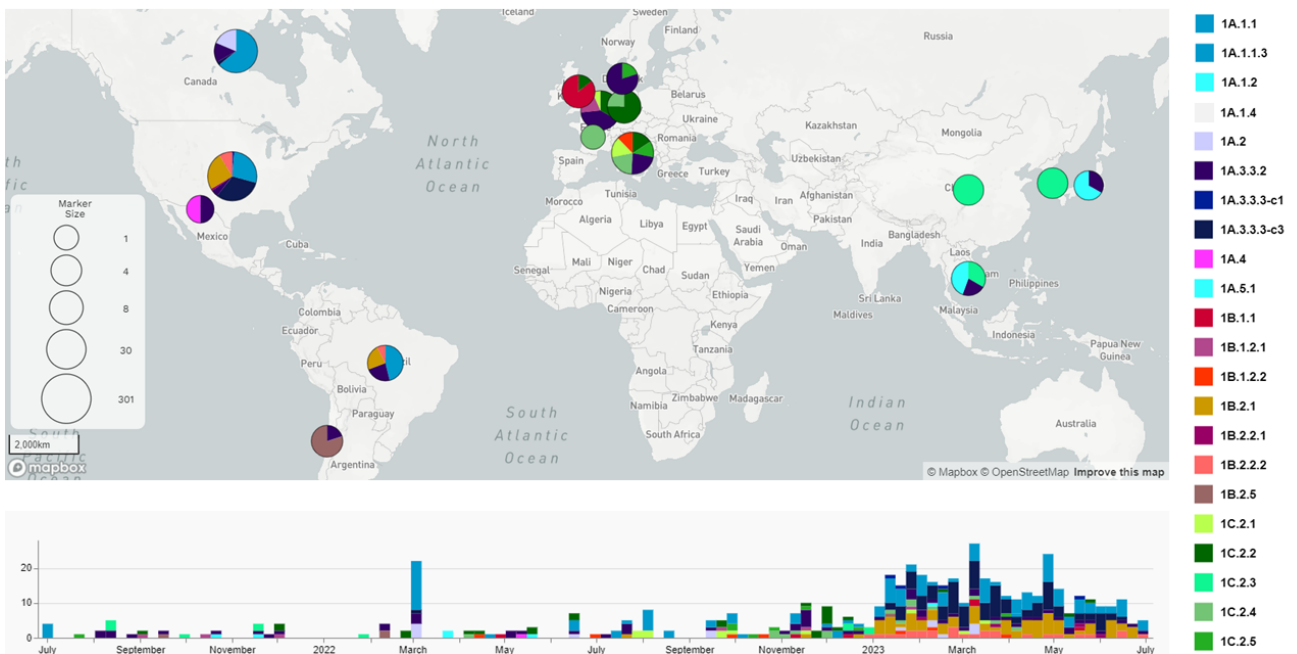


Figure 2. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between June 30, 2021– June 30, 2023 (n=524); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions.

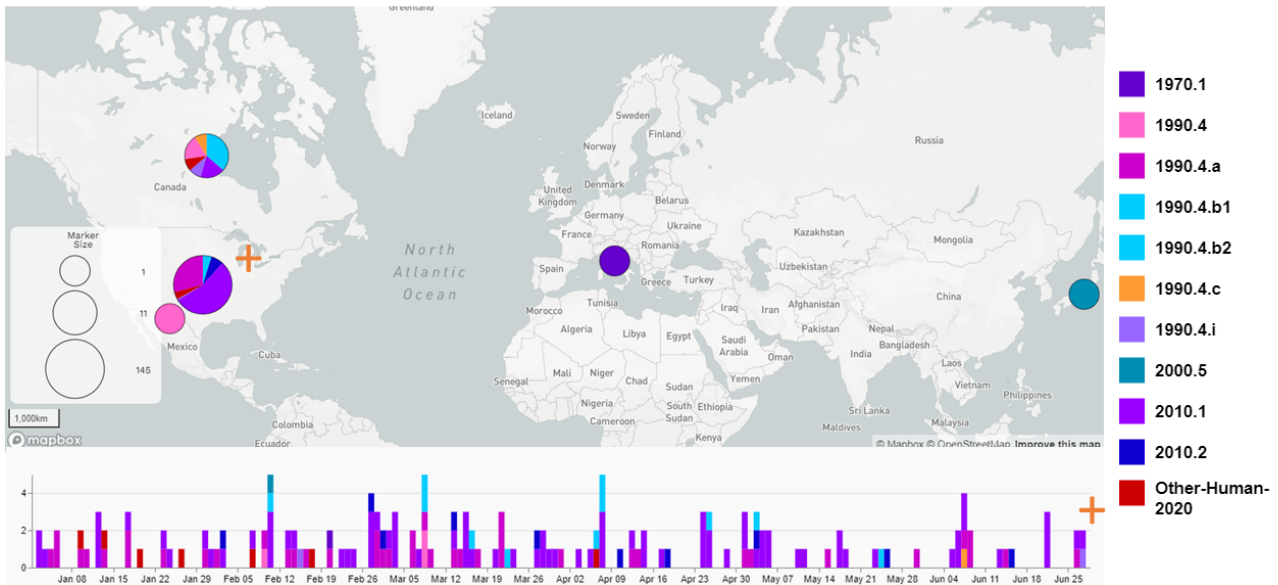


Figure 3. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between January 1, 2023 – June 30, 2023 (n=158). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions. Clades containing variants, location, and date of detection are marked with orange crosses.

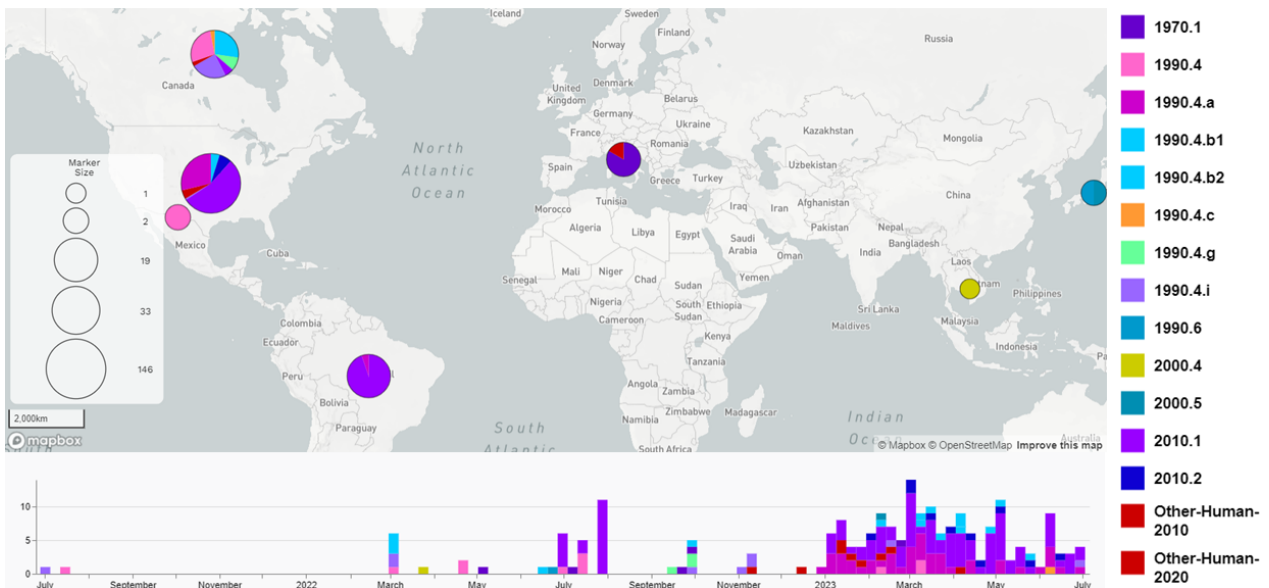
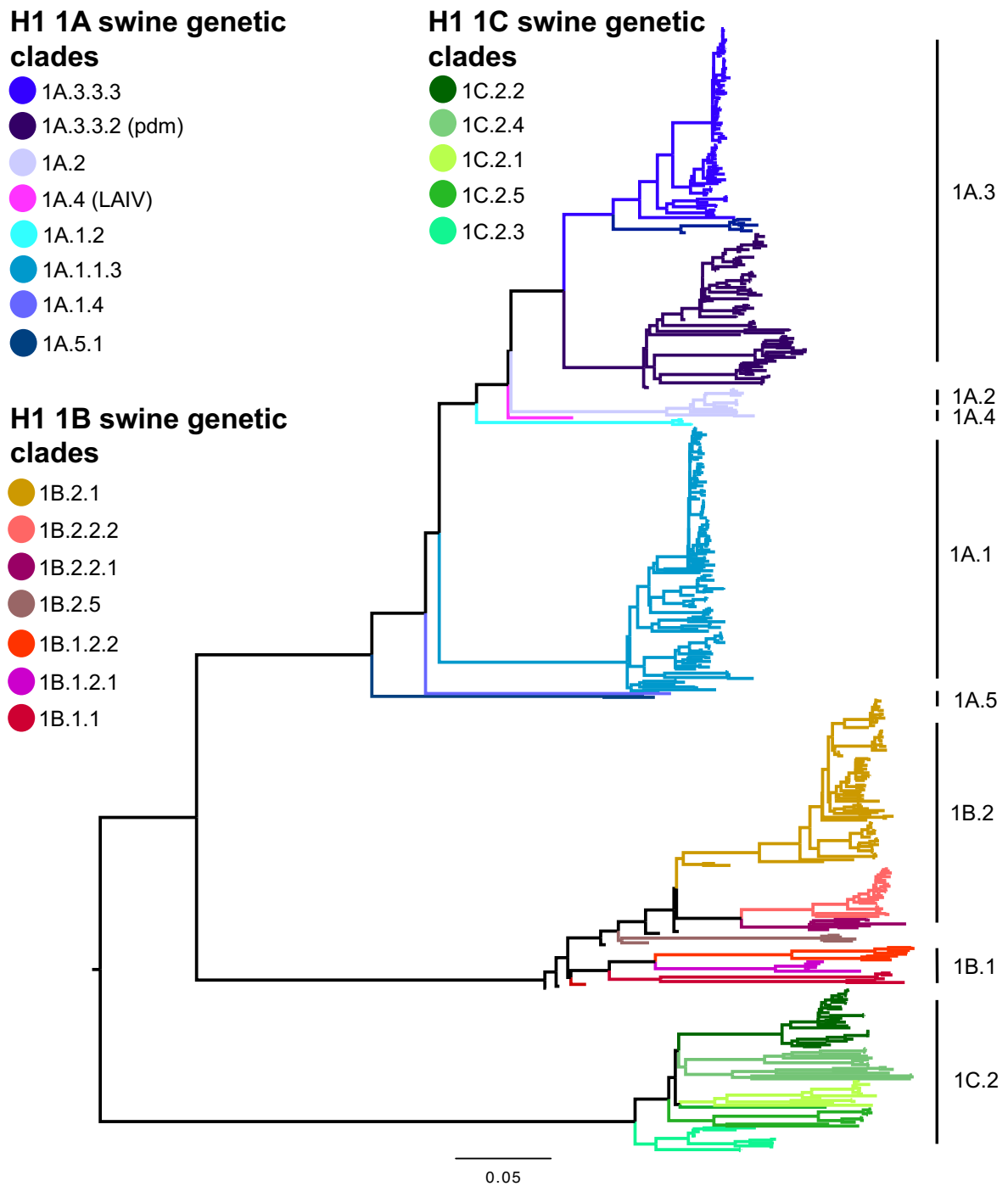


Figure 4. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between June 30, 2021 – June 30, 2023 (n=208). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions.

## Contemporary Global H1 swine IAV: genetic diversity



*n=540, data deposited within last 6 months.*

Figure 5. Global swine H1 phylogenetic tree (n=524) with reference strains (n=16) colored by clade and annotated by global H1 lineage nomenclature. Analyses were conducted with reference sequences and data (all data deposited January 1, 2023 – June 30, 2023) from GISAID or shared via the OFFLU swine IAV working group.

