OFFLU SWINE INFLUENZA REPORT

JANUARY 2021 TO JUNE 2021

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 Jan 1, 2021 – June 30, 2021) were downloaded from GISAID or GenBank were 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. 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). An HA1 consensus sequence was generated for each contemporary clade and the best matched 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

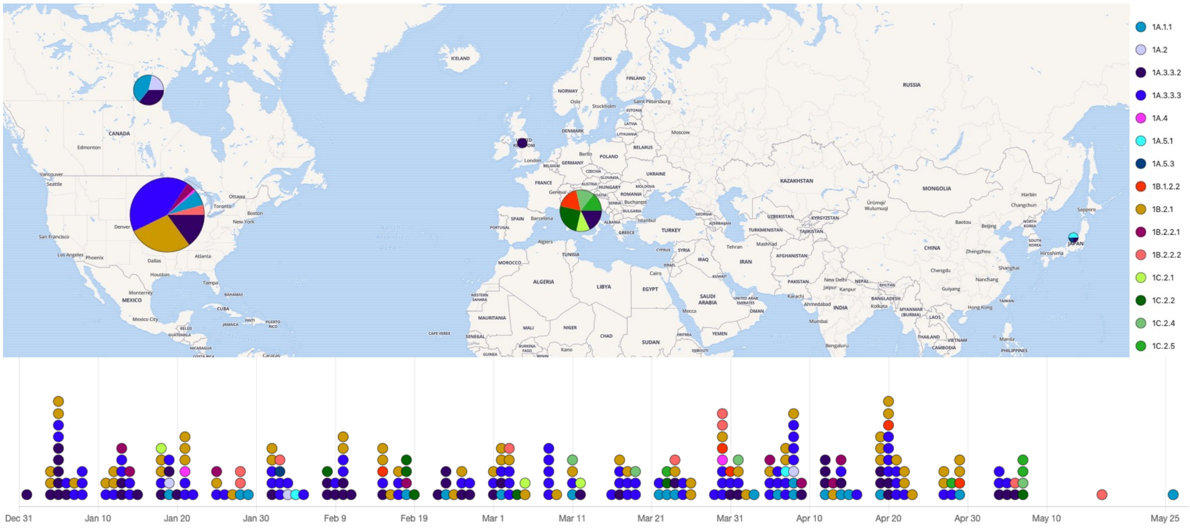


Figure 1. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected January 1 2021 – June 30 2021 (n=258); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters.

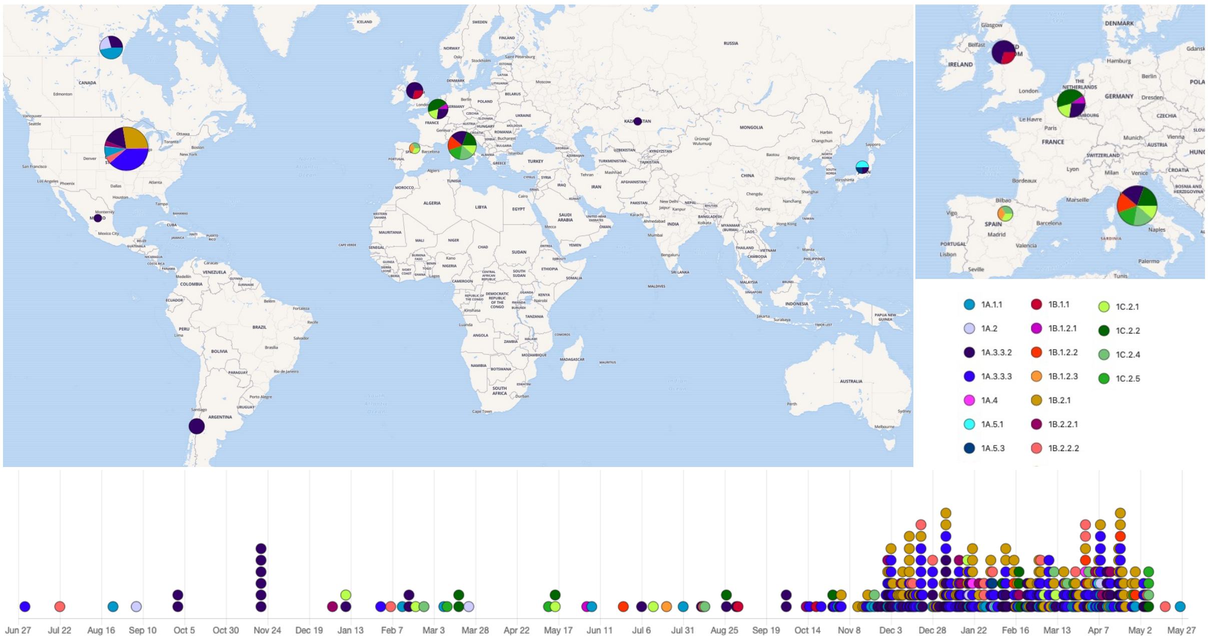


Figure 2. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences deposited to GISAID or GenBank between January 1, 2021 – June 30, 2021 (n=651), data were subsequently filtered to display sequences that were collected between July 1, 2019 – June 30, 2021 (n=373). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. For visualization, the insert demonstrates data in Europe. Geographic nodes scaled to detection proportions.

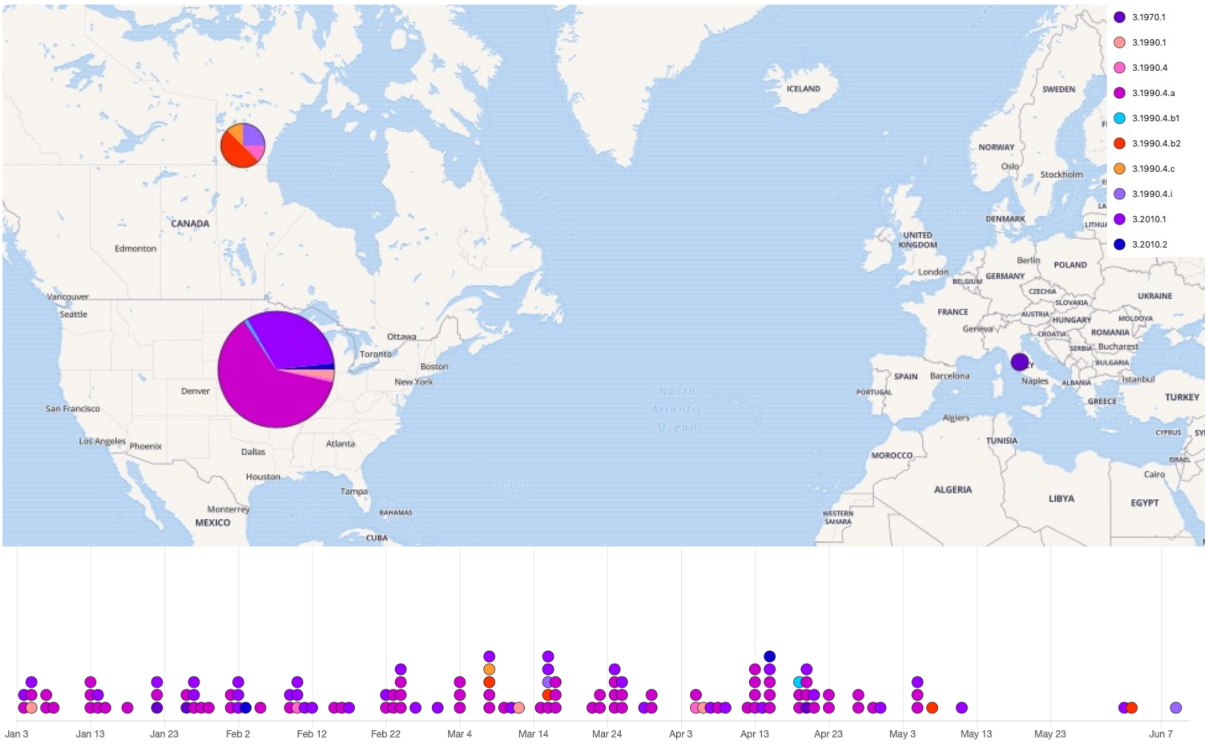


Figure 3. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected January 1, 2021 – June 30, 2021 (n=125); collection date is represented along bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters.

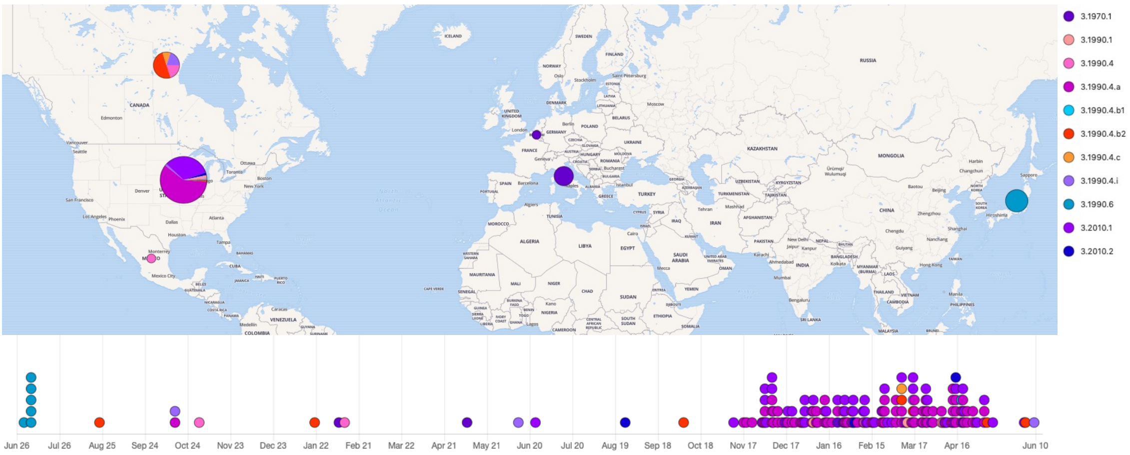


Figure 4. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences deposited to GISAID or GenBank between January 1, 2021– June 30, 2021 (n=179), data were subsequently filtered to display sequences that were collected between July 1, 2019 – June 30, 2021 (n=161). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions.