## Contemporary Global H3 swine IAV: genetic diversity

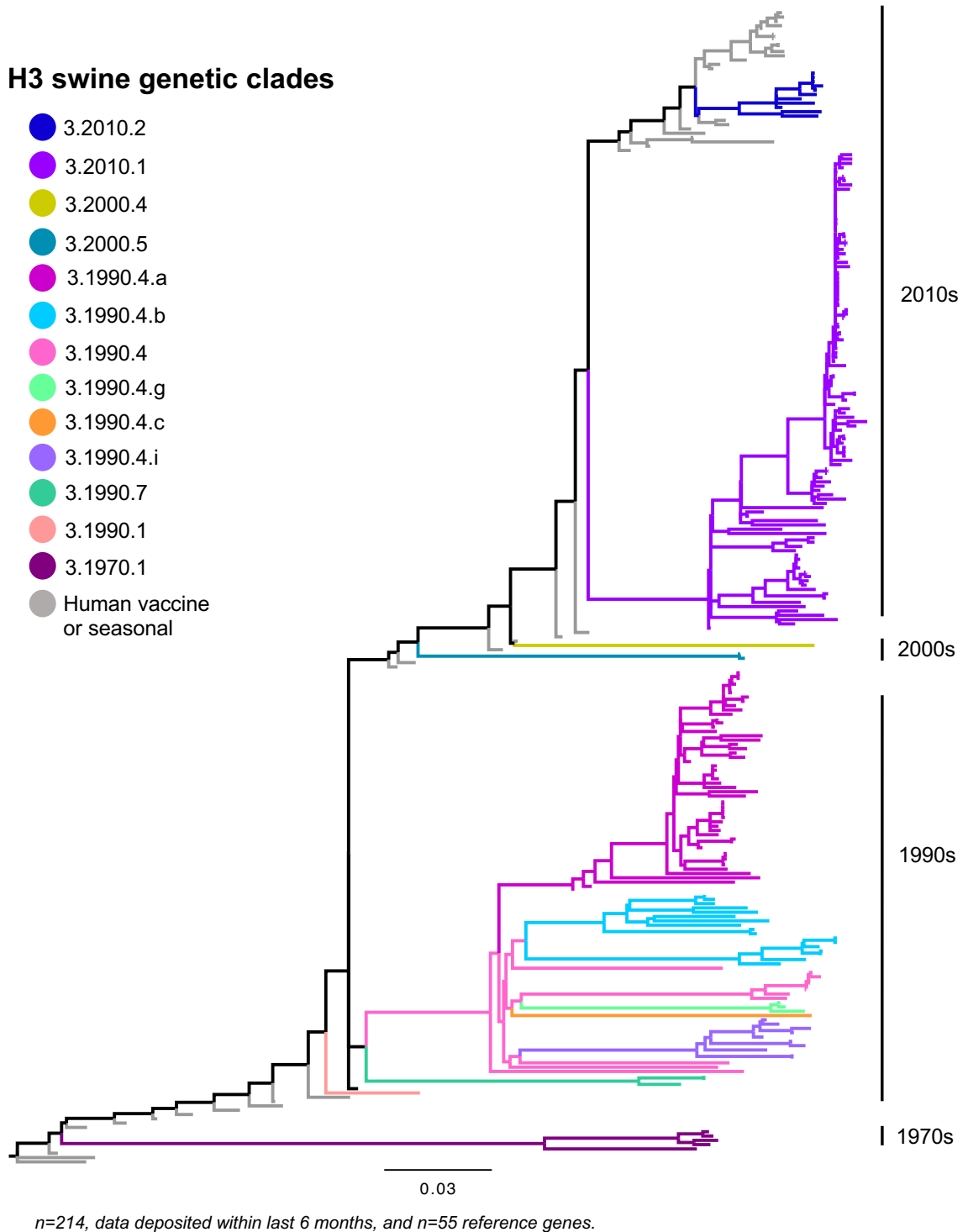


Figure 6. Global swine H3 phylogenetic tree (n=159) with reference strains (n=55) colored by clade and annotated by decade of introduction from human seasonal H3. Analyses were conducted with reference sequences and data (all data deposited January 1, 2023 – June 30, 2023) from GISAID or shared via the OFFLU swine IAV working group.

## Regional geographic summary

Phylogenetic analysis identified 30 genetic clades from H1 and H3 IAV in swine in sequence data deposited between January 1 - June 30, 2023.

19 clades were H1 subtype, with detections from each of the lineages: 1A classical swine lineage (7 clades); 1B human-seasonal lineage (7 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.1.5.1 in Japan. The 1A.3.3.2 (pdm09) circulated in all countries that deposited sequence data during this 6-month period (7 countries).
- A 1A.1.4 variant was detected in Taiwan, but no swine virus data were available.
- 1B.1 human seasonal lineage was only in Europe, and the 1B.2 human seasonal lineage was only in the USA.
- 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.
- Regionally restricted clades had new detections outside of prior geography: 1A.4 (Mexico); 1A.3.3.3-c3 (Canada); and the 1C.2.3 (Cambodia, South Korea).
- In the 2-yr window, undersurveilled regions reported novel clades including: 1A.1.2 (Cambodia), 1A.4 (Mexico), and 1B.2.5 (Chile).

11 H3 clades were detected in swine within 5 distinct lineages derived from human seasonal H3 virus spillovers grouped by the decade of introduction (1970.1; 1990.4; 2000.5; 2010.1; 2010.2) with 2 novel human seasonal spillovers (Other-Human-2010, Other-Human-2020). The 1990.4 lineage diversified into 5 co-circulating genetic clades.

- The 2010.2 lineage was detected in the USA, the 2010.1 lineage was detected in the USA and Canada, the 1970.1 lineage was detected in Europe, and the 2000.5 lineage was detected in Japan. The 1990.4 lineage was detected in the USA, Canada, and Mexico.
- Regionally restricted lineages had new detections outside of prior geography: 2010.1 (Canada); and 1990.4 (Mexico).
- In the 2-yr window, undersurveilled regions reported a novel lineage, 2000.4 (Cambodia).
- Novel human-to-swine spillovers were detected in Italy (Other-Human-2010), and the USA and Canada (Other-Human-2020).

### Global Variant Cases:

**During the reporting period (January 1 – June 30, 2023), 2 variant cases were reported and included with the swine analyses:**

- **Brazil: H1N1v (1A.3.3.2)**
- **Taiwan: H1N2v (1A.1.4)**

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

- **China: 2x H1v (1 1C.2.3 – 1 no sequence data)**
- **USA: H1N2v (1B.2.1), H3v (no sequence data)**
- **Netherlands: H1N1v (1C.2.2)**



# 1A classical swine lineage

- ★ H1v candidate vaccine virus
- A/Victoria/4897/2022  
A/Sydney/5/2021
- H1N1pdm seasonal vaccines
- Reported H1v cases
- + New variant(s)
- # HI tested viruses

## H1 1A swine genetic clades

- 1A.3.3.3-c3 (n=96)
- 1A.3.3.3-c1 (n=4)
- 1A.3.3.2/pdm (n=64)
- 1A.2 (n=15)
- 1A.4/LAIV (n=1)
- 1A.1.2 (n=4)
- 1A.1.1.3 (n=137)
- 1A.1.4 (n=1v)
- 1A.1.5.1 (n=1)

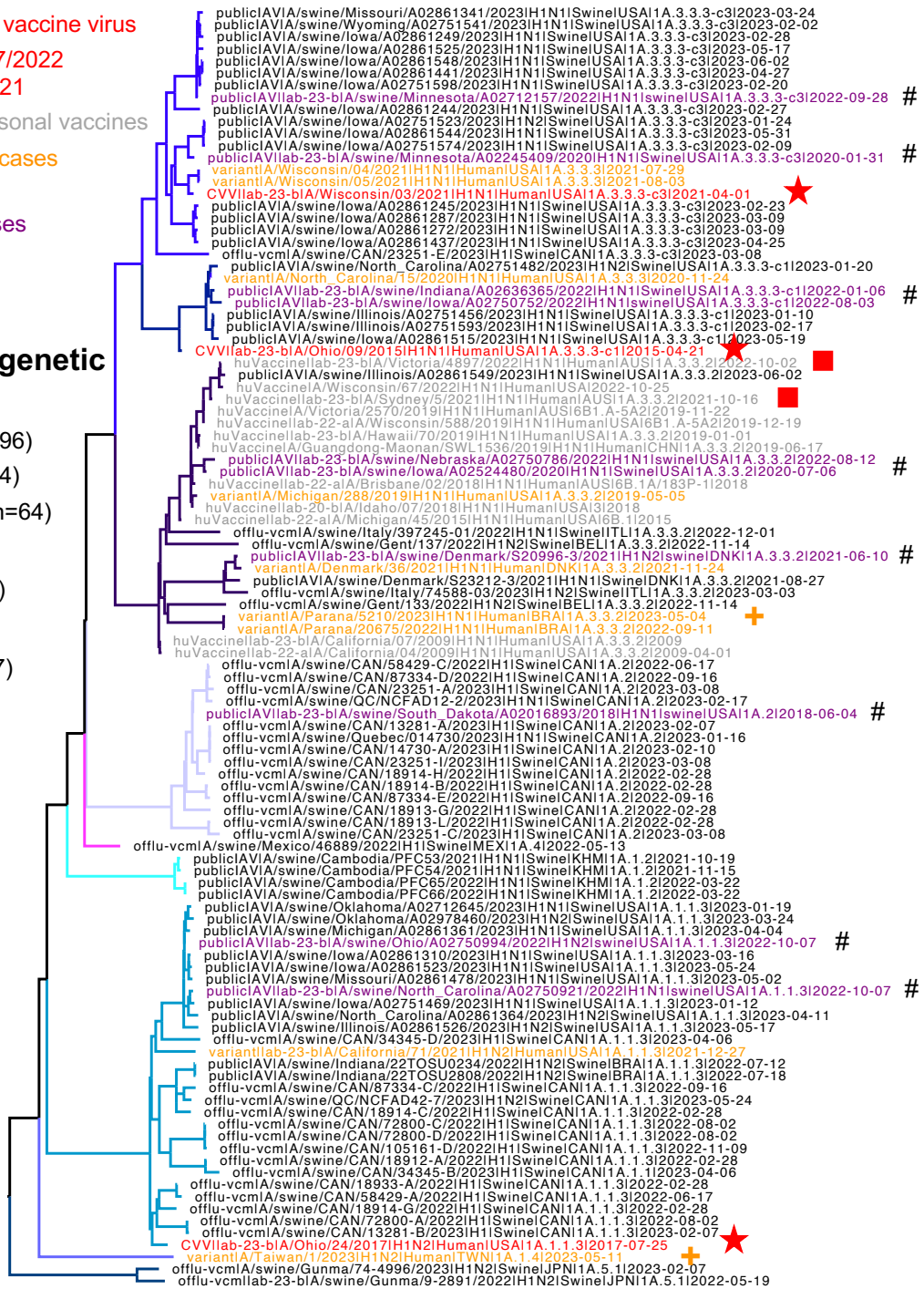


Figure 7. Swine H1 HA genes of the 1A lineage (tree was proportionally down-sampled to 98 swine HA genes, excluding references). Number of detections of each clade from data deposited between January 1 – June 30, 2023 are presented adjacent to the clade name in the figure key.

## 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
A/swine/SouthDakota/A02016893/2018*	1A.2	beta	20	<10	10	20	320	320	<10	20	320	1280	<10	10	20	20	20	640	40
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/Nebraska/A02750786/2022*	1A.3.3.2	pdm09	20	<10	<10	<10	1280	1280	40	40	640	1280	40	<10	<10	<10	20	1280	80
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 vaccine strains in dark red, new swine strains 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 1A.2 swine strain was not significantly detected by any CVV and had a 4-fold drop in cross-reactivity to the A/Hawaii/70/2019-like 6B.1A.5a.1 vaccine but reacted with adult human sera.

- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine retained cross-reactivity to the A/Hawaii/70/2019-like 6B.1A.5a.1 vaccine strain and with adult human sera. The swine strains had >8-fold decrease to the A/Sydney/5/2021 5a.2a and A/Victoria/4897/200 vaccine strains.
- 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 Data: 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	US Clade	A/Ohio/9/2015 CVV	A/California/7/2009	A/Sydney/5/2021	A/Guangdong-Maonan/SWL1536/2019	A/Victoria/2570/2019 (IVR-215)	A/Victoria/4897/2022 (IVR-238)	A/Victoria/4897/2022
A/Ohio/9/2015 CVV	1A.3.3.3-c1	gamma.1	160	80	40	80	40	40	40
A/California/7/2009	1A.3.3.2	pdm09	20	1280	160	1280	160	640	80
A/Guangdong-Maonan/SWL1536/2019	1A.3.3.2, 5a.1	pdm09	40	2560	640	5120	640	320	320
A/Victoria/2570/2019 (IVR-215)	1A.3.3.2, 5a.2	pdm09	160	320	1280	640	5120	1280	640
A/Sydney/5/2021	1A.3.3.2, 5a.2a	pdm09	20	160	640	160	640	320	320
A/Victoria/4897/2022 (IVR-238)	1A.3.3.2, 5a.2a.1	pdm09	160	160	640	320	1280	2560	2560
A/swine/Denmark/S20996-3/2021	1A.3.3.2	pdm09	80	160	160	160	160	80	40

Reference CVV in red, 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 European swine showed variable reactivity within clade, with significant loss against contemporary A/Victoria/4897/2022 vaccine antisera.

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 1A.2 compared to the nearest human H1 vaccine (A/Hawaii/70/2019) in USA HI assays and the most similar vaccine (A/California/07/2009).

Relative to HI				Relative to most similar vaccine			
site	A/Hawaii/70/2019	A/swine/South_Dakota/A02016893/2018	annotations	site	A/California/07/2009	A/swine/South_Dakota/A02016893/2018	annotations
35	D	N		35	D	N	
61	I	L		61	I	L	
71	S	F	Cb	71	S	F	Cb
73	A	R	Cb	73	A	R	Cb
83	S	P		74	S	R	Cb
85	S	P		84	S	N	
104	Q	H		85	S	P	
109	S	L		97	D	N	
120	T	A		104	Q	H	
121	S	N		109	S	L	
128	S	T		120	T	A	
129	D	N		121	S	N	
137	P	S	Ca2	128	S	T	
138	H	Y		137	P	S	Ca2
142	K	N	Ca2	138	H	Y	
162	N	S	Sa	142	K	N	Ca2
163	Q	K	Sa	168	D	N	
164	T	S	Sa	170	G	K	Ca1
168	D	N		176	L	I	
170	G	K	Ca1	183	S	P	
176	L	I		186	A	T	
185	I	S		190	S	T	Sb, RBS
186	A	T		195	A	E	Sb, RBS
187	A	D	RBS	205	R	K	
189	E	Q	Sb, RBS	216	I	A	
190	S	T	Sb, RBS	224	E	A	RBS
195	A	E	Sb, RBS	234	V	I	
203	T	S		235	E	D	
205	R	K		239	K	T	
216	T	A		260	N	G	
224	E	A	RBS	261	A	S	
234	V	I		270	T	K	
235	E	D		273	H	Q	
239	K	T		274	D	N	
256	T	A		276	N	T	
260	D	G		285	A	S	
261	A	S		298	I	V	
270	T	K		302	K	E	
273	H	Q		311	K	N	
274	D	N		314	L	M	
276	N	T		aadiff		40	
283	E	K					
285	A	S					
295	V	I					
298	I	V					
302	K	E					
311	K	N					
314	L	M					
321	V	I					
aadiff		49					

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

Table 5. Amino acid substitutions between representative swine 1A.3.3.2 clade strains compared to human seasonal H1 vaccine strains (A/California/07/2009 and A/Hawaii/70/2019), additional HI tested vaccine strains, and a recent human variant.

cont.

site	A/California/07/2009	A/Hawaii/70/2019	A/Sydney/5/2021	A/Victoria/4897/2022	A/swine/Iowa/A02524480/2020	A/swine/Nebraska/A02750786/2022	A/swine/Denmark/S20996-3/2021	A/Parana/5210/2023	annot.
15	T					N			
19	V					I	I		
43	K					Q			
47	V					I			
51	H					N			
54	K		Q	Q					
69	S						T		
70	L						I		Cb
74	S	R	R	R	R	R	G	R	Cb
82	T						A		
83	P	S	S	S	S	S	S		
84	S	N	N	N	N	N	N		
85	S						P		
94	D		N					X	
97	D	N	N	N	N	N		X	
104	Q						H		
113	R				K				
119	K						N		
120	T							S	
125	N							X	Sa
129	N	D	D	D			S		
130	K		N	N				N	
135	A							S	RBS
137	P			S	S	S			Ca2
139	A						N		
141	A							T	
142	K			R			N	S	Ca2
152	V						I	I	
155	G						E		Sa
156	N		K	K					Sb
161	L		I	I					
162	S	N	N	N	N	N	N		Sa
163	K	Q	Q	Q	Q	Q	I		Sa
164	S	T	T	T	T	T			Sa
166	I						V		Ca1
173	V				I	I	I		
176	L						V		

site	A/California/07/2009	A/Hawaii/70/2019	A/Sydney/5/2021	A/Victoria/4897/2022	A/swine/Iowa/A02524480/2020	A/swine/Nebraska/A02750786/2022	A/swine/Denmark/S20996-3/2021	A/Parana/5210/2023	annot.
183	S	P	P	P	P	P	P	P	
185	S	I	I	I	T	T		N	
186	A		T	T			D	T	
187	D	A					S	S	RBS
189	Q	E	E	E				Y	Sb, RBS
190	S						W		Sb, RBS
194	N						D		RBS
195	A							G	Sb, RBS
196	D						N		
202	G						S		
203	S	T	T	T	T	T	T	T	
205	R					K	K		
210	F						Y		
216	I	T	A	A	T	T	R		
222	D							N	Ca2, RBS
223	Q			R					RBS
224	E		A	A					RBS
225	G							X	RBS
250	V		A	A			A		
256	A	T	T	T	T	T			
259	R		K	K					
260	N	D	D	E					
261	A						T		
270	T						A		
272	V						I		
274	D						N		
277	T			A					
283	K	E	E	E	E	E			
295	I	V	V	V	V	V			
308	K		R	R			R	X	
311	K						R	X	
321	I	V	V	V	V	V	T	X	
aa diff		19	28	31	18	22	39	23	

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

Table 6a. Amino acid substitutions between representative swine 1A.3.3.3-c1 strains compared to the 1A.3.3.3-c3 CVV (A/Wisconsin/03/2021) used in the HI assay and the 1A.3.3.3-c1 CVV (A/Ohio/09/2015).

Relative to HI					Relative to most similar CVV				
site	A/Wisconsin/03/2021	A/swine/Indiana/A02636365/2022	A/swine/Iowa/A02750752/2022	annotations	site	A/Ohio/09/2015	A/swine/Indiana/A02636365/2022	A/swine/Iowa/A02750752/2022	annotations
2	T	K	K		22	K	R	R	
3	L	I	I		48	X	A	A	
22	K	R	R		68	E	D	D	
35	T	N	N		72	T	R	R	
36	R	K	K		82	T	N	N	
45	K	R	R		83	S	P	P	
68	E	D	D		120	T		N	
71	S	A	A	Cb	125	N	K	K	Sa
72	T	R	R		169	R	K	K	
82	T	N	N		194	N		H	RBS
83	S	P	P		204	S	P	P	Ca1
84	N	S	S		205	R		K	
86	E	N	N		222	G	D	D	Ca2, RBS
113	R	K	K		228	N		S	
120	T		N		235	E		A	
125	N	K	K	Sa	269	E	G	G	
129	D	N	N		278	T	I	I	
142	R	N	N	Ca2	283	N	S	S	
146	R	K	K		295	I	V	V	
149	V	I	I		aadiff		14	19	
153	Q	K	K	Sb					
155	G	E	E	Sa					
161	V	I	I						
163	T	I	I	Sa					
166	I	T	T	Ca1					
170	E	G	G	Ca1					
183	S	P	P						
186	A	T	T						
189	R	Q	Q	Sb, RBS					
193	K	Q	Q	Sb, RBS					
194	N		H	RBS					
195	E	A	A	Sb, RBS					
196	D	N	N						
197	A	S	S						
204	S	P	P	Ca1					
205	R		K						
224	S	A	A	RBS					
228	N		S						
235	E		A						
250	A	V	V						
269	D	G	G						
270	A	T	T						
271	S	P	P						
278	T	I	I						
283	N	S	S						
295	I	V	V						
aadiff		41	46						

Reference CVV in red, HI swine strains in purple.



Table 6b. 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.

# 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

# H1 1B swine genetic clades

- 1B.2.1 (n=80)
- 1B.2.2.2 (n=27)
- 1B.2.2.1 (n=4)
- 1B.2.5 (n=4)
- 1B.1.2.2 (n=8)
- 1B.1.2.1 (n=6)
- 1B.1.1 (n=6)

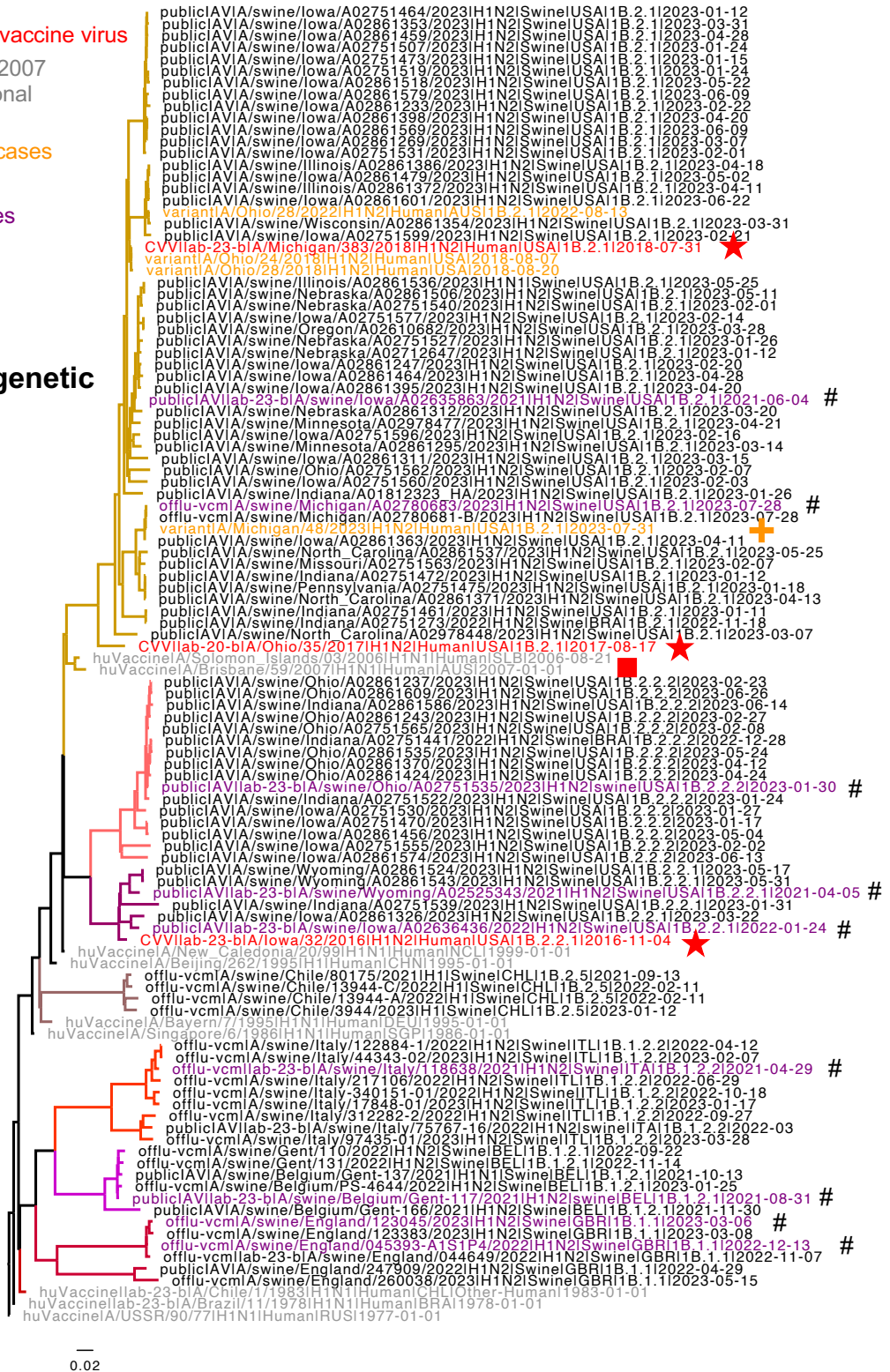


Figure 8. Swine H1 HA genes of the 1B lineage (tree was proportionally down sampled to 95 swine HA genes, excluding references). Number of detections of each clade from data deposited between January 1 - June 30, 2023 are presented adjacent to the clade name in the figure key.

## 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	80	<10	<10	<10	10	20
A/swine/Iowa/A02635863/2021*	1B.2.1	delta-2	80	<10	<10	<10	10	20
A/swine/Michigan/A02780683/2023	1B.2.1	delta-2	40	20	nt	nt	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/Iowa/A02636436/2022	1B.2.2.1	delta-1a	40	20	20	10	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 2021a; 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 major 1B.2.1 (delta-2) clade swine representative A/swine/Iowa/A02635863/2021 had no loss of titer and A/swine/Michigan/A02780683/2023 epidemiologically linked to the variant case had 2-fold loss to the within-clade CVV A/Michigan/383/2018, but both displayed limited titers in adult human sera.
- The 1B.2.2.1 A/swine/Wyoming/A02525343/2021 had a >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 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/Brazil/11/1978	A/Chile/1/1983
A/Michigan/383/2018 RG58A	1B.2.1	delta-2	640	160	80
A/Brazil/11/1978	seasonal		20	640	320
A/Chile/1/1983	seasonal		40	320	1280
<b>A/swine/England/045393/2022</b>	1B.1.1		40	80	10
<b>A/swine/England/123045/2023</b>	1B.1.1		20	40	10
<b>A/swine/Belgium/Gent-117/2021</b>	1B.1.2.1		80	640	640
A/swine/Italy/118638/2021*	1B.1.2.2		20	nt	40

Reference CVV in red, reference seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted gray. \*Previously tested in report 2022b. nt=not tested.

- There are no within-clade CVVs for 1B lineages from Europe.
- The swine 1B.1.1 had significant loss in cross-reactivity with A/Chile/1/1983 and A/Brazil/11/1978.
- The swine 1B.1.2.1 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 1B.2.1 CVV and against the human seasonal ancestral A/Chile/1/1983 reference strain.

Table 9. 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/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 strains compared to the within-clade CVV (A/Iowa/32/2016).

site	A/Iowa/32/2016	A/swine/Wyoming/A02525343/2021	A/swine/Iowa/A02636436/2022	annotations
19	L	V	V	
68	E		D	
69	S		T	
72	S	P		
74	K	E	E	Cb
96	T	A	A	
104	Q		H	
106	S	N		
130	T	D		
132	K	T	V	RBS
141	E		K	
142	G		S	Ca2
157	L	R		Sa
163	K		M	Sa
168	E	D	D	
175	I	V		
179	V		I	
194	T		A	RBS
208	R	K		
215	T	A		
222	D	N	N	Ca2, RBS
241	I	V		
287	N	T		
289	N		S	
310	T	R		
aadiff		16	15	

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

Table 11. Amino acid substitutions between a 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 12. Amino acid substitutions between representative swine 1B.1.1 strains compared to the human seasonal vaccine strains, A/Chile/1/1983 and A/Brazil/11/1978, used in the HI assays.

site	A/Chile/1/1983	A/Brazil/11/1978	A/swine/England/045393- A1S1P4/2022	A/swine/England/123045/2023	annotations
14	D		E	E	
36	N	S	S	S	
43	K	R			
48	A		P	P	
51	Q			H	
54	K		T	T	
66	E		K	K	
74	K		N	N	Cb
82	T		L	L	
85	S		P	P	
89	T		I	I	
94	Y		H	H	
95	F			L	
96	A		S	S	
120	E		D	D	
121	S	R			
125	K		G	G	Sa
128	V	I			
130	K	R			
135	A	S	S	S	RBS
137	S		P	P	Ca2
139	K		D	D	
141	K			E	
142	S			R	Ca2
149	L		V	V	
153	E		G	G	Sb
156	G		N	N	Sb
157	S		L	L	Sa
161	L		V	V	
162	S		N	N	Sa
190	T		A	A	Sb, RBS
193	R		H	H	Sb, RBS
194	K		N	N	RBS
197	A		T	T	
205	H	N			
209	R		K	K	
216	K		R	R	
220	V		I	I	RBS
222	N	G			Ca2, RBS
237	G		D	D	Ca1
238	D		E	E	
239	T		E	E	
252	W		Q	Q	
260	G		S	S	
267	T		I	I	
270	A		T	T	
276	D		N	N	
277	A	T	T	T	
308	R		K	K	
324	I			V	
aadiff		9	39	44	

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



Table 13. Amino acid substitutions between representative swine 1B.1.2 strains compared to the most similar human seasonal vaccine A/Chile/1/1983 used in HI assays.