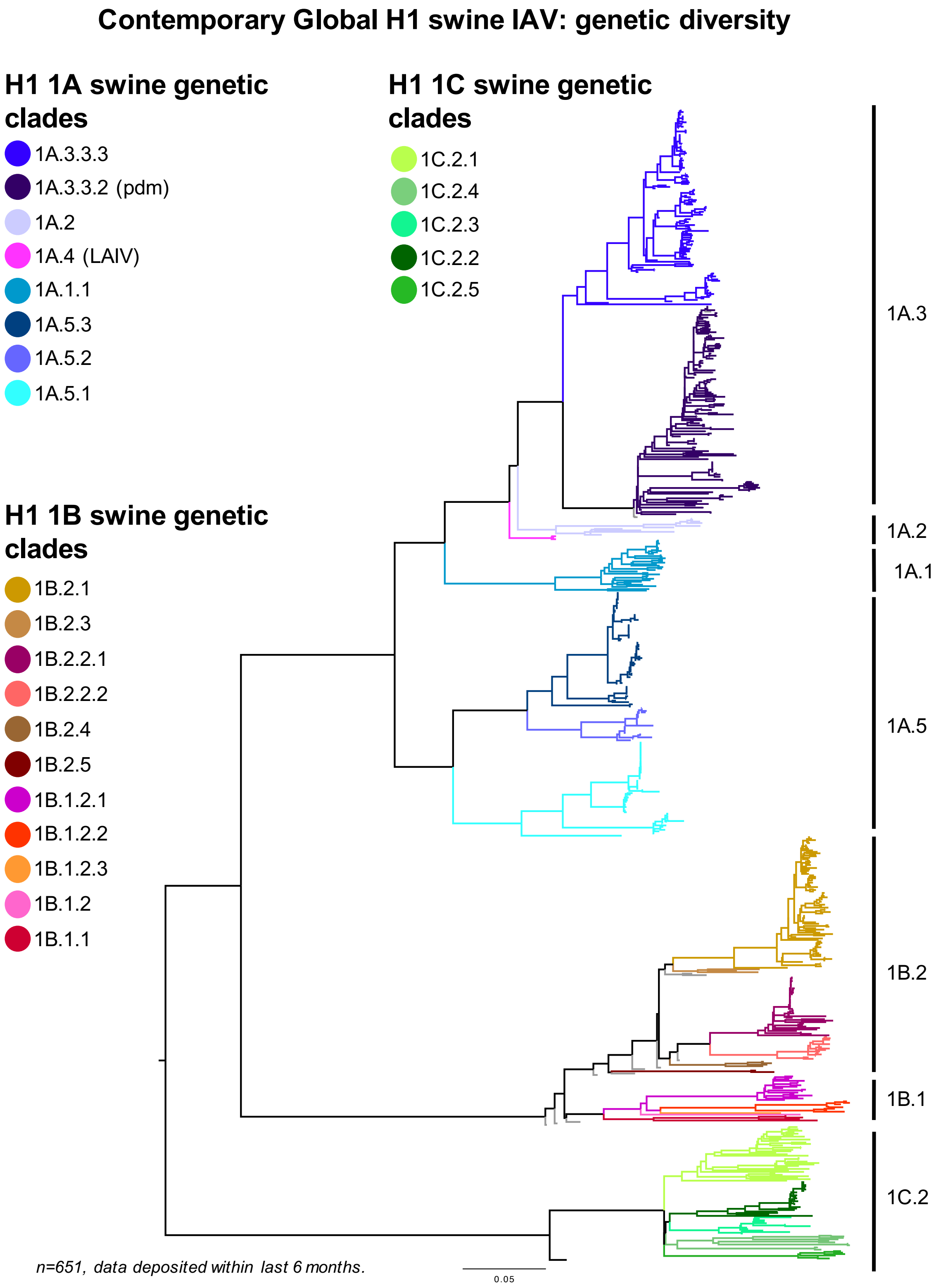
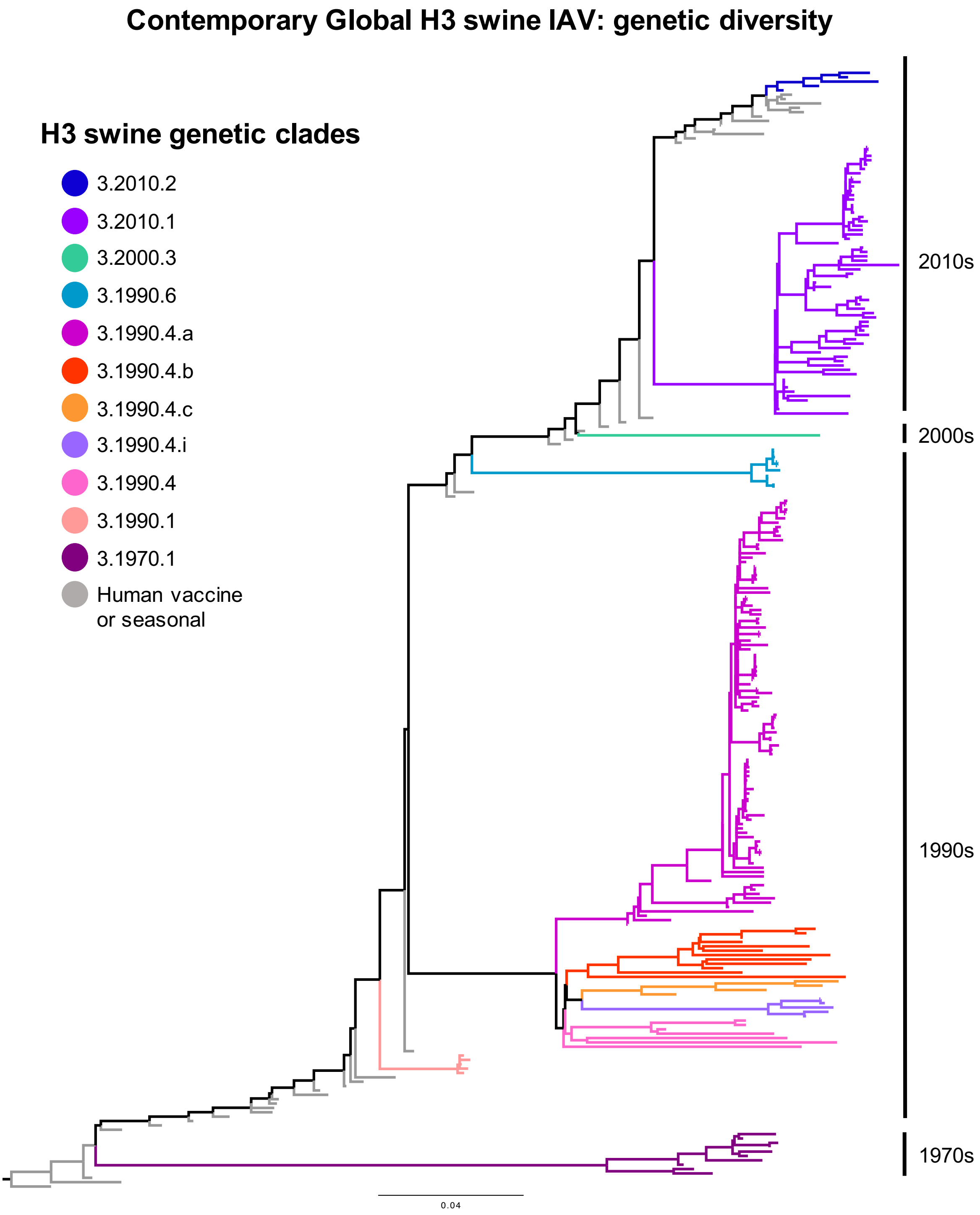


Figure 5. Global swine H1 phylogenetic tree colored by clade and annotated by global H1 lineage nomenclature. Analyses were conducted with reference sequences and new data (deposited January 1, 2021 – June 30, 2021) from GISAID or shared via the OFFLU swine IAV working group.



n=179, data deposited within last 6 months, and n=77 reference genes.

Figure 6. Global swine H3 phylogenetic tree colored by clade and annotated by decade of introduction from human seasonal H3. Analyses were conducted with reference sequences and new data (deposited January 1, 2021 – June 30, 2021) to GISAID or shared via the OFFLU swine IAV working group.

Regional geographic summary

Phylogenetic analysis identified twenty-nine genetic clades from H1 and H3 IAV in swine reported January 1, 2021 – June 30, 2021. Eighteen clades were H1 subtype, with detections from each of the lineages: 1A classical swine lineage (n=8); 1B human-seasonal lineage (n=6); and 1C Eurasian avian lineage (n=4). Eleven clades were within six distinct lineages derived from human seasonal H3 virus spillovers: these lineages are grouped by the decade of introduction into swine (1970.1; 1990.1, 1990.4, and 1990.6; 2010.1; 2010.2). The 1990.4 lineage diversified into six cocirculating genetic clades.

- The 1A classical swine lineage viruses have global detection: 1A.1.1 in USA and Canada; 1A.2 in Canada; 1A.4 in USA; 1A.3.3.3 in USA; and 1A.5 HA genes in Japan. The 1A.3.3.2/pdm circulated in all countries that submitted sequence data during this period.
- 1B.1 human seasonal lineage was only in Europe, and the 1B.2 human seasonal lineage was only in the USA.
- The 1C Eurasian avian lineage was in Europe and Asia. Recent 1C diversification warranted the naming of two additional genetic clades: 1C.2.4 detected in Italy, France, and Russia; and 1C.2.5 detected in Italy. The 1C.2.1 and 1C.2.2 clades were throughout Europe (Belgium, Italy, France, Germany, Netherlands). There were no new sequence data reported from the HA clade 1C.2.3 in China.
- Data from Japan includes two newly identified lineages with four novel genetic clades. 1A.5: a classical swine lineage clade that diversified into three cocirculating genetic clades. 1990.6: a human-to-swine H3 transmission in the 1990s.
- The H3 1990.1, 2010.1, and 2010.2 clades only detected in the USA, the H3 1970.1 clade in Europe, the 1990.6 lineage in Japan. The 1990.4 lineage circulates in the USA, Canada, and Mexico.
- Genetic and antigenic characterization in this report period include unique global H1 and H3 HA genes from under-surveilled areas outside the report window: H1 1A.2 (Canada); 1A.3.3.3 (South Korea); 1B.2.3 (Brazil); H1 1B.2.4 (Brazil); 1B.2.5 (Chile); 1C.2.3 (South Korea). H3 1990.4a (South Korea), 1990.4 (Canada), 1990.4.b2 (Canada), 1990.4.c (Canada).

During this reporting period (January 1 2021 to June 30 2021), variant cases included with the swine analyses were reported in:

- **USA: H3N2v (1 2010.1; 1 1990.4a); H1N1v (1 1A.3.3.2, 2 1A.3.3.3); H1N2v (1 no sequence)**
- **Canada: H3N2v (3.1990.4.i); H1N1v (1A.3.3.2); H1N2v (1A.1.1)**
- **Denmark: H1N1v (1A.3.3.2)**
- **Germany: H1N1v (1C.2.1)**
- **China: H1N1v (4 1C.2.3)**
- **Australia: H3N2v (1990-like)**

***Additional variant cases were reported after this reporting period, and will be included in subsequent reports. Analyses with available data are summarized in Annex 2.**

***New swine data combined with variant detections require updates to the swine H1 clade nomenclature and automated tool on fludb.org. The updates are in progress.**

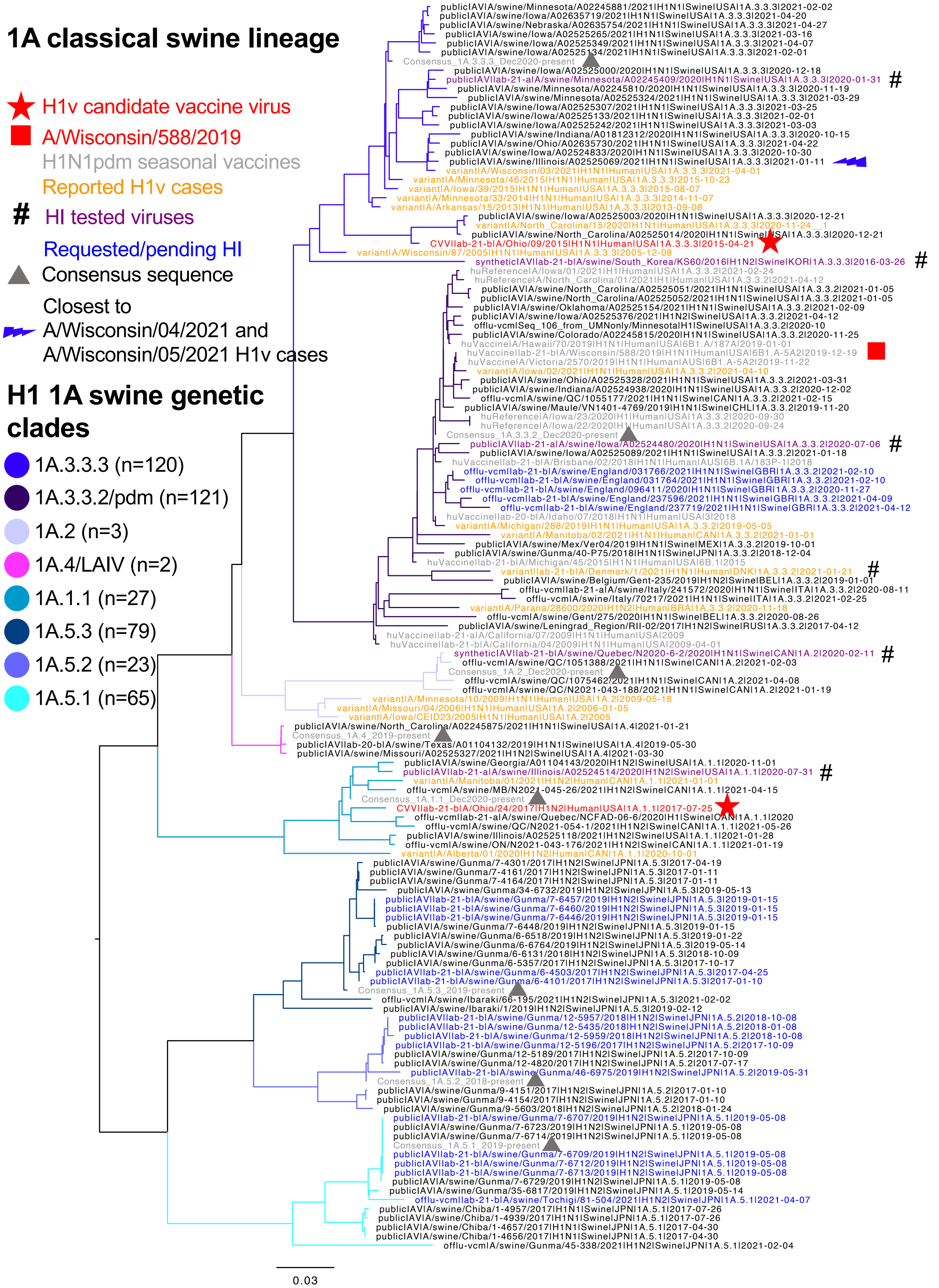


Figure 7. Swine H1 HA genes of the 1A lineage (tree was proportionally down sampled to 98 swine HA genes from 465): detections of each clade from data deposited between January 1 to June 30 2021 are presented adjacent to the clade name.