cont. →

site	A/Chile/1/1983	A/swine/Belgium/Gent-117/2021	A/swine/Italy/118638/2021	annotations
25	T	A		
36	N	S		
43	K	R		
47	I		V	
54	K		R	
71	F		L	Cb
73	K	N		Cb
82	T		A	
83	P	T		
85	S	P	A	
89	T	I	L	
94	Y		H	
96	A	S	S	
111	F		I	
124	P	S		Sa
125	K	N		Sa
127	N	S	S	
128	V		I	
129	T	N	-	
130	K		-	
132	V		A	RBS
135	A	S	S	RBS
138	H		K	
139	K		Q	
141	K		R	
142	S		R	Ca2
146	R		T	
157	S	I		Sa
162	S		T	Sa
163	K		M	Sa
166	V		M	Ca1
168	N		D	
169	K	R		

site	A/Chile/1/1983	A/swine/Belgium/Gent-117/2021	A/swine/Italy/118638/2021	annotations
170	E	D	K	Ca1
171	K	E	R	
175	V	L		
184	N		S	
185	I	M		
189	K	R	R	Sb, RBS
190	T	A	A	Sb, RBS
196	N	T	T	
202	V		T	
208	R	K	K	
209	R		K	
211	T	I		
220	V	I	I	RBS
222	N		D	Ca2, RBS
237	G	K	S	Ca1
248	L	F		
252	W	L		
258	S		N	
262	G	E	E	
267	T	V	V	
270	A		V	
272	M	V		
276	D		N	
277	A	T		
278	K		T	
283	Q		R	
288	S		N	
295	V		I	
298	V		I	
308	R		K	
310	T	K		
313	R		K	
315	V		A	
aadiff		33	49	

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

# 1C Eurasian avian lineage

- ★ H1v candidate vaccine virus
- Reported H1v cases
- + New variant(s)
- # HI tested viruses

## H1 1C swine genetic clades

- 1C.2.2 (n=27)
- 1C.2.4 (n=24)
- 1C.2.1 (n=11)
- 1C.2.5 (n=8)
- 1C.2.3 (n=11)

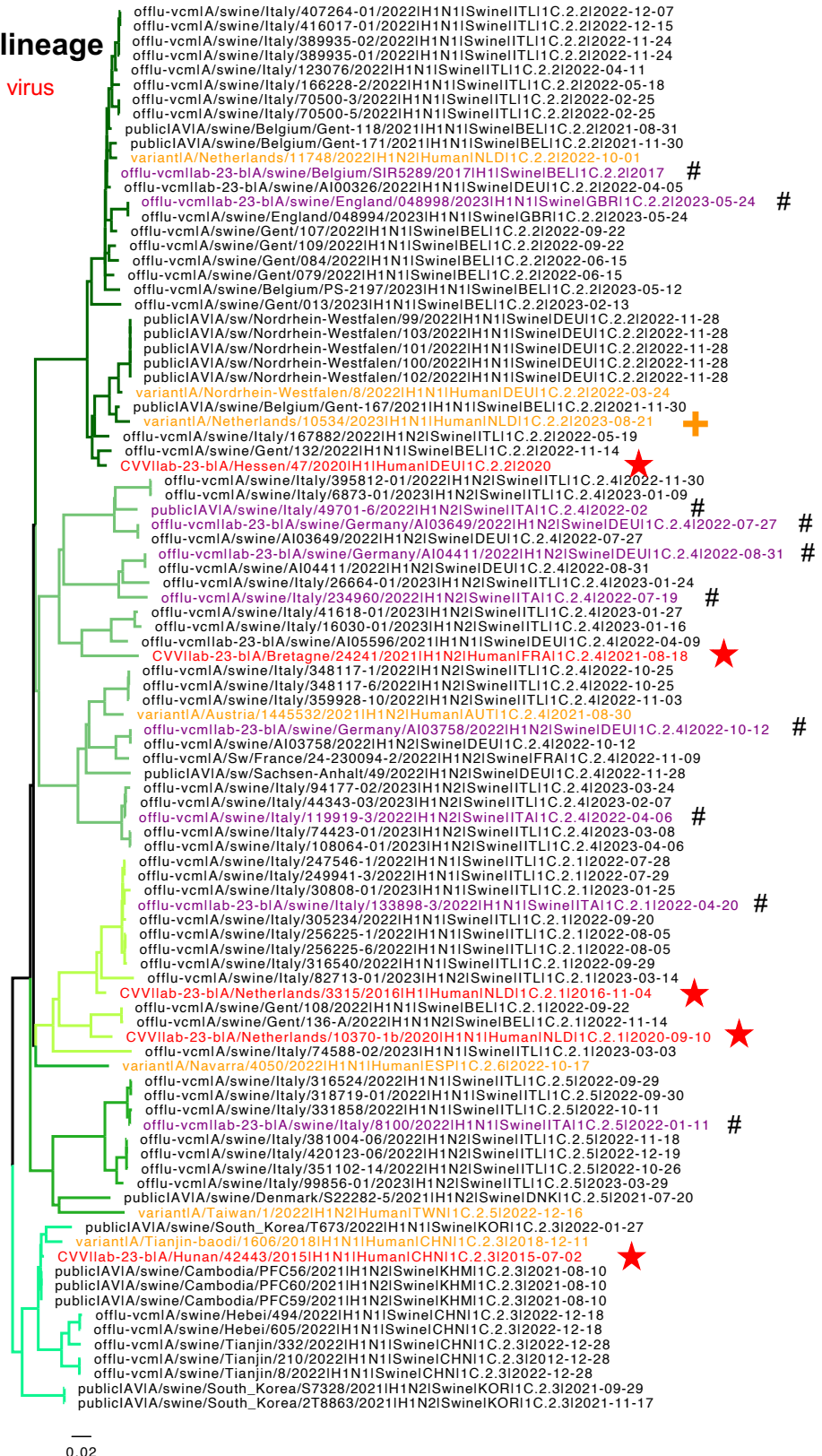


Figure 9. Swine H1 HA genes of the 1C lineage. Number of detections of each clade from data deposited between January 1 - June 30, 2023 are presented adjacent to the clade name in the figure key.

## Antigenic analysis: Swine 1C Lineage

Table 14. UK & EU Data: 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/2424-1/2021 CVV
A/Netherlands/3315/2016 CVV	1C.2.1	1280	320	160	80	40
A/Netherlands/10370-1b/2020 CVV	1C.2.1	320	640	320	320	80
<b>A/swine/Italy/133898-3/2022</b>	1C.2.1	640	160	160	160	80
A/Hessen/47/2020-like CVV	1C.2.2	160	320	640	640	160
<b>A/swine/Belgium/SIR5289/2017</b>	1C.2.2	160	40	160	80	80
<b>A/swine/England/048998/2023</b>	1C.2.2	80	80	640	640	10
A/Hunan/42443/2015 CNIC-1601 CVC	1C.2.3	160	160	320	640	80
A/Bretagne/24241/2021 CVV	1C.2.4	80	20	40	80	1280
A/swine/Italy/49701-6/2022*	1C.2.4	160	80	40	80	80
A/swine/Italy/234960/2022*	1C.2.4	20	20	20	40	20
A/swine/Italy/119919-3/2022*	1C.2.4	80	80	20	80	20
A/swine/Germany/AI04411/2022*	1C.2.4	40	20	20	0	0
A/swine/Germany/AI03758/2022*	1C.2.4	80	40	20	0	0
A/swine/Germany/AI03649/2022*	1C.2.4	80	80	80	<10	<10
<b>A/swine/Italy/8100/2022</b>	1C.2.5	160	40	160	80	80

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

- The 1C.2.1 swine strain had  $\leq 4$ -fold loss in reactivity to the within clade CVV.
- The 1C.2.2 swine strains had  $\leq 4$ -fold to the within-clade CVV (A/Hessen/47/2020).
- The 1C.2.4 swine strains displayed  $> 8$ -fold reduction to the within clade CVV.
- The 1C.2.5 swine strain has no within-clade CVV and had limited cross-reactivity to other 1C CVVs.

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

site	A/Netherlands/3315/2016	A/Netherlands/10370-1b/2020	A/swine/Italy/133898-3/2022	annotations
20	M		L	
31	N	D		
44	L	M		
48	A		P	
71	I	L	T	Cb
74	N	K		Cb
83	S	P		
89	A		T	
97	D	N		
120	E	A	A	
132	S	T		RBS
137	S	P		Ca2
141	I	A	V	
142	K	N		Ca2
155	E	G	N	Sa
161	I	L		
163	T	K		Sa
175	I	V	V	
199	V	I		
202	G	E		
208	K	Q		
215	V	A	A	
227	M		I	
253	Y	H		
258	N	D		
267	M	I		
271	N	H		
311	Q	R		
321	T	I	I	
324	V	I		
326	S		T	
aadiff		26	12	

Reference CVVs in red, HI swine strain in purple.

Table 16. Amino acid substitutions between representative swine 1C.2.2 strains compared to the within-clade CVV (A/Hessen/47/2020 CVV) and a recent 1C.2.2 variant.

site	A/Hessen/47/2020	A/swine/Belgium/SIR5289/2017	A/swine/England/048998/2023	A/Netherlands/10534/2023	annotations
3	I	L	L		
36	S		N		
43	S		N		
53	K	R	R	R	
82	T			A	
84	D	N	N		
97	D	N	H	N	
125	N	D	D		Sa
127	D			E	
130	R			K	
142	N	K			Ca2
161	L	I	I		
162	R		K		Sa
163	N	K	K	K	Sa
175	V			I	
185	D	E	E		
186	S			N	
205	K			E	
211	T		K		
214	T	I	I		
235	D	N	N	K	
253	Y	H	H		
258	N		D		
261	S		P		
267	M		I	R	
269	N	D	D		
271	R		H		
278	M		V		
289	N			D	
302	E	K	K	K	
308	K			R	
310	T		N		
311	H		Y		
aadiff		14	24	14	

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

Table 17. Amino acid substitutions between representative swine 1C.2.4 strains compared to the within-clade CVV (A/Bretagne/24241/2021).

cont.

site	A/Bretagne/24241/2021	A/swine/Germany/AI03649/2022	A/swine/Italy/234960/2022	A/swine/Germany/AI03758/2022	A/swine/Germany/AI04411/2022	A/swine/Italy/19919-3/2022	A/swine/Italy/49701-6/2022	annotations
5	I	V		V		V	V	
20	M	L	L	L	L	L	L	
31	N	D	D				D	
36	N	S	S	S	S	S	S	
46	G				E			
51	Q		N	K	N			
53	G			E				
56	D	N	N	N	N	N	N	
69	L				V			
70	L		I					Cb
71	V	L	L	L	L	L	L	Cb
83	S	P		P				
84	N		D		D			
86	K			D		E		
89	T	I					I	
94	E			K				
96	A	S				S	S	
102	R		K		K		K	
104	L			Q		Q		
106	S						G	
107	T						A	
112	E		K		K			
116	I			M		V	V	
119	K	G	E		E		E	
120	-	A	A	A	A	A	A	
121	N	A	T	T	T	T	T	
124	P	E	S				K	Sa
125	S	N	N	D	N	E	N	Sa
127	E		T		T			
128	A	T	T	T	T	T	T	
129	-	K	G	T	G	T	K	
130	-					R		
132	T				A			RBS
134	V	G		I	A			RBS
135	S			A		A		RBS
137	S					Y		Ca2
138	K	R	H	H	H	H	R	
141	A	T			G		T	
142	N	R	S	R	K		P	Ca2
146	R	Q					Q	
149	L		S		S			
152	T			L		V		
153	S	K	K	R	K	K	K	Sb
155	G	-				E	-	Sa
156	K	N	S	N	T	N	I	Sb
157	P	S	S	S	S	S	S	Sa
159	S	P	P	P	P	P	P	Sa
161	L	I					I	

site	A/Bretagne/24241/2021	A/swine/Germany/AI03649/2022	A/swine/Italy/234960/2022	A/swine/Germany/AI03758/2022	A/swine/Germany/AI04411/2022	A/swine/Italy/19919-3/2022	A/swine/Italy/49701-6/2022	annotations
163	K		R		R			Sa
166	K	T	T	T	T	T	T	Ca1
168	N	D	D			D		
169	K	R	Q		Q			
170	G				E			Ca1
172	E	K					K	
175	V					I		
184	T					N		
185	Y		A		D			
186	S			N		R		
189	Q		L		L			Sb, RBS
190	T	A				A	A	Sb, RBS
193	Q		Y					Sb, RBS
194	N	S					S	RBS
196	H		D					
199	V			I				
200	S		L					
202	V		A	E	A	E		
203	S	T					T	
205	K			N				
208	Q		R	R	K	R		
211	T		K					
215	V					A		
216	P	E	T	T	T	T	D	
219	E	K	K	K	K	K	K	RBS
224	R	A	A	A	A	A	A	RBS
235	D			T				
237	E	G	G	G	G	G	G	Ca1
239	T	N					N	
241	T				A			
249	I					V		
252	R	K		W		W	K	
253	Y		H		H			
256	A						V	
258	E	D	S	D	S	N	D	
259	K	M					M	
260	G	S					S	
261	S			L		L		
262	N	S	I	S	S	S	S	
266	M			V		V	L	
267	K			I				
269	D			E				
271	H				Q			
283	H						Q	
288	S			G		G	G	
289	D	N	N	N		N	S	
290	R			L		L		
298	I					V		
311	Q			K				
324	I				L			
aadiff		45	45	48	45	47	49	

Reference CVV in red, HI swine strains in purple.

Table 18. 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/Netherlands/3315/2016	A/Netherlands/10370-1b/2020	A/Hessen/47/2020	A/Hunan/42443/2015	A/Bretagne/24241/2021	A/swine/Italy/8100/2022	annot.
2	T					I	
5	V				I		
20	M		L	L		L	
31	N	D					
35	N		T		T		
36	S				N		
44	L	M				M	
47	K					N	
48	A			I			
53	G		K				
56	N			S	D	D	
57	V		L				
66	E			K			
69	L					F	
71	I	L	L	L	V	L	Cb
74	N	K					Cb
80	I		V				
82	T					A	
83	S	P	P				
84	N		D				
85	S					P	
89	A		T		T		
94	E					K	
97	D	N					
102	R			K			
104	Q				L		
111	F					L	
120	E	A	A	A	-	R	
121	T				N		
125	N				S	D	Sa
127	E		D	D			
128	T				A		
129	T				-		
130	K		R	R	-		
132	S	T	T	T	T	T	RBS
134	V		I			S	RBS
135	A				S	S	RBS
137	S	P					Ca2
138	H				K		
141	I	A	A	A	A	A	
142	K	N	N	N	N	N	Ca2
152	V				T		
153	K				S		Sb
155	E	G	G	G	G	G	Sa

site	A/Netherlands/3315/2016	A/Netherlands/10370-1b/2020	A/Hessen/47/2020	A/Hunan/42443/2015	A/Bretagne/24241/2021	A/swine/Italy/8100/2022	annot.
156	N				K		Sb
157	S				P		Sa
159	P				S		Sa
161	I	L	L	L	L	L	
162	S		R			N	Sa
163	T	K	N	K	K	K	Sa
166	T				K	I	Ca1
169	K					R	
175	I	V	V	V	V	V	
183	P					S	
184	T					N	
185	D				Y		
186	S					N	
190	T					A	Sb, RBS
199	V	I	I				
202	G	E			V		
208	K	Q	Q		Q	R	
214	I		T				
215	V	A				M	
216	A				P		
219	K				E		RBS
220	V		I		I		RBS
222	E					N	Ca2, RBS
224	A				R		RBS
227	M		I			I	
237	G				E		Ca1
245	T					N	
252	W				R		
253	Y	H		H			
258	N	D		K	E	K	
262	N		S	S		S	
266	M		I				
267	M	I		R	K	I	
269	D		N				
271	N	H	R	Q	H	H	
278	K		M				
287	K					N	
288	S			G		N	
289	N				D	S	
290	L				R		
298	I		V				
310	T					K	
311	Q	R	H				
321	T	I	I	I	I	V	
324	V	I	I	I	I	I	
aadiff		26	34	25	44	44	

Reference CVVs in red, HI swine strain in purple.

### H3 swine lineage

★ H3v candidate vaccine virus

■ A/Darwin/6/2021

Previous H3N2 seasonal vaccines

Reported H3N2v cases

+ New variant(s)

# HI tested viruses

### H3 swine genetic clades

- 3.2010.2 (n=10)
- 3.2010.1 (n=99)
- 3.2000.4 (n=1)
- 3.2000.5 (n=2)
- 3.1990.4.a (n=48)
- 3.1990.4.b (n=16)
- 3.1990.4 (n=11)
- 3.1990.4.g (n=3)
- 3.1990.4.c (n=1)
- 3.1990.4.i (n=9)
- 3.1970.1 (n=5)

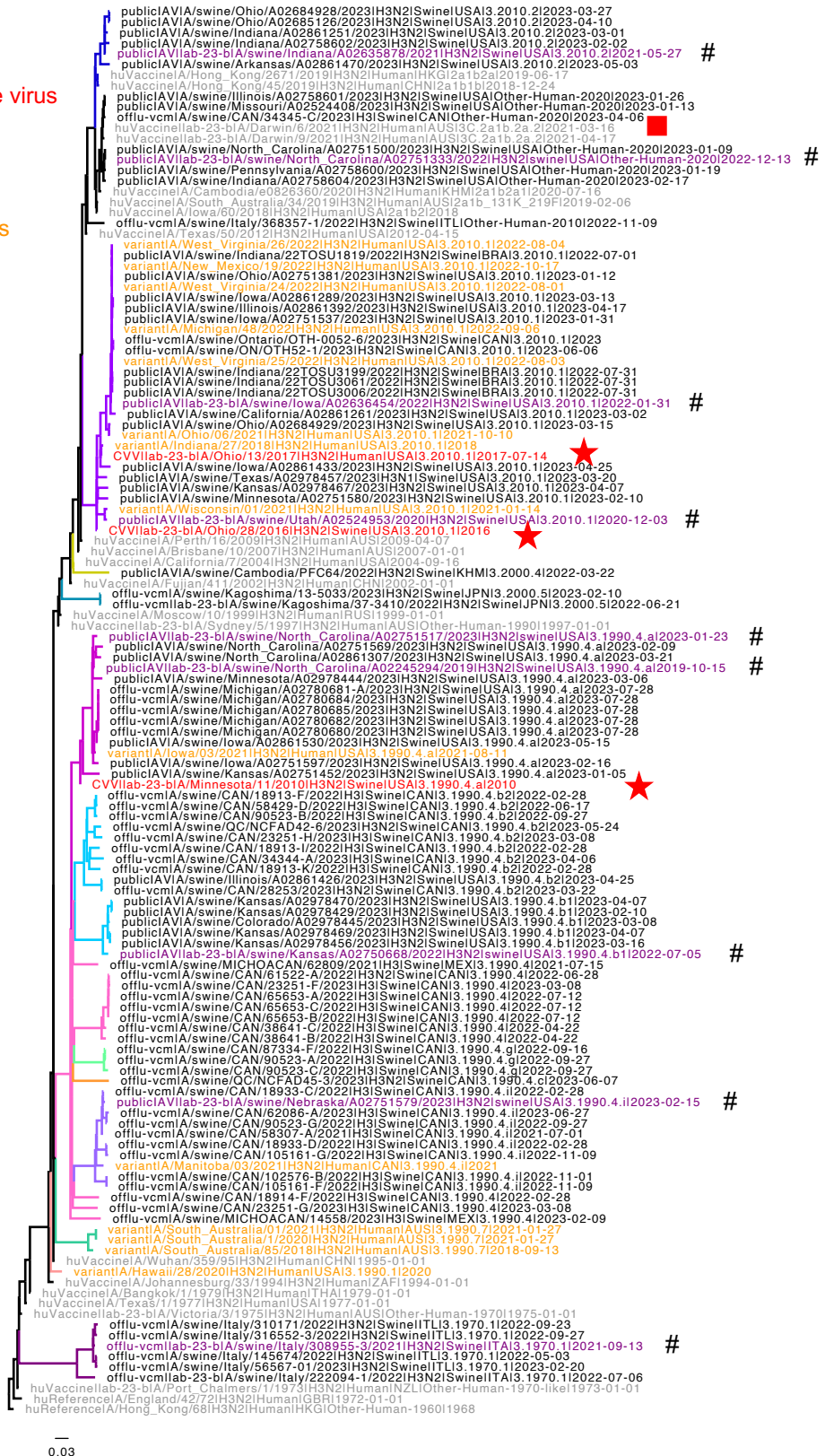


Figure 10. Swine H3 HA genes. Number of detections of each clade from data deposited between January 1 - June 30, 2023 are presented adjacent to the clade name in the figure key. Clades within the tree were proportionally down sampled to 93 swine HA genes (excluding references); tree includes clade consensus sequences, and human vaccine strains.



### Antigenic analysis: Swine H3

Table 19. 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	IDCDC-RG60A A/Ohio/13/2017 CVV	A/swine/Utah/A02524953/2020 (H3N2) #19	A/swine/Utah/A02524953/2020 (H3N2) #20	r A/Darwin/9/2021	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	20	40	80	20	80	10	80	20
A/swine/North Carolina/A02245294/2019*	1990.4.a	80	640	640	20	20	40	40	40	10	160	80
<b>A/swine/North Carolina/A02751517/2023</b>	1990.4.a	40	640	320	10	10	10	80	10	<10	160	40
A/swine/Kansas/A02750668/2022*	1990.4.b1	<10	<10	10	<10	<10	10	<10	10	<10	320	160
<b>A/swine/Nebraska/A02751579/2023</b>	1990.4.i	<10	10	10	<10	10	10	<10	<10	<10	160	40
<b>IDCDC-RG60A A/Ohio/13/2017 CVV</b>	2010.1	<10	20	80	1280	160	160	20	20	10	320	80
A/swine/Utah/A02524953/2020*	2010.1	10	40	40	160	640	640	20	20	10	320	80
A/swine/Iowa/A02636454/2022*	2010.1	10	20	40	160	320	320	10	40	<10	320	80
A/swine/Indiana/A02635878/2021*	2010.2	20	20	20	<10	20	40	20	20	<10	160	20
<b>A/HongKong/45/2019*</b>	3C.2a1b.1b	<10	nt	nt	<10	nt	nt	nt	nt	nt	1280	160
<b>A/Darwin/9/2021</b>	3C.2a1b.2a.2	<10	10	10	10	10	10	1280	320	640	40	10
<b>rg-A/Darwin/6/2021-like*</b>	3C.2a1b.2a.2	<10	<10	10	<10	20	20	320	320	640	40	20
<b>A/swine/North Carolina/A02751333/2022</b>	Other-Human	<10	10	10	<10	<10	<10	320	80	80	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; 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 strains 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 and had >8-fold loss in reactivity with adult human sera.

Table 20. EU 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/Victoria/3/1975	IDCDC-RG55C A/Ohio/28/2016-like CVV	A/Sydney/5/97
A/Victoria/3/1975*	Human seasonal	1280	40	160
IDCDC-RG55C A/Ohio/28/2016-like CVV*	2010.1	160	1280	160
A/Sydney/5/97*	Human seasonal	320	80	2560
<b>A/swine/Italy/308955-3/2021*</b>	1970.1	640	320	320

Reference CVV in red, seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted in gray. \*Previously tested in report 2022b

- The 1970.1 representative swine A/swine/Italy/308955-3/2021 demonstrated reactivity with A/Victoria/3/1975, 4-fold decrease to the 2010.1 CVV, and 8-fold to A/Sydney/5/97.

Table 21. Amino acid substitutions between representative swine 1990.4 strains compared to the within-lineage CVV (A/Minnesota/11/2010 x 203).

cont.

site	A/Minnesota/11/2010	A/swine/North_Carolina/A02245294/2019	A/swine/Kansas/A02750668/2022	A/swine/Nebraska/A02751579/2023	A/swine/North_Carolina/A02751517/2023	annotations
1	Q			K		
2	K		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			
91	S				R	
96	N	S	D		S	
104	D		E			
107	T		S	S		
117	N		T	T		
119	E	K			K	
121	T			N		
122	Q			P		Site A
124	S	I		N	I	
131	A	T	N	G	N	
133	D			N		Site A
135	S		A			
137	Y		F	F		Site A
138	A	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
146	S				G	Site A

site	A/Minnesota/11/2010	A/swine/North_Carolina/A02245294/2019	A/swine/Kansas/A02750668/2022	A/swine/Nebraska/A02751579/2023	A/swine/North_Carolina/A02751517/2023	annotations
155	Y		H	H		Site B
156	N	H	S	H	H	
158	N		K			
159	Y			H		
163	E		A	A		
164	Q		L			
172	D		G			
174	F		S			
189	K		A	M		Site B
192	T		I			
193	N		S	S		Site B
196	V		T			
198	A			E		
201	R		G			Site D
202	V		I			
203	I		T	T		
207	K	R			R	Site D
210	Q			L		
216	N		T			
217	I		V			Site D
228	S		G			
259	K				R	
260	I			M		
261	Q			R		
262	S			I	G	
269	R			K		
273	H			L		
275	D		G	G		Site C
276	E		N	N		
278	N		E			Site C
289	P	S			S	
299	K		R	R		
304	A		P			
312	N		S	G		
323	V	I			I	
aadiff		9	43	38	13	

Reference CVV in red, HI swine strains in purple.

Table 22. Amino acid substitutions between representative swine 2010.1 strains compared to the within-clade CVV (DCDC-RG60A A/Ohio/13/2017).

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

Reference CVV in red, HI swine strains in purple.

Table 23. Amino acid substitutions between a representative 2010.2 strain compared to two human seasonal vaccines used in HI assays (A/Darwin/6/2021 and A/Darwin/9/2021 3C.2a1b.2a.2).

site	A/Darwin/6/2021	A/Darwin/9/2021	A/swine/Indiana/A02635878/2021	annotations
9	S		G	
31	N		D	
53	G	D	D	Site C
58	I		V	
62	G		E	
83	E		Q	
94	N		Y	
106	A		I	
131	K		T	
135	T		I	
138	A		S	
144	S		N	Site A
156	S		H	
159	N		Y	
160	I		K	
164	Q		L	
167	T		I	
171	K		N	
186	D	N	G	Site B
189	K		N	Site B
190	N		D	
192	I		T	
195	F		Y	
197	Q		R	
203	T		I	
225	D	G		
291	D		N	
aadiff		3	26	

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

Table 24. Amino acid substitutions between a representative swine 1970.1 strain compared to the putative ancestral human seasonal H3 strain (H3 A/Port Chalmers/1/1973); HI-tested human seasonal vaccines (A/Victoria/3/1975 and A/Sydney/5/1997) are also included.

cont.

site	A/Sydney/5/1997	A/Port_Chalmers/1/1973	A/Victoria/3/1975	A/swine/Italy/308955-3/2021	annotations
2	K	D	D	D	
3	I	F	L	F	
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	R	K	K	K	
53	D	N	N	K	Site C
54	S	N	N	N	Site C
62	E	I	I	A	
67	I			V	
69	A			S	
82	K	E	E	E	
83	E	T	K	K	
88	V			I	
92	K			R	
94	Y	F	F	F	
104	D			E	
106	A			T	
112	V			I	
117	T			S	
121	N	I	I	T	
124	S	G	G	N	
126	N	T			
131	A	T	T	T	
135	T	G	G	G	
137	Y	N	N	S	Site A
142	S	G	G	G	
143	S	P	P	P	Site A
144	I	D	D	N	Site A
145	K	S	S	N	Site A
146	S	G	G		Site A
155	H	Y	Y	Y	Site B

site	A/Sydney/5/1997	A/Port_Chalmers/1/1973	A/Victoria/3/1975	A/swine/Italy/308955-3/2021	annotations
156	Q	K	K	K	
157	L	S	S	S	
158	K	G	G	G	
159	Y	S	S	N	
160	K	A	T	T	
163	A	V	V	M	
164	L		Q		
171	N			S	
173	K	N	N	D	
174	F		S		
189	S	Q	K	R	Site B
190	D	E	E	E	
193	S	N	N	N	Site B
194	I	L	L	L	
196	A	V	V	I	
198	A			T	
201	R		K	K	Site D
202	V			I	
203	T			I	
213	V	I	I	I	
217	I		V		Site D
226	I	L	L	L	
233	H	Y	Y	Y	
242	I			T	
244	L	V	V	I	
248	T	N	N	N	
260	I	M	M		
261	R			Q	
262	S	T	T	I	
267	I			V	
269	R			K	
276	K	T	T	T	
278	N	I	S	D	Site C
299	R	K	K		
307	R	K	K	H	
309	V			I	
323	V			I	
aadiff		44	48	66	

Reference vaccines in gray, HI swine strain in purple.