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/Wisconsin/588/2019**	**A/Ohio/9/2015 CVVRG48A**
IDCDC-RG59 A/Ohio/24/2017-like CVV	1A.1.1	alpha-del H1N2v	640	<10	10
A/swine/Illinois/A02524514/2020	1A.1.1	alpha-del H1N2	<10	<10	<10
rg-A/swine/Quebec/N2020-6-2/2020*	1A.2	beta H1N1	10	<10	20
A/Wisconsin/588/2019 6B1.A-5A2	1A.3.3.2	H1N1 pdm09	<10	1280	20
A/swine/Iowa/A02524480/2020	1A.3.3.2	H1N1 pdm09	20	80	<10
A/Ohio/9/2015 CVV	1A.3.3.3	gamma.1 H1N1v	<10	10	320
A/swine/Minnesota/A02245409/2020	1A.3.3.3	gamma.3 H1N1	<10	40	40
rg-A/swine/South Korea/KS60/2016*	1A.3.3.3	gamma H1N2	10	10	10

Reference CVV in red, seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted in grey. *synthetic HA/NA on PR8 backbone.

- The contemporary swine 1A.1.1 (alpha-del) was not detected by the 1A.1.1 CVV A/Ohio/24/2017 ferret anti-sera.
- The contemporary Canada 1A.2 (beta) does not have a CVV contained within clade and demonstrated limited reactivity to any CVV or vaccine anti-sera.
- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine had significant 16-fold decrease in cross-reactivity to the A/Wisconsin/588/2019 6B1.A-5A2 vaccine strain.
- The contemporary swine 1A.3.3.3-clade 3 virus had an 8-fold decrease and a 1A.3.3.3 from South Korea had a 32-fold decrease in cross-reactivity with the 1A.3.3.3-clade 1 CVV A/Ohio/09/2015.

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	A/Brisbane/02/2018	A/California/7/2009	A/Michigan/45/2015
A/Brisbane/02/2018	1A.3.3.2	320	320	160
A/California/4/2009	1A.3.3.2	160	320	80
A/Michigan/45/2015	1A.3.3.2	80	80	80
A/Denmark/1/2021	1A 3.3.2	40	80	40

Reference seasonal vaccine strains in dark red, variant in orange, new swine strains in bold. Homologous titers highlighted gray. ND=Not Done.

- The swine variant 1A.3.3.2 strain demonstrate reduced cross-reactivity to A/Brisbane/02/2018 and A/California/7/2009 and good cross-reactivity to A/Michigan/45/2015.

Table 3. Amino acid substitutions between recent swine 1A.1.1 lineage strains compared to the nearest CVV (A/Ohio/24/2017|CVV|1A.1.1).

site	A/Ohio/24/2017	**A/swine/Illinois/A02524514/2020**	Annotations
48	A	S	
132	V	E	RBS
138	D	Y	
141	A	Q	
149	I	M	
155	G	D	Sa
156	N	D	Sb
170	R	G	Ca2
209	E	K	
224	T	A	RBS
252	K	R	
310	T	R	
311	K	R	
aadiff		**13**	

***Reference CVV in red, swine strains in black**

Table 4. Amino acid substitutions between 1A.2 compared to the nearest human H1 vaccine (A/Wisconsin/588/2019) in HI assays and the most similar vaccine (A/California/07/2009).

Relative to HI

site	A/Wisconsin/588/2019	A/swine/Quebec/N2020-6-	Annotations
35	D	N	
61	I	L	
71	S	F	
73	A	R	Cb
83	S	P	
85	S	P	
104	Q	H	
109	S	L	
120	T	A	
121	S	N	
128	S	T	
129	D	N	
130	N	K	
137	P	S	
138	H	Y	
142	K	N	
156	K	N	Sb
161	I	L	
162	N	S	Sa
163	Q	K	Sa
164	T	S	Sa
168	D	N	
170	G	K	Ca2
176	L	I	
185	I	S	

Relative to most similar vaccine

site	A/California/07/2009	A/swine/Quebec/N2020-6-	Annotations
35	D	N	
61	I	L	
71	S	F	
73	A	R	Cb
74	S	R	
84	S	N	
85	S	P	
97	D	N	
104	Q	H	
109	S	L	
120	T	A	
121	S	N	
128	S	T	
137	P	S	
138	H	Y	
142	K	N	
168	D	N	
170	G	K	Ca2
176	L	I	
183	S	P	
186	A	T	
190	S	T	Sb, RBS
195	A	E	Sb, RBS
205	R	K	
216	I	A	

186	A	T	
190	S	T	Sb, RBS
195	A	E	Sb, RBS
203	T	S	
205	R	K	
216	T	A	
224	E	A	RBS
234	V	I	
235	E	D	
239	K	T	
250	A	V	
256	T	A	
260	D	G	
261	A	S	
270	T	K	
273	H	Q	
274	D	N	
276	N	T	
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		**51**	

224	E	A	RBS
234	V	I	
235	E	D	
239	K	T	
260	N	G	
261	A	S	
270	T	K	
273	H	Q	
274	D	N	
276	N	T	
285	A	S	
298	I	V	
302	K	E	
311	K	N	
314	L	M	
aadiff		**40**	

***Reference vaccine strain in gray, swine strains in black**

Table 5. USA: Amino acid substitutions between recent swine 1A.3.3.2 lineage strains compared to the prior human seasonal H1 vaccine strain (A/Wisconsin/588/2019) used in HI assays.

site	**A/Wisconsin/588/2019**	A/swine/England/096411/2020	A/swine/England/031766/2021	A/swine/England/237596/2021	A/swine/Iowa/A02524480/2020	**A/Brisbane/02/2018**	Annotations
19	V			I			
45	R					G	
54	K			N			
69	S		L				Cb
113	R				K		
129	D	N	N	N	N	N	
130	N	K	K	K	K	K	
137	P				S		
156	K	N	N	N	N	N	Sb
161	I	L	L	L	L	L	
168	D		N				
173	V				I		
185	I	T	T	T	T	T	
216	T	E	E				
223	Q					R	RBS
233	L	I	I	I			
250	A	V	V	V	V	V	
260	D	N	N	N	N	N	
278	T		I				
282	P					A	
298	I					V	
313	R			K			
aadiff		**9**	**12**	**11**	**10**	**11**	

***Reference human seasonal vaccine in gray, swine strains in black**

Table 6. Amino acid substitutions between recent swine 1A.3.3.3 and 1A.3.3.3-c3 lineage strain compared to the nearest CVV (A/Ohio/09/2015|CVV|1A.3.3.3).

site	**A/Ohio/09/2015**	A/swine/South_Korea/KS60/2016	A/swine/Minnesota/A02245409/2020	Annotations
2	K	T	T	
3	I	L	L	
35	N	D		
36	K	R	R	
38	N	D		
40	K	R		
48	X	A	A	
71	A	S	S	
83	S		P	
84	S	N	N	
86	N		E	
113	K	R	R	
119	K	N		
120	T	A	A	
127	E	T	D	
129	N	D		
130	K		R	
137	P		S	
141	T	K		
142	N	P		
146	K		R	
149	I		V	
153	K		Q	Sb
155	E	R	G	Sa
159	P	Q		Sa
161	I		V	
162	N	S		Sa

163	I	K	K	Sa
166	T		I	Ca1
169	R	K		
170	G		E	Ca2
178	A	G		
183	P	S	S	
184	T	N		
186	T	D	A	
189	Q	R		Sb, RBS
190	S	V		Sb, RBS
193	Q		K	Sb, RBS
195	A	E		Sb, RBS
196	N		D	
197	S	A	A	
205	R		K	
208	R	K		
211	E	T		
222	G		D	RBS
224	A	E		RBS
250	V	A	A	
269	E		D	
270	T	A		
271	P	S	S	
278	T	N		
283	N	K		
302	E	K		
321	I	T		
aadiff		**39**	**31**	

***Reference CVV in red, swine strains in black**

Table 7. Amino acid substitutions between recent swine 1A.5.1 lineage strain and the most similar vaccine (A/California/04/2009).

site	**A/California/04/2009**	A/swine/Tochigi/81-504/2021	A/swine/Gunma/7-6707/2019	**A/Michigan/45/2015**	**A/Brisbane/02/2018**	Annotations
2	T	I	I			
19	V	I	I			
36	K	T	T			
39	G	E	E			
45	R				G	
47	V	M	M			
54	K	R	R			
56	N	T				
61	I	L	L			
72	T	S	N			Cb
73	A	V	V			Cb
74	S				R	
83	P	S	S	S	S	
84	S	N	N	N	N	
87	N	Y				
94	D	E				
96	I	S	S			
97	D	N	N	N	N	
119	K	N	N			
120	T	A	A			
124	P		T			Sa
127	D	K	T			
128	S	T	T			
129	N	D	D			
130	K	T	T			
137	P	S	S			
138	H	K	K			
139	A	S	S			
141	A	K	K			
142	K	S	R			

152	V	L	L			
156	N	D	D			Sb
161	L	I	I			
162	S			N	N	Sa
163	K			Q	Q	Sa
164	S				T	Sa
166	I		V			Ca1
168	D		N			
173	V	I	I			
183	S	P	P		P	
185	S	N		T	T	
186	A	D	D			
187	D	S	S			RBS
189	Q	R	R			Sb, RBS
190	S	W	W			Sb, RBS
191	I	L	L	L	L	RBS
195	A	E	E			Sb, RBS
196	D	N	N			
197	T			A	A	
203	S	T	T	T	T	
205	R	T	T			
207	S		N			
208	K	Q	Q			
210	F		I			
211	K	M	M			
216	I	E	E	T	T	
223	Q			R	R	RBS
224	E	A	A			RBS
239	K	T	T			
250	V		A			
256	A			T	T	
257	M	V	V			
258	E	D	D			
260	N	G	G			
261	A	S	S			
270	T	V	V			
272	V	L	L			
273	H		Y			
274	D	N	N			
276	N	T	T			
278	T	E	E			
282	P				A	

283	K			E	E	
295	I	V	V		V	
298	I	V	V		V	
302	K	E	E			
310	T	A	A			
314	L	M	M			
321	I			V	V	
aadiff		**61**	**64**	**14**	**21**	

***Reference CVV in red, swine strains in black**

Table 8. Amino acid substitutions between recent swine 1A.5.2 lineage strain and the most similar vaccine (A/California/04/2010).

site	A/California/04/2010	A/swine/Gunma/12-5959/2018	A/swine/Gunma/46-6975/2019	A/California/04/2009	A/Michigan/45/2015	Annotations
9	A	S	S			
19	V	I				
35	D	T	T			
36	K	T	T			
45	R	G	G			
47	V	K	K			
61	I	V				
68	E	G				
69	S	I	I			Cb
70	L	T				Cb
71	S	P	P			
72	T		A			Cb
83	S			P		
84	S	N	N		N	
96	I	A	A			
97	D	N	N		N	
108	V	M	M			
113	R	Q	Q			
125	N	H	H			Sa
127	D	E	E			
128	S	T	T			
141	A	T	T			
146	K	R	R			
162	S				N	Sa
163	K				Q	Sa
166	I	V	V			Ca1
168	D	N	N			
183	S	P	P			
185	S				T	

186	A	T	T			
191	L			I		RBS
195	A	N	N			Sb, RBS
197	A	T	T	T		
203	T			S		
205	R	T	T			
211	K	T	M			
216	I	A	A		T	
223	Q				R	RBS
224	E	A	A			RBS
234	V	I	I			
239	K	T	T			
256	A				T	
258	E	N	N			
260	N	G	G			
261	A	S	S			
264	G	S				
267	I	N	N			
270	T	V	V			
272	V	L	L			
276	N	T				
278	T	K	K			
283	K				E	
286	I	L	L			
298	I	V	V			
302	K	E	E			
314	L	M	M			
321	V			I		
aadiff		**46**	**41**	**5**	**9**	

***Reference CVV in red, swine strains in black**

Table 9. Amino acid substitutions between recent swine 1A.5.3 lineage strain and the most similar vaccine (A/California/07/2009).

site	A/California/07/2009	A/swine/Gunma/6-4101/2017	A/swine/Gunma/7-6446/2019	A/Michigan/45/2015	A/Brisbane/02/2018	Annotations
2	T	M	M			
35	D	T	T			
36	K	T	T			
45	R	E	E		G	
47	V	M	M			
69	S	I	I			Cb
73	A	V	V			Cb
74	S	N	N		R	
83	P			S	S	
84	S	N	N	N	N	
96	I	A	S			
97	D	N	N	N	N	
120	T	S	S			
125	N	L	L			Sa
127	D	E	E			
128	S	T	T			
137	P	S	S			
141	A	T	T			
142	K		N			
146	K	R				
156	N	T	T			Sb
162	S	N	N	N	N	Sa
163	K			Q	Q	Sa
164	S				T	Sa
166	I	V	V			Ca1
183	S	P	P		P	
185	S			T	T	
186	A	T	T			
195	A	N	N			Sb, RBS
197	A	S	P			