Table 25. Amino acid substitutions between a representative swine Other-Human-2020 strain compared to most similar human seasonal H3 strain (A/Darwin/9/2021) used in the HI assays.

site	A/Darwin/9/2021	A/swine/North_Carolina/A02751333/2022	annotations
50	E	K	
79	F	V	
140	I	K	
156	S	H	
186	N	D	Site B
225	G	D	
aadiff		6	

Reference vaccine in red, HI swine strain in purple.

## Summary and Risk Assessment

### Global Variant Cases:

During the reporting period (January 1 – June 30, 2023), 2 variant cases were reported and included with the swine analyses:

- Brazil: H1N1v (1A.3.3.2)
- Taiwan: H1N2v (1A.1.4)

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

- China: 2x H1v (1 1C.2.3 – 1 no sequence data)
- USA: H1N2v (1B.2.1), H3v (no sequence data)
- Netherlands: H1N1v (1C.2.2)

### Europe Swine:

- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in European swine had variable cross-reactivity with human vaccine antisera and a trend for loss in cross-reactivity with more recent human vaccine strains.
- There are no within-clade CVVs for 1B lineages from Europe. The swine 1B.1.1 had significant loss in cross-reactivity with A/Chile/1/1983. The swine 1B.1.2.1 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 both the 1B.2.1 CVV and against the human seasonal A/Chile/1/1983 reference strain.
- The 1C.2.1 swine strain had  $\leq 4$ -fold loss in reactivity to the within clade CVV. The 1C.2.2 swine strains had  $\leq 4$ -fold to the within-clade CVV (A/Hessen/47/2020). The 1C.2.4 swine strains displayed  $> 8$ -fold reduction to the within clade CVV. The 1C.2.5 swine strain has no within-clade CVV and had limited cross-reactivity to other 1C CVVs.
- The representative swine H3.1970.1 demonstrated reactivity with A/Victoria/3/1975, 4-fold decrease to the 2010.1 CVV, and 8-fold to A/Sydney/5/97.

### North America Swine:

- 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 1A.2 swine strain was not significantly detected by any CVV and had a 4-fold drop in cross-reactivity to the A/Hawaii/70/2019-like 6B.1A.5a.1 vaccine but reacted with adult human sera. The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine retained cross-reactivity to A/Hawaii/70/2019-like 6B.1A.5a.1 vaccine strain and with adult human sera. The swine strains had  $> 8$ -fold decrease to the A/Sydney/5/2021 5a.2a and A/Victoria/4897/200 vaccine strains. 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.
- The contemporary swine 1B.2.1 (delta-2) had no loss of titer to the within-clade CVV A/Michigan/383/2018 but limited titers in adult human sera. The 1B.2.2.1 A/swine/Wyoming/A02525343/2021 had a  $> 8$ -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.



- 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 strains 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 >8-fold loss to human vaccine strains and had >8-fold loss in reactivity with adult human sera.

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

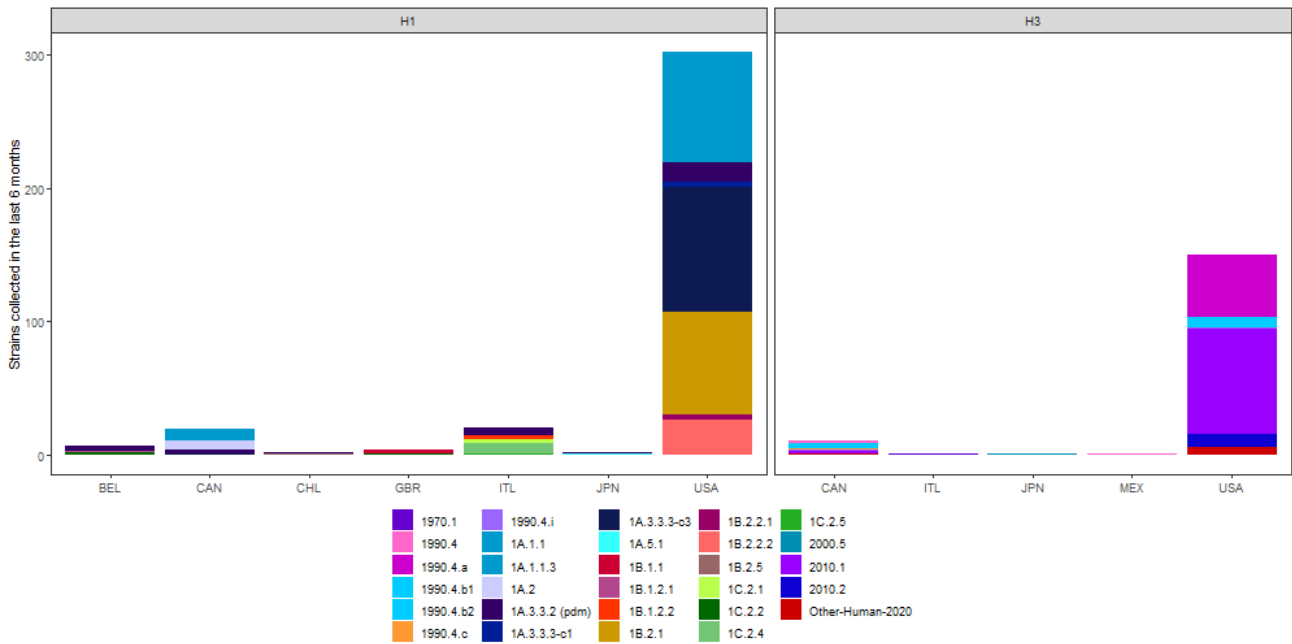


Figure A1. Summary of swine HA genes colored by phylogenetic clade for sequences deposited January 2023 – July 2023 and truncated to those collected within the last 6 months (n=534).

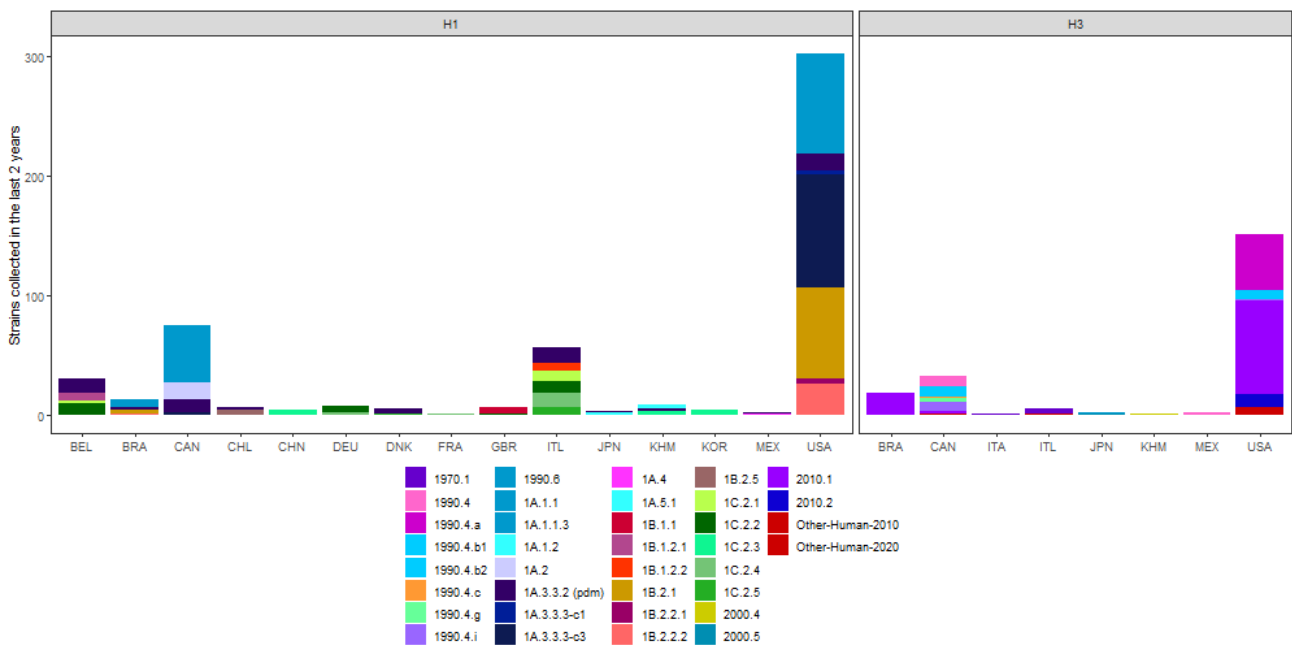


Figure A2. Summary of swine HA genes colored by phylogenetic clade for sequences deposited July 2021 – July 2023 and truncated to those collected within the last 24 months (n= 742).

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

<b>clade</b>	<b>country</b>	<b>count</b>
1A.3.3.2	BEL	4
1B.1.2.1	BEL	1
1C.2.2	BEL	2
1A.1.1	CAN	1
1A.1.1.3	CAN	7
1A.2	CAN	7
1A.3.3.2	CAN	3
1A.3.3.3-c3	CAN	1
1B.2.5	CHL	1
1B.1.1	GBR	3
1C.2.2	GBR	2
1A.3.3.2	ITA	5
1B.1.2.2	ITA	3
1C.2.1	ITA	3
1C.2.4	ITA	8
1C.2.5	ITA	1
1A.3.3.2	JPN	1
1A.5.1	JPN	1
1A.1.1.3	USA	83
1A.3.3.2	USA	14
1A.3.3.3-c1	USA	4
1A.3.3.3-c3	USA	94
1B.2.1	USA	75
1B.2.2.1	USA	4
1B.2.2.2	USA	26

Table A1b. Summary of swine H1 clades by country collected in the past 24 months and sequences deposited July 2021 – June 2023.

<b>clade</b>	<b>country</b>	<b>count</b>
1A.3.3.2	BEL	12
1B.1.2.1	BEL	6
1C.2.1	BEL	2
1C.2.2	BEL	10
1A.1.1	CAN	1
1A.1.1.3	CAN	47
1A.2	CAN	14
1A.3.3.2	CAN	11
1A.3.3.3-c3	CAN	2
1A.3.3.2	CHL	1
1B.2.5	CHL	4
1C.2.3	CHN	5
1C.2.2	DEU	6
1C.2.4	DEU	5
1A.3.3.2	DNK	4
1C.2.5	DNK	1
1C.2.4	FRA	1
1B.1.1	GBR	6
1C.2.2	GBR	2
1A.3.3.2	ITA	13
1B.1.2.2	ITA	8
1C.2.1	ITA	9
1C.2.2	ITA	10
1C.2.4	ITA	15
1C.2.5	ITA	7
1A.3.3.2	JPN	1
1A.5.1	JPN	2
1A.1.2	KHM	4
1A.3.3.2	KHM	2
1C.2.3	KHM	3
1C.2.3	KOR	4
1A.3.3.2	MEX	1
1A.4	MEX	1
1A.1.1.3	USA	90
1A.3.3.2	USA	17
1A.3.3.3-c1	USA	4
1A.3.3.3-c3	USA	94
1B.2.1	USA	78
1B.2.2.1	USA	4
1B.2.2.2	USA	27

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

<b>clade</b>	<b>country</b>	<b>count</b>
1990.4	CAN	2
1990.4.b2	CAN	4
1990.4.c	CAN	1
1990.4.i	CAN	1
2010.1	CAN	1
Other-Human-2020	CAN	1
1970.1	ITA	1
2000.5	JPN	1
1990.4	MEX	1
1990.4.a	USA	42
1990.4.b1	USA	6
1990.4.b2	USA	1
1990.4.i	USA	1
2010.1	USA	79
2010.2	USA	10
Other-Human-2020	USA	6

Table A2b. Summary of swine H3 clades by country collected in the past 24 months and sequences deposited July 2021 – June 2023.

<b>clade</b>	<b>country</b>	<b>count</b>
1990.4	CAN	9
1990.4.b2	CAN	9
1990.4.c	CAN	1
1990.4.g	CAN	3
1990.4.i	CAN	8
2010.1	CAN	1
Other-Human-2020	CAN	1
1970.1	ITA	5
Other-Human-2010	ITA	1
2000.5	JPN	2
2000.4	KHM	1
1990.4	MEX	2
1990.4.a	USA	43
1990.4.b1	USA	6
1990.4.b2	USA	1
1990.4.i	USA	1
2010.1	USA	97
2010.2	USA	10
Other-Human-2020	USA	7

## Annex 2. Phylogenies of swine HA lineages with tree backbone annotated by inferred amino acid mutations.



Figure A3. A phylogeny of the **1A.3.3.3-c3** clade displaying sequences deposited January 1, 2023 - June 30, 2023 and collected in 2023 (n=95) 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.