203	S			T	T	
205	R	T	T			
208	K	R	R			
211	K	T	T			
212	P	I	I			
216	I	A	S	T	T	
223	Q			R	R	RBS
224	E	A	A			RBS
239	K	T	T			
256	A			T	T	
258	E	N	N			
260	N	G	G			
261	A	S	S			
270	T	V	M			
272	V	L	L			
278	T	K	K			
282	P				A	
283	K			E	E	
286	I	L	L			
295	I	V	V		V	
298	I				V	
302	K	E	E			
310	T	A	A			
314	L	M	M			
321	I	V	V	V	V	
aadiff		**44**	**44**	**12**	**19**	

***Reference CVV in red, swine strains in black**

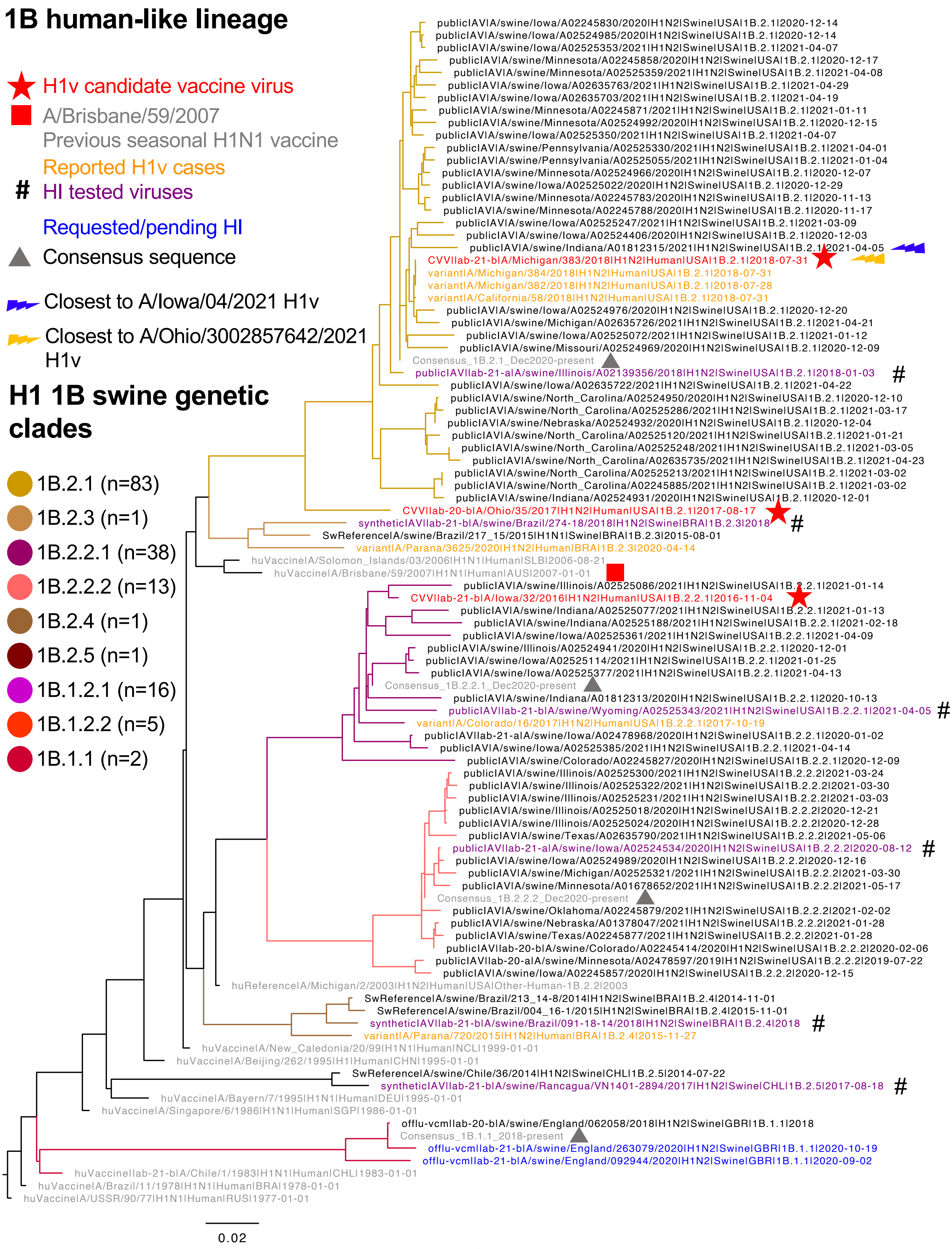


Figure 8. Swine H1 HA genes of the 1B lineage (tree was proportionally down sampled to 72 swine HA genes from 178): detections of each clade from data deposited between January 1 to June 30, 2021 are presented adjacent to the clade name.

Antigenic analysis: Swine 1B Lineage

Table 10. 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/Michigan/383/201RG58A	1B.2.1	d2 H1N2v	160	20
A/swine/Illinois/A02139356/2018	1B.2.1	d2 H1N2	160	10
A/Iowa/32/2016	1B.2.2.1	d1a H1N2v	10	320
A/swine/Wyoming/A02525343/2021	1B.2.2.1	d1a H1N2	10	80
A/swine/Iowa/A02524534/2020	1B.2.2.2	d1b H1N2	10	40
rg-A/swine/Brazil/274-18/2018*	1B.2.3	H1N2	160	40
rg-A/swine/Brazil/091-18-14/2018*	1B.2.4	H1N2	80	40
rg-A/swine/Rancagua/VN1401-2894/2017*	1B.2.5	H1N2	<10	20

Reference CVV in red, new swine strains in bold. Homologous titers highlighted grey. *synthetic HA/NA on PR8 backbone.

- The contemporary swine 1B.2.1 (delta-2) was cross-reactive to CVV A/Michigan/383/2018.
- The contemporary swine 1B.2.2.1 (delta-1a) had a 4-fold decrease from the within clade CVV A/Iowa/32/2016.
- The contemporary swine 1B.2.2.2 (delta-1b) does not have a CVV contained within clade and had a significant 8-fold decrease from CVV A/Iowa/32/2016.
- The Brazil swine 1B.2.3 does not have a within clade CVV but was cross-reactive to CVV A/Michigan/383/2018.
- The Brazil swine 1B.2.4 does not have a within clade CVV but was cross-reactive to CVV A/Michigan/383/2018.
- The Chile swine 1B.2.5 does not have a within clade CVV and demonstrated a significant 16-fold decrease from CVV A/Iowa/32/2016 and no reactivity CVV A/Michigan/383/2018.

Table 11. UK & 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	A/Michigan/383/2018	A/Chile/1/1983
A/Michigan/383/2018	1B.2.1	320	40
A/Chile/1/1983	H1 seasonal	160	80
A/swine/Italy/118638/2021	1B.1.2.2	20	40

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

- The contemporary swine 1B.1.2.2 demonstrate poor cross-reactivity to A/Michigan/383/2018 and good cross-reactivity to A/Chile/1/1983.

Table 12. Amino acid substitutions between recent swine 1B.2.1 strains compared to the nearest CVV (A/Michigan/383/2018).

site	**A/Michigan/383/2018**	A/swine/Illinois/A02139356/2018	Annotations
71	N	T	
169	E	K	
170	G	E	Ca2
173	V	I	
259	K	R	
260	S	G	
aadiff		**6**	

***Reference CVV in red, swine strains in black**

Table 13. Amino acid substitutions between recent swine 1B.2.2.1 and 1B.2.2.2 strains compared to the nearest CVV (A/Iowa/32/2016).

site	**A/Iowa/32/2016**	1B.2.2.1|A/swine/Wyoming/A02525343/2021	1B.2.2.2|A/swine/Iowa/A02524534/2020	Annotations
2	T		M	
19	L	V	V	
50	L		I	
72	S	P		Cb
74	K	E		
82	A		T	
85	S		P	
86	D		E	
94	Q		D	
96	T	A	A	
106	S	N		
119	R		K	
129	V		T	
130	T	D		
132	K	T		RBS
149	I		V	
153	V		E	Sb
157	L	R		Sa
168	E	D	N	
170	G		E	Ca2
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	K	E	
215	T	A		
222	D	N	N	RBS
228	N		K	
236	P		A	
241	I	V		
244	T		A	
249	I		V	
256	A		T	
269	K		N	
271	P		S	
273	D		N	
274	E		K	
277	A		T	
283	K		Q	
287	N	T		
289	N		S	
310	T	R	A	
314	M		L	
aadiff		**16**	**38**	

***Reference CVV in red, swine strains in black**

Table 14. Amino acid substitutions between recent swine 1B.2.3, 1B.2.4, and 1B.2.5 compared to the nearest CVV (A/Iowa/32/2016|CVV|1B.2.2.1). A direct comparison to the human seasonal vaccine with the highest amino acid similarity A/New Caledonia/20/1999 is included.

Relative to HI

site	**A/Iowa/32/2016**	1B.2.3|A/swine/Brazil/274-18/2018	1B.2.4|A/swine/Brazil/091-18-14/2018	1B.2.5|A/swine/Rancagua/VN1401-2894/2017	**A/Michigan/383/2018**	Annotations
5	I				V	
19	L	V	V	I	V	
30	V			I		
40	K		R	R		
43	L	S		R		
45	K			R		
54	N				S	
56	S		N			
69	S	L			L	Cb
71	I			F	N	
74	K	E		E	E	
80	V			A		
82	A	K	K	T	T	
83	P				S	
85	S	P	P	P	P	
86	D	E	E	E	E	
89	A		T	K	T	
94	Q	Y	Y	Y	Y	
96	T	A	A	A	E	
106	S			G		
112	E	K		K	K	
113	R				K	
119	R	K	K	K	K	

Relative to nearest vaccine

site	**A/New_Caledonia/20/1999**	1B.2.3|A/swine/Brazil/274-18/2018	1B.2.4|A/swine/Brazil/091-18-14/2018	1B.2.5|A/swine/Rancagua/VN1401-2894/2017	Annotations
19	V			I	
30	V			I	
40	K		R	R	
43	L	S		R	
45	K			R	
56	S		N		
69	L		S	S	Cb
71	I			F	
74	E		K		
80	V			A	
82	T	K	K		
89	T	A		K	
106	S			G	
112	E	K		K	
125	T	N	N	N	Sa
130	-			N	
134	A			V	RBS
137	S			P	
138	H		Y		
142	S		P		
143	S		N		
146	R		K		
153	G	R	R	E	Sb

120	E				K	
130	-			N		
132	K	V	V	V	V	RBS
134	A			V	S	RBS
137	S			P		
138	H		Y			
141	E	K	K	K	N	
142	G	S	P	S	S	
143	S		N			
146	R		K			
149	I	L	L	L	L	
153	V	R	R	E		Sb
156	G				N	Sb
160	N	S				Sa
166	K	A	T	V	T	Ca1
168	E	N	N	D	K	
169	K		R		E	
170	G	E	E	D		Ca2
171	K			E		
175	I	V		V	V	
179	V	I				
185	I				M	
186	G	E		R	E	
190	T	A	I	A	A	Sb, RBS
191	L			I		RBS
193	H			Q		Sb, RBS
202	M	V	V	V	V	
204	S		T			Ca2
206	Y			F		
207	S			N		
208	R			K		
209	R			K		
211	T	I				
215	T	I		A	A	
216	K			R		
222	D			N	N	RBS
230	Y	H				
233	L			I		
243	E			K		

160	N	S			Sa
166	V	A	T		Ca1
168	N			D	
169	K		R		
170	E			D	Ca2
171	K			E	
175	V		I		
179	V	I			
183	P	S	S	S	
186	G	E		R	
190	A		I		Sb, RBS
191	L			I	RBS
193	H			Q	Sb, RBS
204	S		T		Ca2
206	Y			F	
207	S			N	
208	R			K	
209	R			K	
211	T	I			
215	A	I	T		
216	K			R	
222	D			N	RBS
230	Y	H			
233	L			I	
243	E			K	
252	W	R			
253	Y	F		H	
261	F		L		
267	T			I	
271	P			S	
272	M			V	
273	D			E	
274	E			G	
276	D		E		
283	Q		R	K	
310	A			T	
314	M			I	
321	I			N	
aadiff		**16**	**21**	**41**	

244	T	A	A	A	A	
245	N				S	
252	W	R			R	
253	Y	F		H		
256	A				E	
259	R				K	
260	G				S	
261	L	F		F	F	
267	T			I		
269	K	N	N	N	N	
271	P			S		
272	M			V		
273	D			E	G	
274	E			G		
276	D		E		N	
277	A				T	
283	K	Q	R		Q	
289	N	S	S	S	S	
310	T	A	A		A	
314	M			I		
321	I			N	T	
aadiff		**36**	**31**	**56**	**48**	

***Reference CVV in red, vaccine in gray, swine strains in black**

Table 15. Amino acid substitutions between recent swine 1B.1 lineage strains compared to the vaccine A/Chile/1/1983.

site	A/Chile/1/1983	1B.1.1|A/swine/England/092944/2020	1B.1.1|A/swine/England/263079/2020	1B.1.2.1|A/swine/Gent/249/2020	1B.1.2.1|A/swine/Belgium/Gent-114/2019	1B.1.2.1|A/swine/Belgium/Gent-178/2019	1B.1.2.2|A/swine/Italy/110217-1/2021	1B.1.2.2|A/swine/Italy/135572/2021	1B.1.2.2|A/swine/Italy/118638/2021	1B.1.2.3|A/Sw/France/29-200240/2020	Annotations
14	D									E	
35	D	N					N				
36	N	S	S		S	S					
43	K	R	R	R	R						
47	I						V	V	V	T	
48	A									T	
51	Q			H							
54	K						R	R	R		
57	I	V	V								
68	E	K	T								
71	F						L	L	L		
73	K	R	R								Cb
82	T					A	A	A	A		
83	P			T	T	T					
84	N									S	
85	S	P	P	P	P	P	A	A	A	P	
88	G	D	D								
89	T	V	V	I	I	I	I	L	L	I	
94	Y	H	H				H	H	H		
96	A	S	S	S	S	S	S	S	S	S	
106	S	N	N							G	
111	F						I	I	I	L	
124	P			S	S	F					Sa
125	K	Q	Q	N	N	N					Sa
127	N			S	S	S	S	S	S	S	
128	V			X			I	I	I	I	

129	T			X	N	N	-	-	-	N	
130	K			X			-	-	-		
132	V			G	I		A	A	A		RBS
134	A	V	V								RBS
135	A	S	S	S	S	S	S	S	S	S	RBS
138	H						K	K	K		
139	K	N	N				Q	Q	Q		
141	K						R	R	R	X	
142	S			G				R	R	R	
143	S									N	
146	R	Q	Q				T	T	T		
149	L						I				
153	E	G	G								Sb
155	N	D	D								Sa
157	S			I	I	I					Sa
161	L									I	
162	S	N	N				T	T	T		Sa
163	K						M	M	M		Sa
166	V						M	M	M		Ca1
168	N	D	D				D	D	D	D	
169	K			R	R	R					
170	E	K	K	D	D	N	K	K	K		Ca2
171	K			E	E	E	R	R	R		
175	V		I		L	L					
179	V	I	I							I	
183	S	A	A								
184	N							S	S		
185	I			V	M	M					
189	K		R	R	R	R	R	R	R	R	Sb, RBS
190	T	A	A	A	A	A	A	A	A	A	Sb, RBS
193	R	H	H								Sb, RBS
196	N	T	T	T	T	T	T	T	T	T	
202	V	M					M	I	T		
203	S									A	
204	S					T					Ca2
207	N									S	
208	R			K	K		K	K	K		
209	R						K	K	K		
211	T	I	I	I	I	I					
215	A	S	T		T						
216	K	R								R	
220	V			I	I	I	I	I	I	I	RBS

222	N						D	D	D	D	RBS
232	T		I								
235	E	K								G	
237	G			N	K	K	S	S	S	K	Ca1
238	D						E				
241	I	M									
248	L			F	F	F					
252	W	R	R	L	L	L					
256	A	S	S								
258	S			I			N	N	N		
260	G		N								
262	G	E	E	E	E	E	E	E	E	K	
267	T	V	V	V	V	V	V	V	V	I	
270	A			V			V	V	V		
272	M	L	L	V	V	V					
276	D	N	N		N		N	N	N		
277	A	T	T	T	T	T				T	
278	K							T	T		
283	Q						R	R	R		
288	S						N	N	N		
290	L	R	R								
295	V						I	I	I		
298	V						I	I	I		
300	I		V								
308	R						K	K	K		
310	T			K	K	K		I			
313	R						K	K	K		
315	V						A	A	A		
321	I	V	V								
aadiff		**42**	**42**	**36**	**34**	**31**	**49**	**50**	**49**	**32**	

***Reference vaccine strain in gray, past HI strain in blue, current swine strains in black**

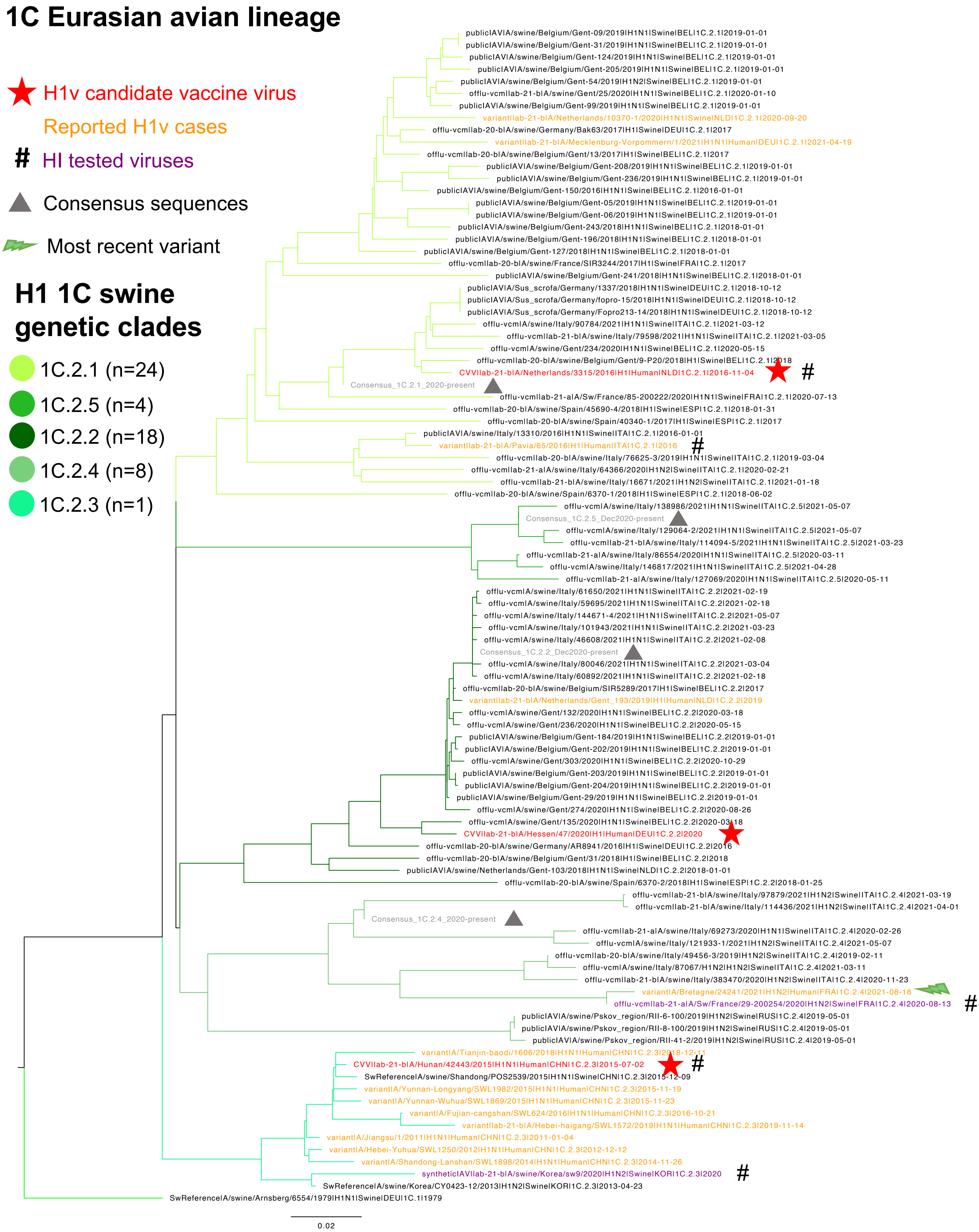


Figure 9. Swine H1 HA genes of the 1C lineage: detections of each clade from data deposited between January 1 to June 30 2021 are presented adjacent to the clade name.

Antigenic analysis: Swine 1C Lineage

Table 16. South Korea Data: Hemagglutination inhibition of CVV vaccine ferret antisera against contemporary swine 1C lineage strains selected to represent clade consensus.

	Global Clade	US Clade	A/Hunan/42443/2015 CVV
A/Hunan/42443/2015 CVV	1C.2.3	H1N1	320
rg-A/swine/Korea/sw9/2020*	1C.2.3	H1N2	320

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

Reference CVV in red, new swine strains in bold. Homologous titers highlighted grey. *synthetic HA/NA on PR8 backbone.

- The South Korea swine 1C.2.3 was cross-reactive to the within clade CVV A/Hunan/42443/2015.

Table 17. 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/Hunan/42443/2015 CNIC-1601– CVV	A/Netherlands/ 3315/2016 - CVV	A/PAVIA/65/2016
A/Hunan/42443/2015 CNIC-1601 CVV	1C.2.3	320	40	40
A/Netherlands/ 3315/2016 CVV	1C.2.1	20	80	<20
A/PAVIA/65/2016	1C.2.1	<20	40	640
A/swine/France/29-200254/2020	1C.2.4	80	80	80

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

- The A/swine/France/29-200254/2020 1C.2.4 strain was cross-reactive to the nearest 1C.2.1 clade CVV A/Netherlands/ 3315/2016 and 1C.2.3 A/Hunan/42443/2015 CNIC-1601. This swine strain has high sequence similarity to the recent variant A/Bretagne/24241/2021 (see Annex 2).

Table 18. EU Data: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine 1C lineage strains.

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

	Global Clade	A/Hunan/42443/2015	A/Netherlands/10370-1b/2020	A/Pavia/65/2016	A/Netherlands/3315/2016 (APHA)	A/Netherlands/3315/2016 (crick)	A/Hessen/47/2020-C2/MDCK1	A/Hebei-Haigang/1572/2019
A/Hunan/42443/2015	1C.2.3	320	160	80	40	20	320	320
A/Netherlands/10370-1b/2020	1C.2.1	160	160	40	80	40	20	40
A/PAVIA/65/2016	1C.2.1	80	80	320	40	40	40	40
A/Netherlands/3315/2016 (APHA)	1C.2.1	40	40	40	80	80	40	80
A/Netherlands/3315/2016 (Crick)	1C.2.1	40	40	40	80	80	40	80
A/Hessen/47/2020-C2/MDCK1	1C.2.2	320	160	80	80	40	160	160
A/Hebei-Haigang/1572/2019	1C.2.3	160	160	160	80	80	80	160
A/Netherlands/Gent-193/2019	1C.2.2	320	320	640	80	80	320	320
A/swine/Italy/79598/2021	1C.2.1	40	80	40	160	80	20	80
A/swine/Italy/16671/2021	1C.2.1	40	40	40	40	20	20	40
A/Mecklenburg-Vorpommern/1/2021	1C.2.1	40	160	80	40	40	80	160
A/swine/Italy/97879/2021	1C.2.4	20	20	40	40	20	20	20
A/swine/Italy/114436/2021	1C.2.4	80	40	40	40	40	20	40
A/swine/Italy/383470/2020	1C.2.4	160	40	40	20	20	40	80
A/swine/Italy/114094-5/2021	1C.2.5	320	40	80	80	40	40	40

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

- The contemporary swine clades 1C.2.1, 1C.2.2, 1C.2.4 and 1C.2.5 demonstrate good cross-reactivity to CVV A/Netherlands/3115/2016 (APHA), apart from A/swine/Italy/383470/2020 (1C.2.4)
- The swine 1C.2.2 variant virus A/Netherlands/Gent-193/2019 demonstrate good cross-reactivity to all the tested strains.
- The contemporary swine clades 1C.2.1, 1C.2.2, 1C.2.4 and 1C.2.5 demonstrate variable cross-reactivity to CVV A/Hunan/42443/2015 and other CVVs in development: A/ A/Netherlands/10370-1b/2020 and A/Hessen/47/2020-C2/MDCK1.