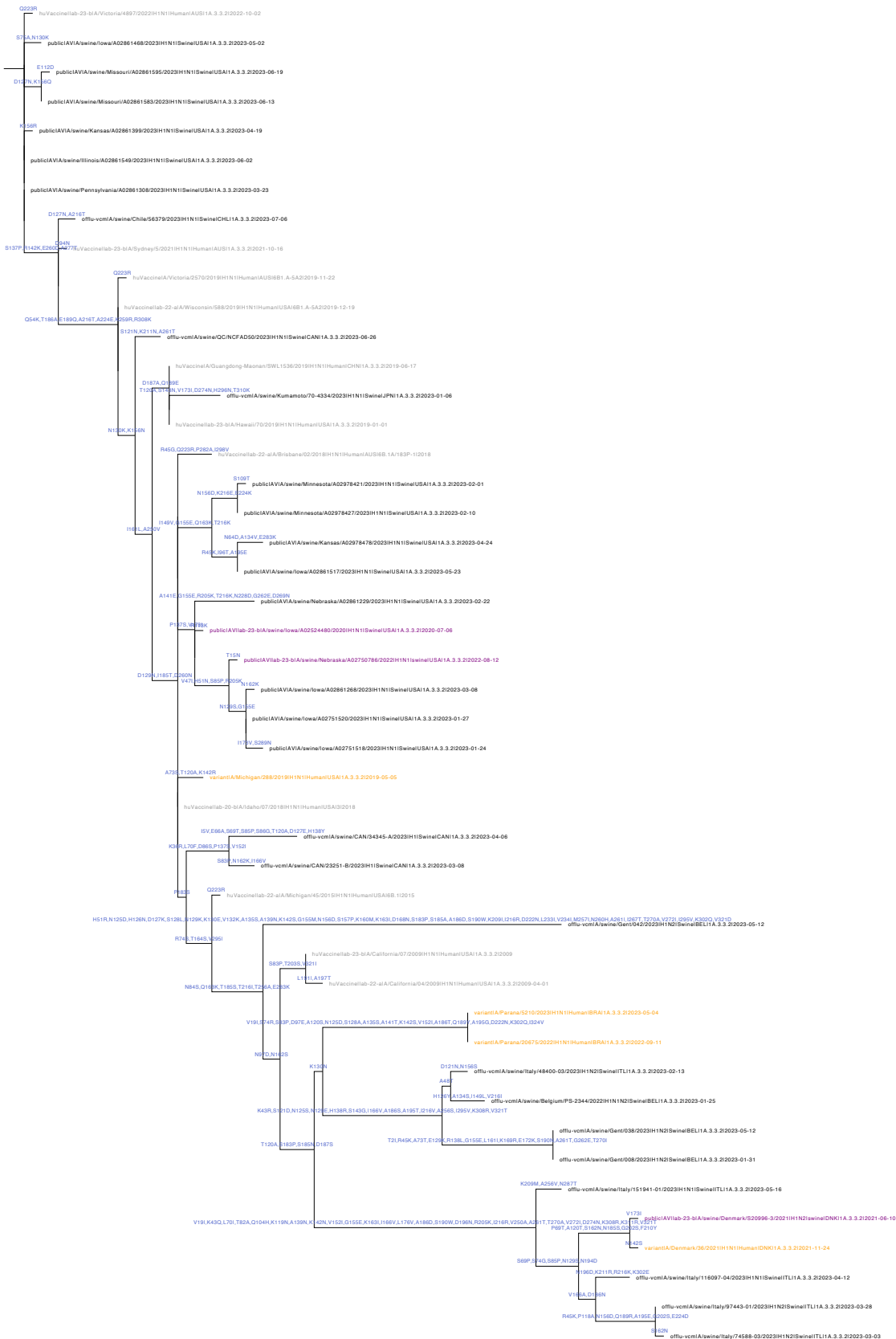


Figure A4. A phylogeny of the **1A.3.3.2 (pdm)** clade displaying n=28 sequences deposited January 1, 2023 - June 30, 2023 and collected in 2023, and n=18 reference genes. The clade is rooted relative to the most recent human vaccine strain (A/Victoria/4897/2022) and all branches are annotated with the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.



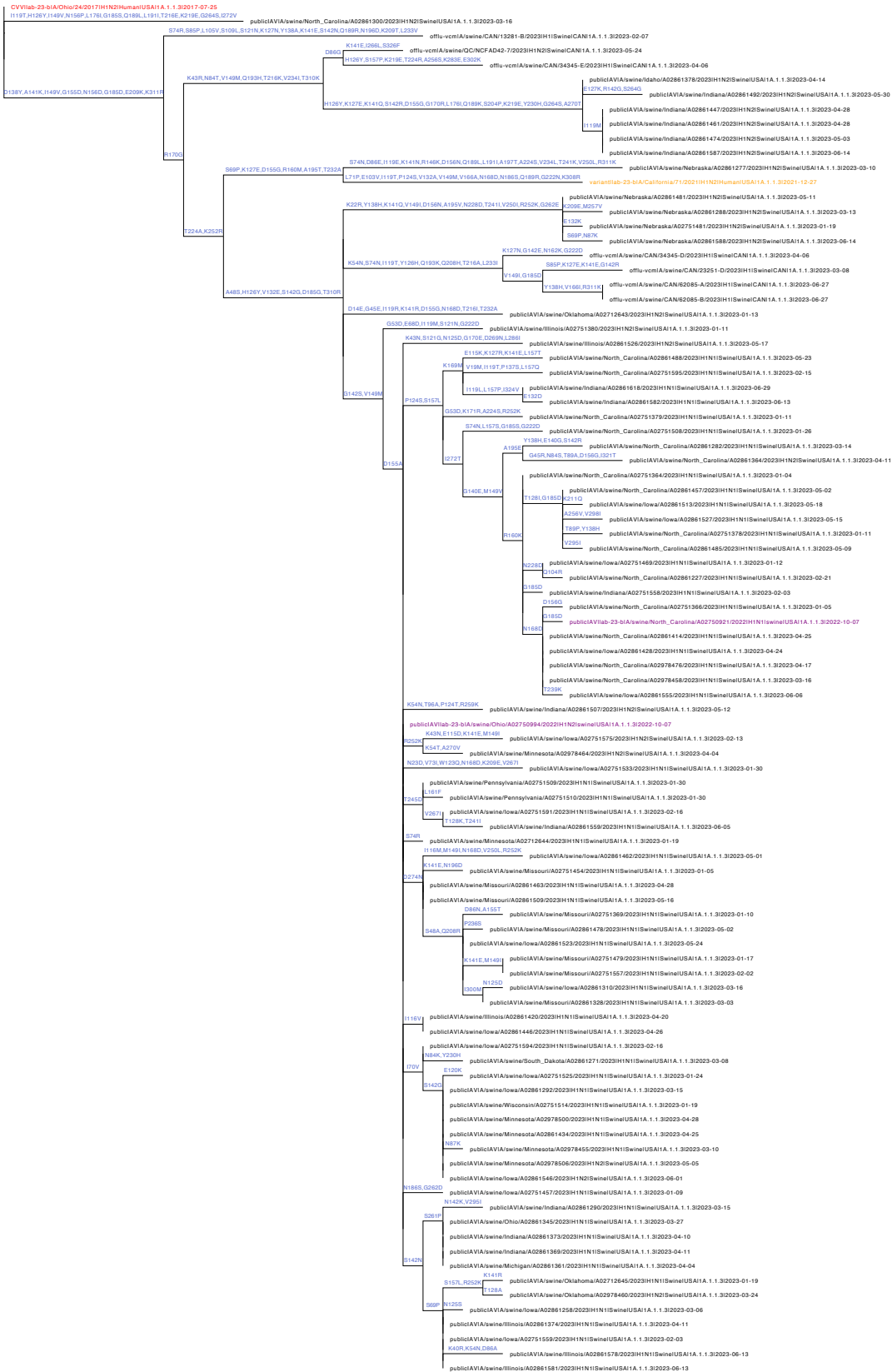


Figure A5. A phylogeny of the **1A.1.1.3** clade displaying n=90 sequences deposited January 1, 2023 - June 30, 2023 and collected in 2023, and n=4 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Ohio/24/2017) and all branches are annotated with the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 7.

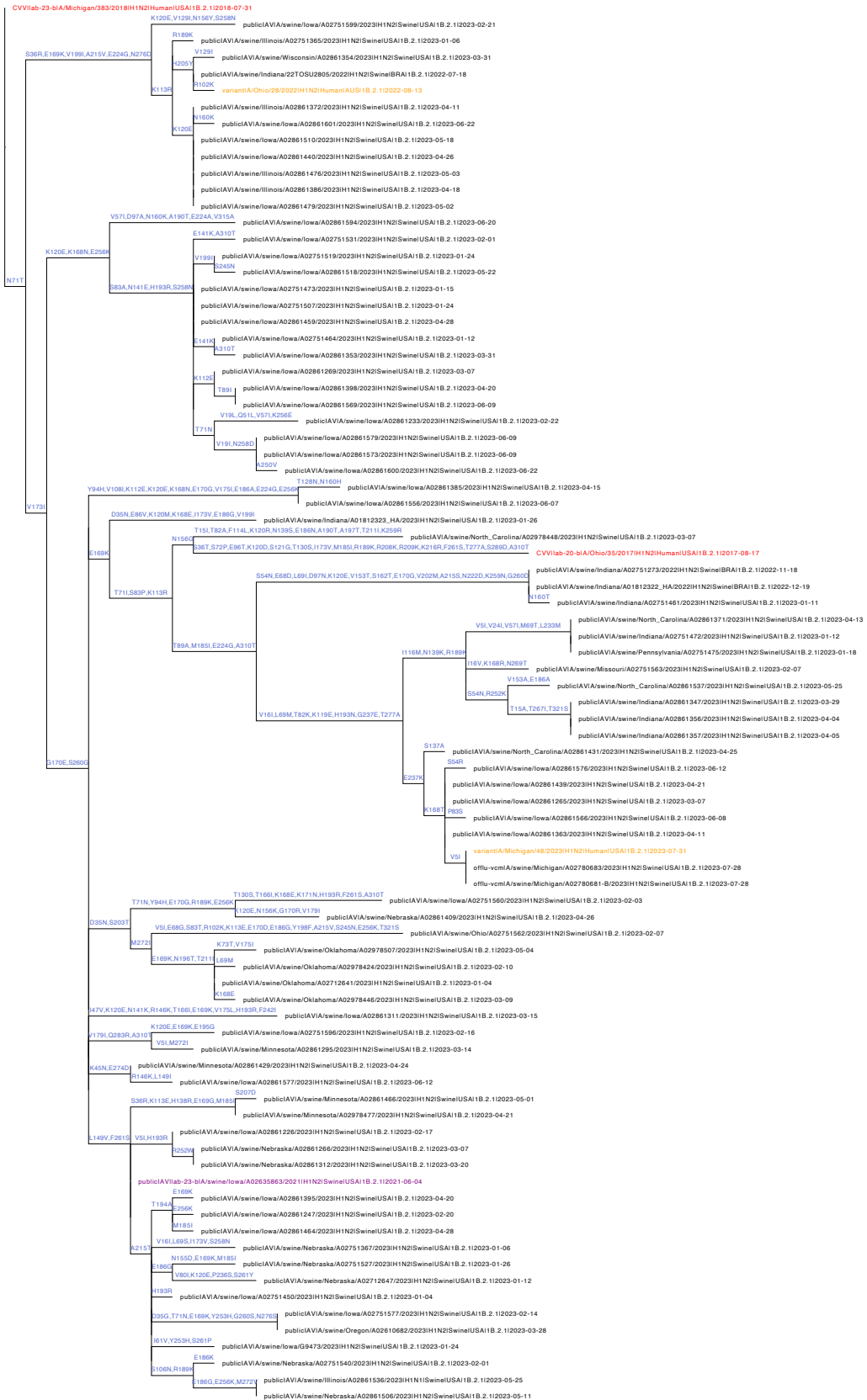


Figure A6. A phylogeny of the **1B.2.1** clade displaying n=80 sequences deposited January 1, 2023 - June 30, 2023 and collected in 2022-2023, and n=5 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Michigan/383/2018) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 8.

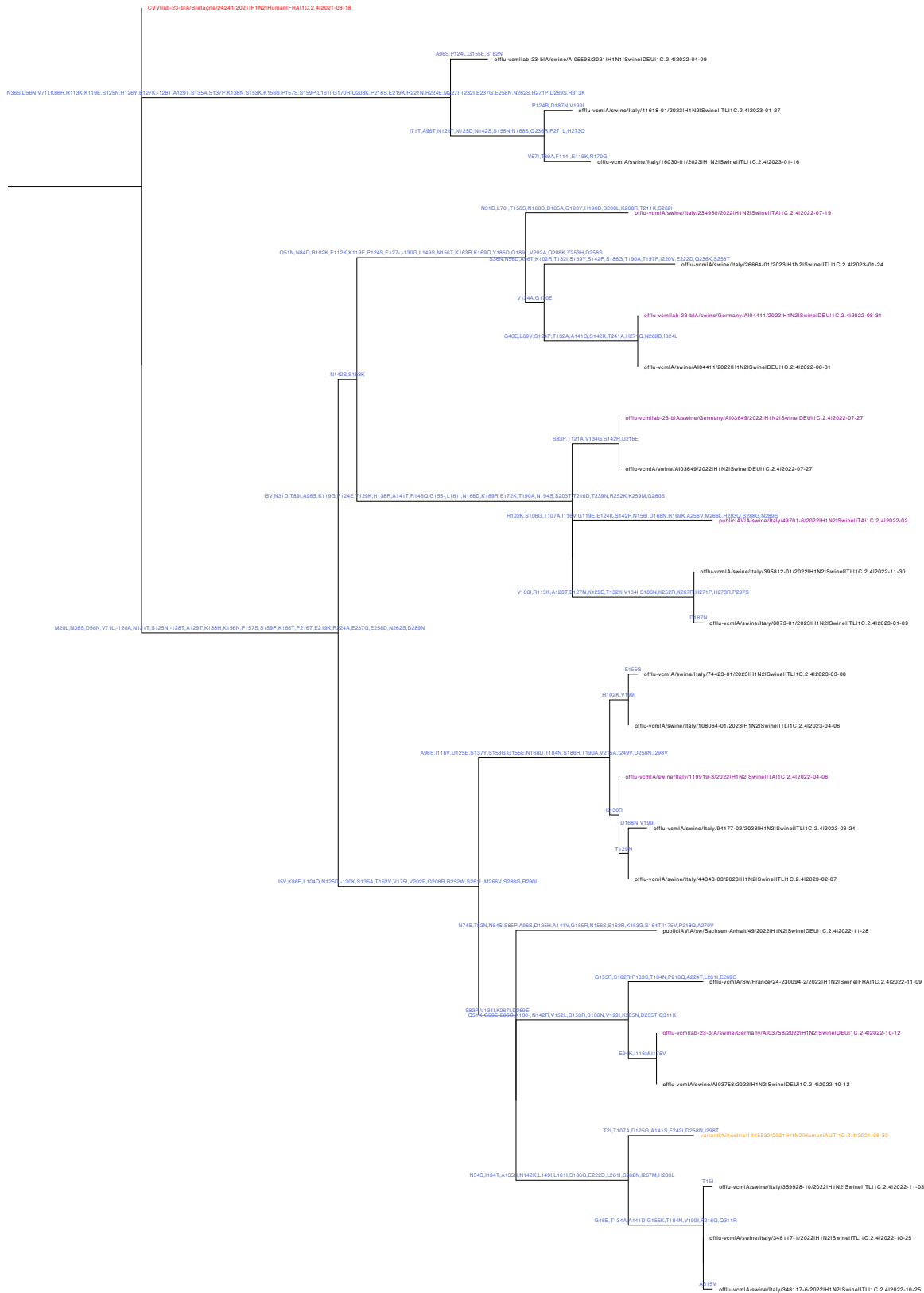


Figure A7. A phylogeny of the **1C.2.4** clade displaying n=24 sequences deposited January 1, 2023 - June 30, 2023 and collected in 2022-2023, and n=2 reference genes. The clade is rooted relative to the closest CVV (A/Britagne/24241/2021) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.