Table 19. Amino acid substitutions between recent swine 1C.2.1 strains compared to the nearest CVV (A/Netherlands/3315/2016|CVV|1C.2.1).

site	**A/Netherlands/3315/2016**	A/swine/Gent/25/2020	A/swine/Italy/79598/2021	A/swine/Italy/16671/2021	**A/Hunan/42443/2015**	**A/Hessen/47/2020**	A/Mecklenburg-Vorpommern/1/2021	A/Pavia/65/2016	A/Netherlands/10370-1/2020	Annotations
20	M		L	L	L	L		L		
22	K			R						
31	N	D					D		D	
35	N					T				
44	L	I					M		M	
48	A				I					
53	G			E		K		E		
56	N				S					
57	V					L				
61	I		L							
66	E				K					
69	L			F				F		Cb
71	I	L		L	L	L	L	L	L	
74	N	K					K	Y	K	
80	I					V				
83	S					P	P		P	
84	N					D				
86	K		N							
87	N		D				S			
89	A			T		T		T		
96	A			T				N		
97	D			N			G	N	N	
102	R			K	K					
111	F		L					L		
120	E	A	A	T	A	A	A	A	A	
125	N			D						Sa
127	E				D	D		D		
129	T	N								

130	K				R	R				
132	S	T		T	T	T	T	T	T	RBS
134	V					I	A			RBS
135	A						S			RBS
137	S	P					P		P	
141	I	A	N	A	A	A	A	A	A	
142	K	N		N	N	N	N	N	N	
155	E	G		G	G	G	G		G	Sa
157	S	F								Sa
161	I	L		L	L	L	L	L	L	
162	S	N				R				Sa
163	T	N		K	K	N	K	K	K	Sa
175	I			V	V	V	V	V	V	
186	S		N							
189	Q		L							Sb, RBS
190	T						A			Sb, RBS
196	H							N		
199	V					I	I		I	
202	G		E					E	E	
208	K	Q		Q		Q	Q	Q	Q	
214	I					T				
215	V	A					A		A	
220	V		I			I				RBS
222	E							K		RBS
227	M					I				
232	T			I				I		
237	G		E							Ca1
253	Y	H		H	H			X	H	
258	N	D		D	K		D	D	D	
260	G		S							
262	N		S		S	S		S		
266	M					I				
267	M	I		V	R				I	
269	D					N	S			
270	A	S								
271	N	H		H	Q	R	L	H	H	
273	H			Q				Q		
278	K					M				
287	K		T							
288	S	G			G					
298	I				V					
311	Q	R				H	R		R	

321	T	I	I	I	I	I	I	I	I	
324	V	I		I	I	I	I	I	I	
aadiff		**26**	**16**	**27**	**25**	**34**	**28**	**29**	**26**	

***Reference CVV in red, swine strains in black**

Table 20. Amino acid substitutions between recent swine 1C.2.3 strains compared to the nearest CVV (A/Hunan/42443/2015).

site	1C.2.3|A/Hunan/42443/2015	A/swine/Korea/sw9/2020	Annotations
35	N	T	
48	I	N	
56	S	N	
57	V	I	
66	K	E	
73	A	S	Cb
84	N	S	
89	A	T	
130	R	K	
185	D	Y	
190	T	S	Sb, RBS
199	V	I	
216	A	T	
224	A	S	RBS
258	K	E	
267	R	M	
288	G	S	
298	V	I	
aadiff		**18**	

***Reference CVV in red, swine strains in black**

Table 21. Amino acid substitutions between recent swine 1C.2.4 strains compared to the nearest CVV (A/Hunan/42443/2015).

site	**A/Hunan/42443/2015**	A/swine/Italy/97879/2021	A/swine/Italy/383470/2020	A/swine/Italy/114436/2021	**A/Netherlands/3315/2016**	**A/Hessen/47/2020**	Annotations
5	V	I	I	I			
20	L		M		M		
35	N	T	T	T		T	
48	I	A	A	A	A	A	
51	Q	N		N			
53	G					K	
56	S	N	N	N	N	N	
57	V					L	
66	K	E	E	E	E	E	
69	L			P			Cb
71	L		T		I		
80	I					V	
83	S					P	
84	N	D		D		D	
86	K		R				
89	A	T	T	T		T	
96	A		T				
102	K	R	R	R	R	R	
104	Q	L	L	L			
112	E	K		K			
113	R		K				
119	K	E	E	E			
120	A		-		E		
124	P	S	L	S			Sa
126	H		Y				
127	D	-	K	-	E		
130	R	G	-	G	K		
132	T				S		RBS
134	V	A		A		I	RBS
135	A	S		S			RBS
137	S		P				

138	H		N				
141	A				I		
142	N	S	S	S	K		
149	L	S		S			
152	V	T	T	T			
155	G				E		Sa
156	N	S		S			Sb
161	L		I		I		
162	S					R	Sa
163	K	R		R	T	N	Sa
166	T		K				Ca1
169	K	Q		Q			
170	G	E	I	E			Ca2
175	V				I		
185	D		Y				
186	S	R		R			
189	Q	L		L			Sb, RBS
195	N	D		D			Sb, RBS
199	V					I	
202	G	A	V	A			
205	K	T		T			
208	K					Q	
214	I					T	
216	A	T	S	T			
220	V	I	I	I		I	RBS
221	R		N				RBS
222	E	D		D			RBS
224	A		E				RBS
227	M		I			I	
232	T		I				
236	Q		R				
252	W	K	K	K			
253	H		Y		Y	Y	
258	K	S	N	S	N	N	
262	S				N		
266	M					I	
267	R	K	K	K	M	M	
269	D					N	
271	Q	N	L	N	N	R	
273	H		Q				
278	K					M	
288	G	S	S	S	S	S	

289	N		S				
290	L	R	R	R			
298	V	I	I	I	I	I	
311	Q					H	
313	R		K				
321	I				T		
324	I				V		
aadiff		**39**	**46**	**40**	**25**	**29**	

***Reference CVV in red, swine strains in black**

Table 22. Amino acid substitutions between recent swine 1C.2.5 strains compared to the nearest CVV (A/Hunan/42443/2015).

site	**A/Hunan/42443/2015**	A/swine/Italy/114094-5/2021	**A/Netherlands/3315/2016**	**A/Hessen/47/2020**	Annotations
2	T	I			
20	L		M		
35	N			T	
44	L	M			
47	K	N			
48	I	A	A	A	
53	G			K	
56	S	D	N	N	
57	V			L	
66	K	E	E	E	
69	L	F			Cb
71	L		I		
80	I			V	
82	T	A			
83	S			P	
84	N			D	
85	S	P			
89	A			T	
102	K	R	R	R	
111	F	L			
120	A	G	E		
125	N	D			Sa
127	D	E	E		
130	R	K	K		
132	T		S		RBS
134	V	S		I	RBS
135	A	S			RBS
141	A		I		
142	N	S	K		
155	G		E		Sa

161	L		I		
162	S	N		R	Sa
163	K	N	T	N	Sa
169	K	R			
170	G	E			Ca2
175	V		I		
179	V	I			
190	T	A			Sb, RBS
196	H	R			
199	V			I	
208	K	R		Q	
214	I			T	
215	V	I			
220	V			I	RBS
222	E	N			RBS
224	A	K			RBS
227	M	I		I	
236	Q	R			
245	T	N			
253	H	Y	Y	Y	
258	K		N	N	
259	K	R			
262	S		N		
266	M			I	
267	R	I	M	M	
269	D			N	
270	A	V			
271	Q	H	N	R	
278	K			M	
287	K	N			
288	G	N	S	S	
298	V	I	I	I	
310	T	K			
311	Q			H	
313	R	K			
321	I		T		
324	I		V		
aadiff		**42**	**25**	**29**	

***Reference CVV in red, swine strains in black**

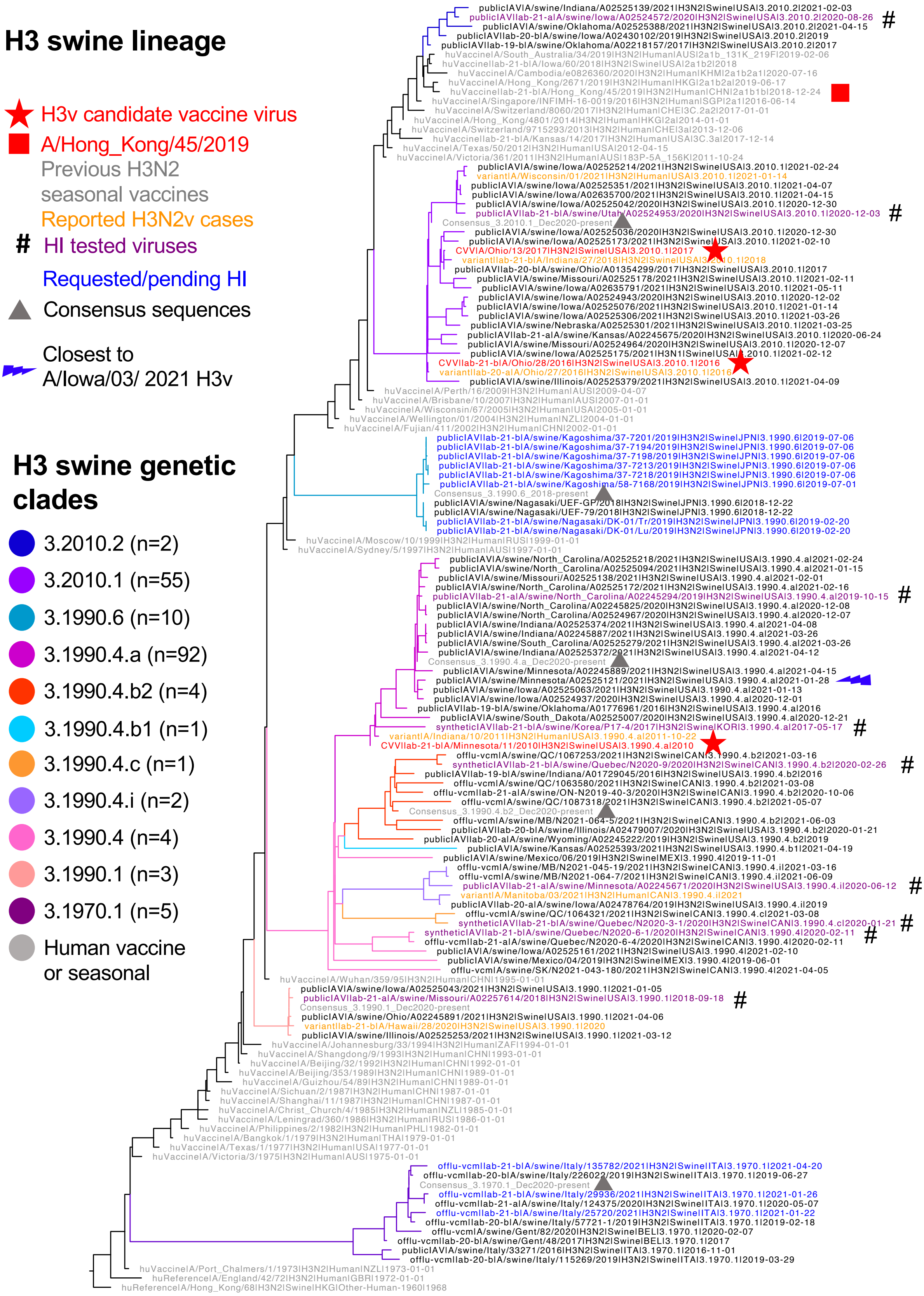


Figure 10. Swine H3 HA genes: detections of each clade from data deposited between January 1 to June 30, 2021 are presented adjacent to the clade name. Clades within the tree were proportionally down sampled to 95 swine HA genes from 212; tree includes clade consensus sequences, and human vaccine strains.

Antigenic analysis: Swine H3

Table 23. 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/Hawaii/28/2020	A/Minnesota/11/2010 x 203	IDCDC-RG55C A/Ohio/28/2016-like CVV	A/Indiana/27/2018	A/Kansas/14/2017	A/Iowa/60/2018	A/Hong Kong/45/2019
A/Hawaii/28/2020	3.1990.1 H3N2v	640	20	20	80	<10	<10	<10
A/swine/Missouri/A02257614/2018	3.1990.1	160	10	<10	20	<10	10	<10
A/Minnesota/11/2010 x 203	3.1990.4. H3N2v	40	640	20	20	<10	<10	<10
rg-A/swine/Quebec/N2020-6-1/2020*	3.1990.4 H3N2	40	40	10	20	<10	<10	<10
A/swine/North Carolina/A02245294/2019	3.1990.4.a H3N2	20	40	20	40	20	10	<10
rg-A/swine/Korea/P17-4/2017*	3.1990.4.a H3N2	80	160	20	80	20	10	10
rg-A/swine/Quebec/N2020-9/2020*	3.1990.4.b 2 H3N2	20	40	40	20	10	<10	<10
rg-A/swine/Quebec/N2020-3-1/2020*	3.1990.4.c H3N2	20	40	20	20	10	<10	<10
A/swine/Minnesota/A02245671/2020	3.1990.4.i H3N2	40	20	20	20	20	20	<10
IDCDC-RG55C A/Ohio/28/2016-like CVV	3.2010.1 H3N2v	20	20	1280	40	10	10	<10
A/Indiana/27/2018	3.2010.1 H3N2v	10	10	<10	640	10	10	<10
A/swine/Utah/A02524953/2020	3.2010.1 H3N2	10	10	40	40	10	<10	<10
A/swine/Iowa/A02524572/2020	3.2010.2 H3N2	20	20	20	40	40	40	<10
A/Kansas/14/2017	3C.3a H3N2	10	10	<10	10	80	20	20
A/Iowa/60/2018	3C.2a1b2 H3N2	<10	<10	<10	<10	20	160	40
A/Hong Kong/45/2019	3C.2a1b H3N2	<10	10	<10	<10	20	80	160

Reference CVV in red, seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted in grey. A/Indiana/27/2018 is surrogate for CVV A/Ohio/13/2017 2010.1 H3N2v. *synthetic HA/NA on PR8 backbone.