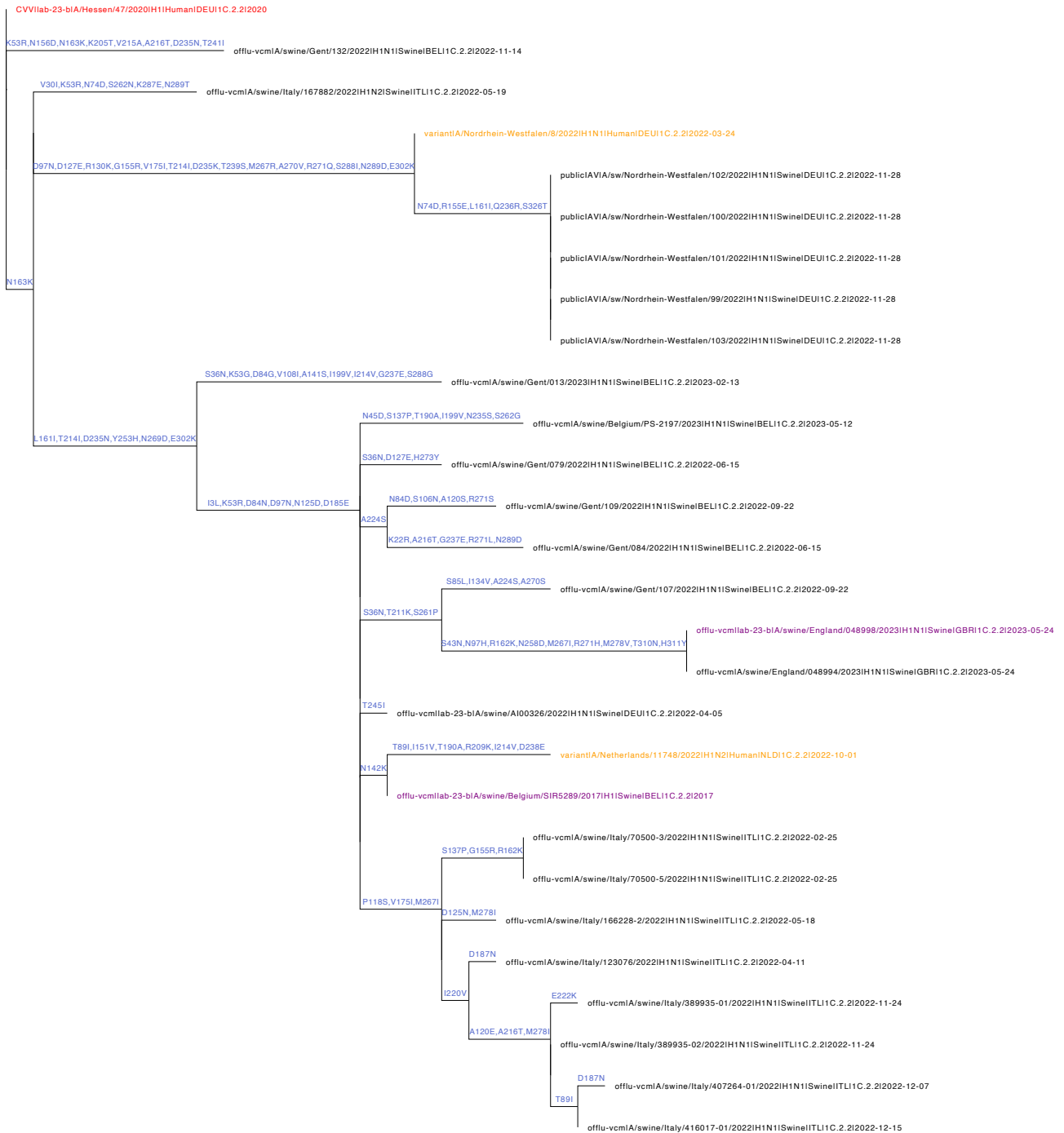


Figure A8. A phylogeny of the **1C.2.2** clade displaying n=24 sequences deposited January 1, 2023 - June 30, 2023 and collected in 2022-2023, and n=4 reference genes. The clade is rooted relative to the closest CVV (A/Hessen/47/2020) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 9.

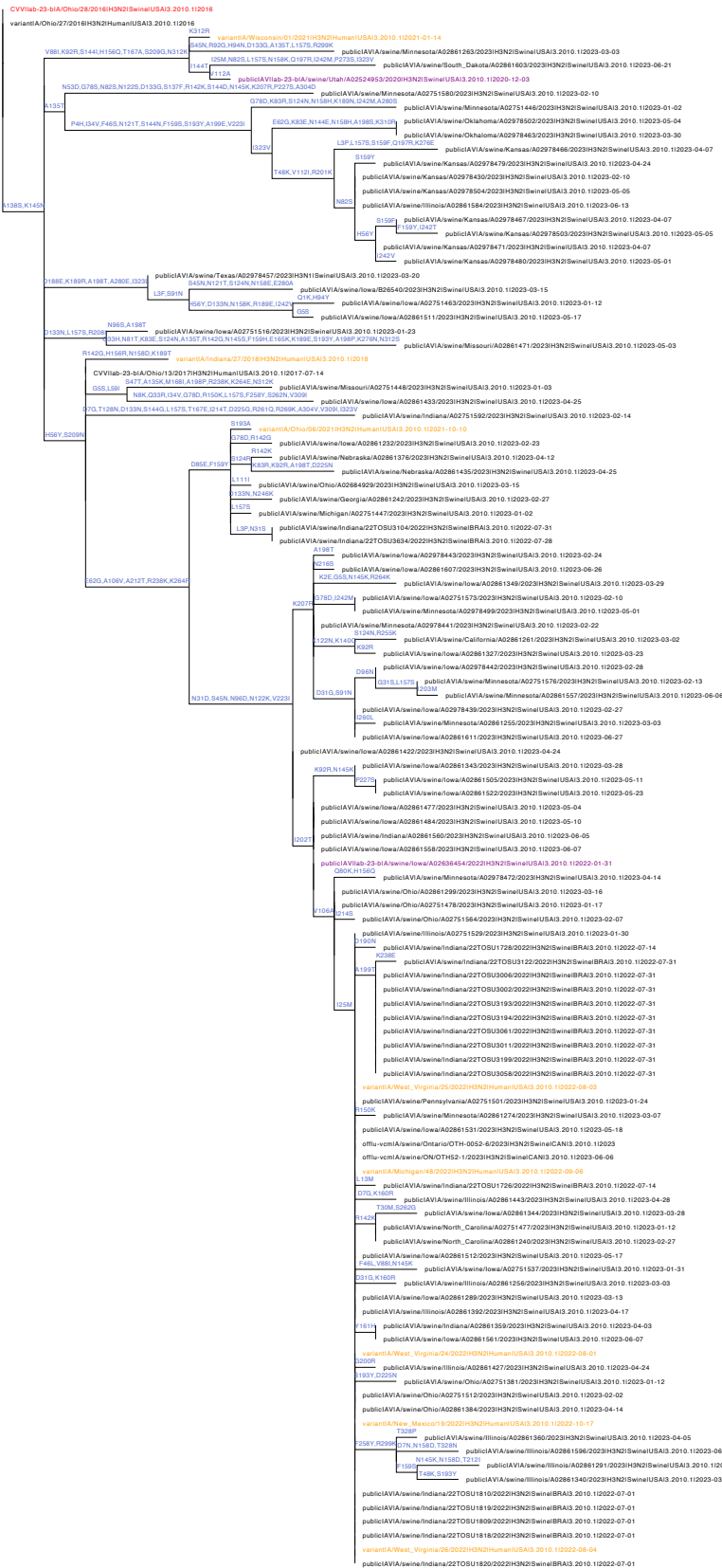


Figure 9. A phylogeny of the **3.2010.1** clade displaying n=100 sequences deposited January 1, 2023 - June 30, 2023 and collected in 2022-2023, and n=12 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Ohio/28/2016) and all branches are annotated with the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 10.

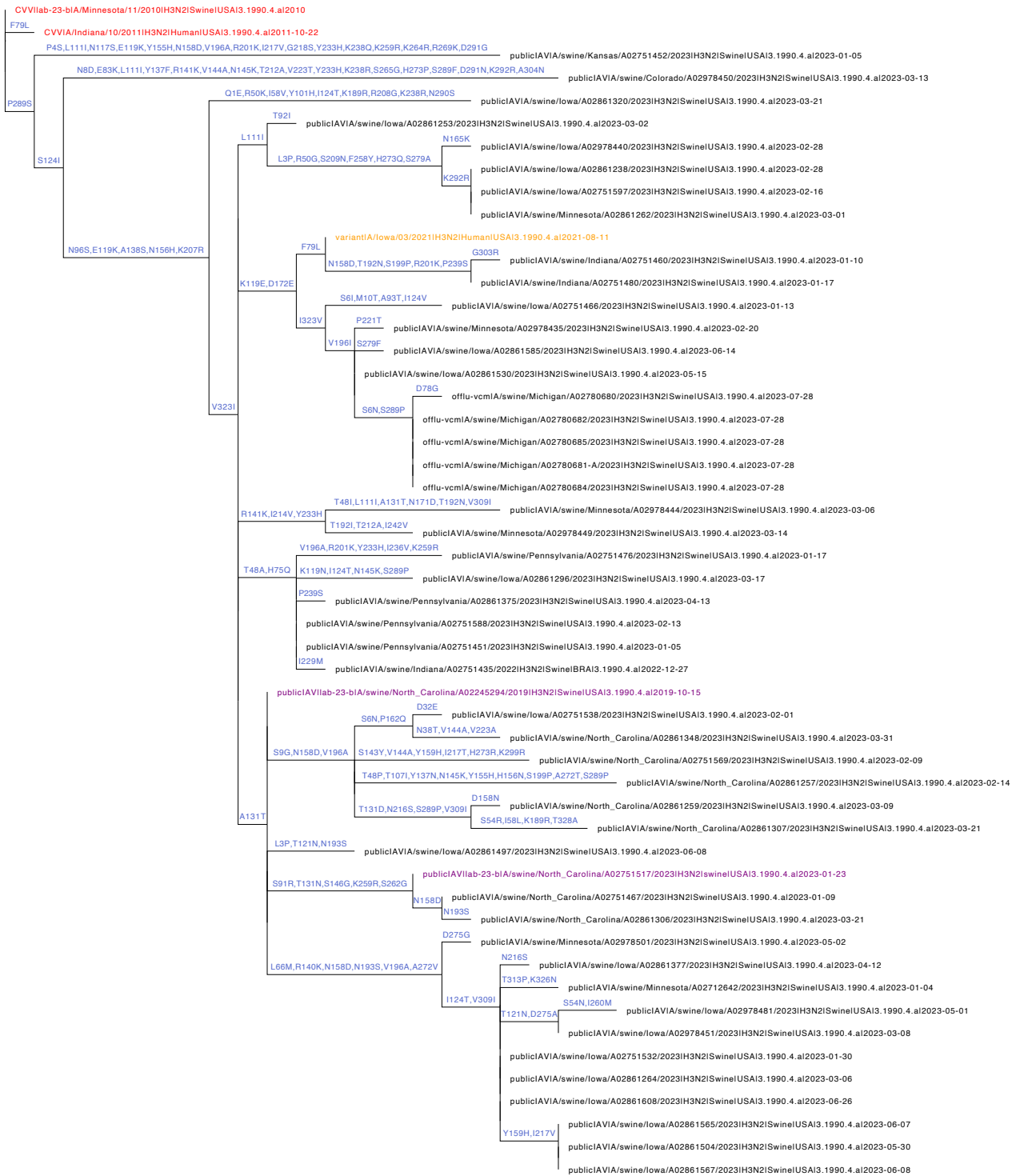


Figure A10. A phylogeny of the **3.1990.4.a** clade displaying n=48 swine sequences deposited January 1, 2023 - June 30, 2023 and collected in 2022-2023, and n=4 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Minnesota/11/2010) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored to be consistent with Figure 10.