- The contemporary swine 1990.1 does not have a CVV contained within clade and had limited reactivity with any CVV or vaccine anti-sera.
- The Canada 1990.4 strain demonstrated a significant 16-fold decrease in cross-reactivity from CVV A/Minnesota/11/2010 x 203.
- The contemporary swine 1990.4.a demonstrated a significant 16-fold decrease, and the South Korea 1990.4.a demonstrated a 4-fold decrease in cross-reactivity from CVV A/Minnesota/11/2010 x 203.
- The Canada swine 1990.4.b2 does not have a CVV contained within clade and demonstrated a significant 16-fold decrease from CVV A/Minnesota/11/2010 x 203.
- The Canada swine 1990.4.c does not have a CVV contained within clade and demonstrated a significant 16-fold decrease from CVV A/Minnesota/11/2010 x 203.
- The contemporary swine 1990.4.i does not have a CVV contained within clade and demonstrated a significant 32-fold decrease from CVV A/Minnesota/11/2010 x 203.
- The contemporary swine 2010.1 representative strain (A/Indiana/27/2018) did not react to CVV (IDCDC-RG55C A/Ohio/28/2016-like) anti-sera. The contemporary swine 2010.1 representative strain (A/swine/Utah/A02524953/2020) had significant 32-fold decrease to CVV (IDCDC-RG55C A/Ohio/28/2016-like).
- The contemporary swine 2010.2 representative strain does not have a CVV contained within clade but reacted to A/Kansas/14/2017 antisera, had 4-fold decrease to HuVacc (A/Iowa/60/2018), and no reactivity to HuVacc (A/Hong Kong/45/2019).

Table 24. EU Data: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine H3 strains selected to represent clade consensus.

	Global Clade	A/Victoria/3/1975	A/Ohio/28/2016	A/Sydney/5/97
A/Victoria/3/1975	Human seasonal	320	80	20
A/Ohio/28/2016	H3.2010.1	80	320	80
A/Port Chalmers/1/1973	Human seasonal	80	20	<10
A/Sydney/5/97	Human seasonal	40	80	160
A/swine/Italy/25720/2021	3.1970.1	80	80	10

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

Reference CVV in red, seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted in gray. ND=not done.

- The contemporary swine 3.1970.1 demonstrated moderate to poor cross-reactivity to the historical human vaccine and 2010.1 CVV strains.

Table 25. Amino acid substitutions between recent swine 1970.1 strains compared to the nearest vaccine by amino acid similarity (A/Victoria/3/1975) and to the putative ancestral human seasonal H3 strain (H3 A/Port Chalmers/1/1973).

site	**A/Victoria/3/1975**	A/swine/Italy/135782/2021	A/swine/Italy/25720/2021	A/swine/Italy/29936/2021	**A/Port_Chalmers/1/1973**	Annotations
3	L	F	F	F	F	
5	G	R	R	R		
6	N	K	K	K		
7	D	G	G	G		
7+1	N	-	-	-	-	
8	N		D			
31	N	D	D	D		
45	S	N	N	N		
46	S	F	V	F		
48	T	M	I	M		
53	N	K	K			Site C
56	H	Y				
62	I	A	A	A		
69	A	S	S	S		
83	K	R	R	R	T	
88	V	I	I	I		
92	K	R	R			
104	D	E	E	E		
106	A	T	T	T		
112	V	I	I	I		
121	I	T	T	T		
124	G	N	N	N		
126	N				T	
132	Q			P		
133	N	D		D		Site A
137	N	S	S	S		Site A
138	A		T	S		
143	P	I	I	I		Site A
144	D		N	N		Site A
145	S	K	K	K		Site A

146	G	S	S	S		Site A
159	S	N	N	N		
160	T				A	
163	V	M		M		
164	Q				L	
171	N	S	S	S		
173	N	D	D	D		
174	S	Y	Y	Y	F	
189	K				Q	Site B
193	N	D	D	D		Site B
196	V	I	I	I		
198	A	E				
201	K				R	Site D
202	V	I	I	I		
203	T	I	I	I		
212	T	I	I	I		
213	I	V	V			
217	V	I	I	I	I	Site D
219	S			P		
242	I	T	T	T		
244	V	I	I	I		
248	N	K				
260	M	I	I	I		
261	R	Q	Q	Q		
267	I	V	V	V		
278	S	N	N	N	I	Site C
299	K	R	R	R		
307	K	H	H	H		
309	V	I	I	I		
323	V	I	I	I		
aadiff		**50**	**48**	**48**	**11**	

***Reference vaccine in gray, swine strains in black**

Table 26. Amino acid substitutions between swine 1990.1 strain compared to the most similar vaccine (A/Wuhan/359/1995).

site	**A/Wuhan/359/95**	A/swine/Missouri/A02257614/2018	Annotations
88	V	I	
106	A	S	
124	G	D	
135	T	G	
138	A	S	
190	D	E	
194	I	L	
199	S	I	
208	R	S	Site D
246	N	S	
262	S	N	
275	G	D	Site C
278	N	Y	Site C
307	R	K	
312	N	K	
aadiff		**15**	

***Reference vaccine strain in gray, swine strains in black**

Table 27. Amino acid substitutions between recent swine 1990.4, 1990.4.a, 1990.4.b2, 1990.4.c, and 1990.4.i compared to the nearest CVV (H3 A/Minnesota/11/2010|CVV|1990.4.a).

site	**A/Minnesota/11/2010**	3.1990.4|A/swine/Quebec/N2020-6-1/2020	3.1990.4.a|A/swine/North_Carolina/A02245294/2010	3.1990.4.a|A/swine/Korea/P17-4/2017	3.1990.4.b2|A/swine/Quebec/N2020-9/2020	3.1990.4.c|A/swine/Quebec/N2020-3-1/2020	3.1990.4.i|A/swine/Minnesota/A02245671/2020	Annotations
2	K			E				
5	G				R	E		
6	S	G		N				
7	D				G			
8	N						K	
9	S			R	N	N	N	
10	M	T					T	
12	T						M	
21	P					S		
27	K				R			
49	G						D	
50	R						K	
53	N				S			Site C
54	S	R						Site C
57	Q						K	
58	I						V	
62	K						G	
75	H	N						
78	D	E						
80	Q						E	
81	N					D		
82	K	E						

83	E			K	K			
96	N		S				D	
103	P					R		
107	T	S			S	S	S	
112	V	I						
117	N	T			T	T	T	
119	E	K	K					
121	T				N		N	
122	Q	P				P	L	Site A
124	S		I		N	N	N	
131	A		T				D	
137	Y				N	F	F	Site A
138	A		S			S	S	
140	R						K	
142	G	K			E		N	
144	V	I			N			Site A
145	N	K			K	K		Site A
155	Y				H		H	Site B
156	N	H	H		H	H	Q	
159	Y			H	N	H	H	
163	E	A			A	A	A	
164	Q	L			L	L	L	
171	N					D		
173	K	E				N		
189	K						M	Site B
192	T					I		
193	N				Y	S	S	Site B
194	L			I				
196	V	I		A		A		
198	A			E			E	
199	S				P	P		
200	G					E		
202	V				I			
203	I	S			T		T	
204	V				I			
207	K		R					Site D
213	V	I						
217	I			V	V			Site D
233	Y				H			
236	I					V		
260	I						M	
261	Q			L			R	

262	S						I	
266	S			T				
267	I			V				
269	R						K	
273	H	P		Y	P	P	L	
275	D	G					G	Site C
276	E	I			N	N	N	
279	S				F			
289	P		S					
299	K	R			R	R	R	
304	A					T		
312	N			H				
323	V		I					
aadiff		**25**	**9**	**14**	**30**	**28**	**39**	

***Reference CVV in red, swine strains in black**

Table 28. Amino acid substitutions between swine 3.1990.6 strain compared to the most similar vaccine (A/Moscow/10/1999).

site	A/Moscow/10/1999	A/swine/Kagoshima/37-7201/2019	A/swine/Kagoshima/37-7194/2019	A/swine/Kagoshima/37-7198/2019	A/swine/Kagoshima/37-7213/2019	A/swine/Kagoshima/37-7218/2019	A/swine/Kagoshima/58-7168/2019	A/swine/Nagasaki/DK-01/Tr/2019	A/swine/Nagasaki/DK-01/Lu/2019	Annotations
1	Q	R	R	R	R	R	R	R	R	
3	L	H	H	H	H	H	H	H	H	
5	G	L	L	L	L	L	L	L	L	
7	D	S	S	S	S	S	S	S	S	
10	T	M	M	M	M	M	M	M	M	
12	T	M	M	M	M	M	M	M	M	
33	Q	H	H	H	H	H	H	H	H	
48	T	M	M	M	M	M	M	M	M	
53	D	N	N	N	N	N	N	N	N	Site C
56	H	Y	Y	Y	Y	Y	Y	Y	Y	
78	G	A	A	A	A	A	A	A	A	
92	K	T	T	T	T	T	T	T	T	
102	V							I	I	
110	S	A	A	A	A	A	A	A	A	
124	S	N	N	N	N	N	N	N	N	
135	T	A	A	A	A	A	A	A	A	
137	S	T	T	T	T	T	T	T	T	Site A
144	I	S	S	S	S	S	S	S	S	Site A
155	H	Y	Y	Y	Y	Y	Y	Y	Y	Site B
156	Q						H			
158	K	R	R	R	R	R	G	R	R	
159	Y						H			
160	R	K	K	K	K	K	K	K	K	
172	D	E	E	E	E	E	E	E	E	
186	S	G	G	G	G	G	G	G	G	Site B
189	S	N	N	N	N	N	N	N	N	Site B
192	T	I	I	I	I	I	I	I	I	
196	T	A	A	A	A	A	A	A	A	

198	A						T	T	T	
203	T	I	I	I	I	I	I	I	I	
226	I	V	V	V	V	V	V	V	V	
229	R	I	I	I	I	I	I	I	I	
233	Y	H	H	H	H	H	H	H	H	
242	I	T	T	T	T	T	T	T	T	
262	S	N	N	N	N	N	N	N	N	
265	S	G	G	G			G	G	G	
271	D	N	N	N	N	N	N	N	N	
273	P	L	L	L	L	L				
276	K	R	R	R	R	R	R	R	R	
279	S	A	A	A	A	A	A	A	A	
280	E	G	G	G	G	G	G	G	G	
289	P	S	S	S	S	S	S	S	S	
312	N	K	K	K	K	K	K	K	K	
315	K	R	R	R	R	R	R	R	R	
aadiff		**40**	**40**	**40**	**39**	**39**	**42**	**41**	**41**	

***Reference vaccine strain in gray, swine strains in black**

Table 29. Amino acid substitutions between recent swine 2010.1 strains compared to the nearest CVV (A/Ohio/13/2017).

site	A/Ohio/13/2017	A/swine/Utah/A02524953/2020	Annotations
56	Y	H	
88	V	I	
92	K	R	
112	V	A	
144	S	T	Site A
156	H	Q	
167	T	A	
209	N	G	
312	N	K	
aadiff		**9**	

***Reference CVV in red, swine strains in black**

Table 30. Amino acid substitutions between 2010.2 strains compared to the most similar human vaccine (A/Kansas/14/2017).

site	A/Kansas/14/2017	A/swine/Iowa/A02524572/2020	Annotations
9	S	G	
31	N	D	
58	I	V	
92	K	R	
121	N	K	
128	A	T	
135	T	E	
144	K	S	Site A
145	S	R	Site A
159	S	Y	
167	T	I	
189	K	N	Site B
190	N	D	
197	Q	R	
203	T	I	
227	P	S	
326	R	K	
aadiff		**17**	

***Reference vaccine strain in gray, swine strains in black**

Summary and Risk Assessment

During this reporting period, variant cases were reported in:

- **USA: H3N2v (1 2010.1; 1 1990.4a); H1N1v (1 1A.3.3.2, 2 1A.3.3.3); H1N2v (1 no sequence)**
- **Canada: H3N2v (3.1990.4.i); H1N1v (1A.3.3.2); H1N2v (1A.1.1)**
- **Denmark: H1N1v (1A.3.3.2)**
- **Germany: H1N1v (1C.2.1)**
- **China: H1N1v (4 1C.2.3)**
- **Australia: H3N2v (1990-like)**

Europe:

- The swine variant 1A.3.3.2 strain demonstrate reduced cross-reactivity to A/Brisbane/02/2018 and A/California/7/2009 and good cross-reactivity to A/Michigan/45/2015 (Table 1) but we note continued genetic heterogeneity in circulating 1A.3.3.2 lineage viruses in European pigs (Figure 7)
- The contemporary swine 1B.1.2.2 demonstrate poor cross-reactivity to A/Michigan/383/2018 and good cross-reactivity to A/Chile/1/1983 (Table 11).
- The A/swine/France/29-200254/2020 1C.2.4 strain (closest to the recent variant case – see Annex 2) was cross-reactive to the nearest 1C.2.1 clade CVV A/Netherlands/ 3315/2016 and 1C.2.3 A/Hunan/42443/2015 CNIC-1601 (Table 17).
- The contemporary swine clades 1C.2.1, 1C.2.2, 1C.2.4 and 1C.2.5 demonstrate good cross-reactivity to CVV A/Netherlands/3115/2016 (APHA), apart from A/swine/Italy/383470/2020 (1C.2.4) (Table 18)
- The swine 1C.2.2 variant virus A/Netherlands/Gent-193/2019 demonstrate good cross-reactivity to all the tested strains (Table 18).
- The contemporary swine clades 1C.2.1, 1C.2.2, 1C.2.4 and 1C.2.5 demonstrate variable cross-reactivity to CVV A/Hunan/42443/2015 and other CVVs in development: A/ A/Netherlands/10370-1b/2020 and A/Hessen/47/2020-C2/MDCK1 (Table 18).
- The contemporary swine 3.1970.1 demonstrate moderate to poor cross-reactivity to all the tested strains (Table 24).

North America:

- 1A.1.1 lineage viruses demonstrated a significant loss of cross-reactivity against the CVV A/Ohio/24/2017 (Table 1) and represent a consistent proportion of 1A lineage viruses detected in U.S. pigs (Figure 7 and A1).
- Global 1A.3.3.2 swine strains exhibit loss in cross-reactivity with current human seasonal vaccine strains.
- There was one confirmed variant case within the 1A.3.3.3 clade-3, A/Wisconsin/03/2021 H1N1v. 1A.3.3.3 clade-3 is a substantial proportion of 1A lineage viruses detected in U.S. pigs (Figure 7) and demonstrated loss in cross-reactivity against the 1A.3.3.3-clade 1 CVV A/Ohio/24/2015 (Table 1).

- 1B.2.2.2 (delta-1b) demonstrated a significant loss in cross-reactivity against the CVV A/Iowa/32/2016 (Table 10) and represents a consistent proportion of 1B lineage viruses detected in U.S. pigs (Figure 8 and A1).
- H3.1990.1 lineage viruses demonstrated a significant loss of cross-reactivity against the CVV A/Minnesota/ 11/2010 (Table 22) and represent a consistent proportion of H3 viruses detected in U.S. pigs (Figure 10 and A1).
- H3.1990.4.a lineage viruses demonstrated a significant loss of cross-reactivity against the CVV A/Minnesota/ 11/2010 (Table 22) and represent a substantial proportion of H3 viruses detected in U.S. pigs (Figure 10 and A1).
- H3.1990.4.b2 lineage viruses demonstrated a significant loss of cross-reactivity against the CVV A/Minnesota/ 11/2010 (Table 22) and represent a consistent proportion of H3 viruses detected in U.S. pigs (Figure 10 and A1).
- H3 2010.1 and 2010.2 lineage viruses had significant loss in cross-reactivity with both the 2010.1 CVV and to the human vaccine strain A/Iowa/60/2018. H3 2010.1 lineage viruses are a substantial proportion of H3 viruses detected in U.S. pigs (Figure 10 and A1).
- Intermittent evidence of sustained transmission was the basis for selecting swine strains from outside the U.S. for synthesis of HA/NA on a PR8 backbone for antigenic characterization. Many of these antigens showed low cross-reactivity to CVV or human seasonal vaccine strains.

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

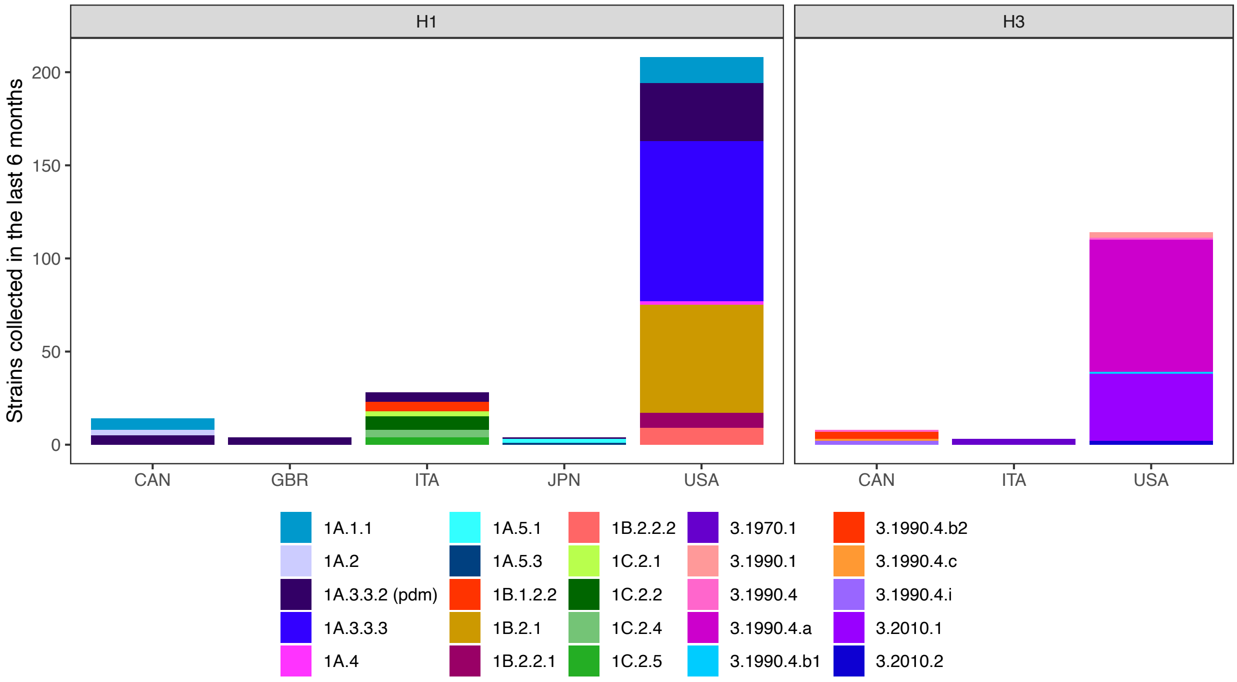


Figure A1. Summary of swine HA clades by country, colored by phylogenetic clade for sequences collected January 1, 2021 – June 30, 2021 (n=383).

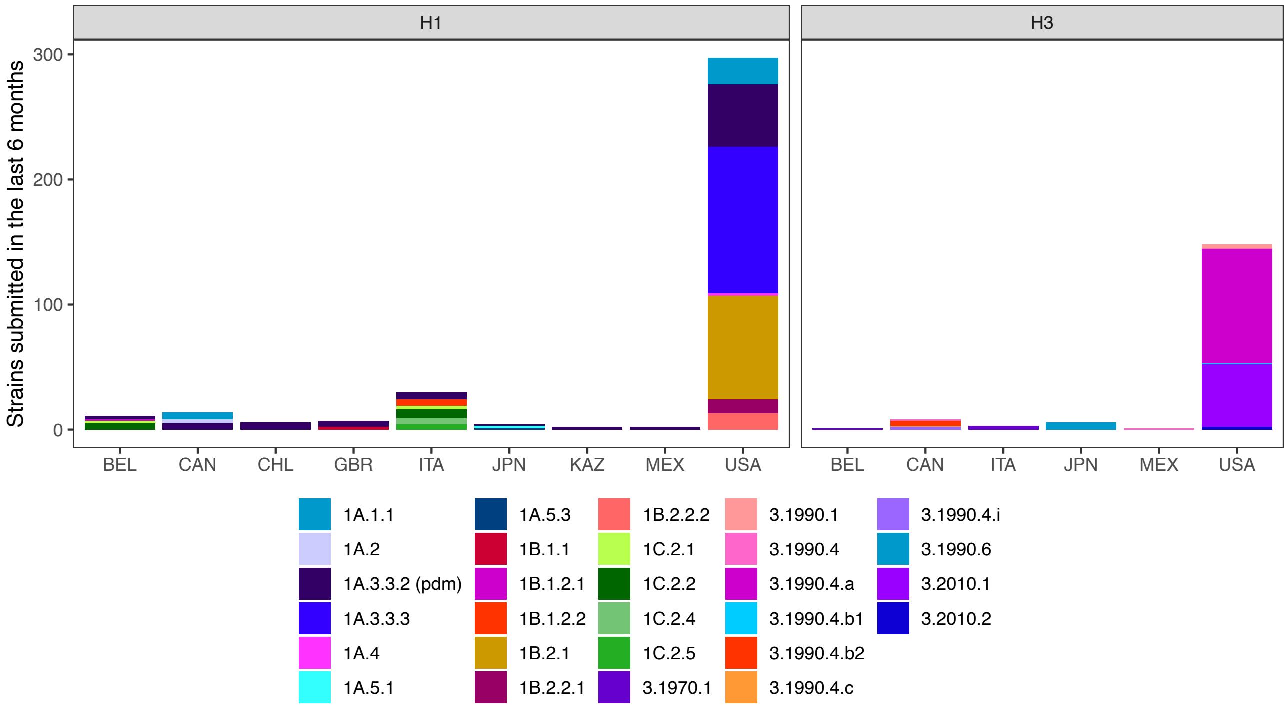


Figure A2. Summary of swine HA genes colored by phylogenetic clade for sequences deposited January 1, 2021 – June 30, 2021 and truncated to collected the last 24 months (n=540).

Table A1. Summary of swine H1 clades by country, data deposited January 2021 - June 2021.

clade	country	count
1A.1.1	CAN	6
1A.1.1	USA	21
1A.2	CAN	3
1A.3.3.2	BEL	7
1A.3.3.2	CAN	5
1A.3.3.2	CHL	6
1A.3.3.2	GBR	5
1A.3.3.2	ITA	6
1A.3.3.2	JPN	33
1A.3.3.2	KAZ	2
1A.3.3.2	MEX	2
1A.3.3.2	RUS	4
1A.3.3.2	USA	51
1A.3.3.3	USA	120
1A.4	USA	2
1A.5.1	JPN	65
1A.5.2	JPN	23
1A.5.3	JPN	79
1B.1.1	GBR	2
1B.1.2.1	BEL	15
1B.1.2.1	NLD	1
1B.1.2.2	ITA	5
1B.2.1	USA	83
1B.2.2.1	USA	38
1B.2.2.2	USA	13
1C.2.1	BEL	17
1C.2.1	DEU	3
1C.2.1	ITA	4
1C.2.2	BEL	10
1C.2.2	ITA	7
1C.2.2	NLD	1
1C.2.4	ITA	5
1C.2.4	RUS	3
1C.2.5	ITA	4

Table A2 Summary of swine H3 clades by country by country, data deposited January 2021 -June 2021.

clade	country	count
3.1970.1	BEL	1
3.1970.1	ITA	4
3.1990.1	USA	3
3.1990.4	CAN	1
3.1990.4	MEX	2
3.1990.4	USA	1
3.1990.4.a	USA	92
3.1990.4.b1	USA	1
3.1990.4.b2	CAN	4
3.1990.4.c	CAN	1
3.1990.4.i	CAN	2
3.1990.6	JPN	10
3.2010.1	USA	55
3.2010.2	USA	2

Annex 2. Late Breaking Variant Detections

USA

A/Wisconsin/04/2021 (H1N1v) 1A.3.3.3 in July 2021
A/Wisconsin/05/2021 (H1N1v) 1A.3.3.3 in August 2021
A/Iowa/03/2021(H3N2v) H3.1990.4a in August 2021
A/Iowa/04/2021 (H1N2v) 1B.2.1 in August 2021
A/Ohio/3002857642/2021 (H1N2v) 1B.2.1 in September 2021

Taiwan

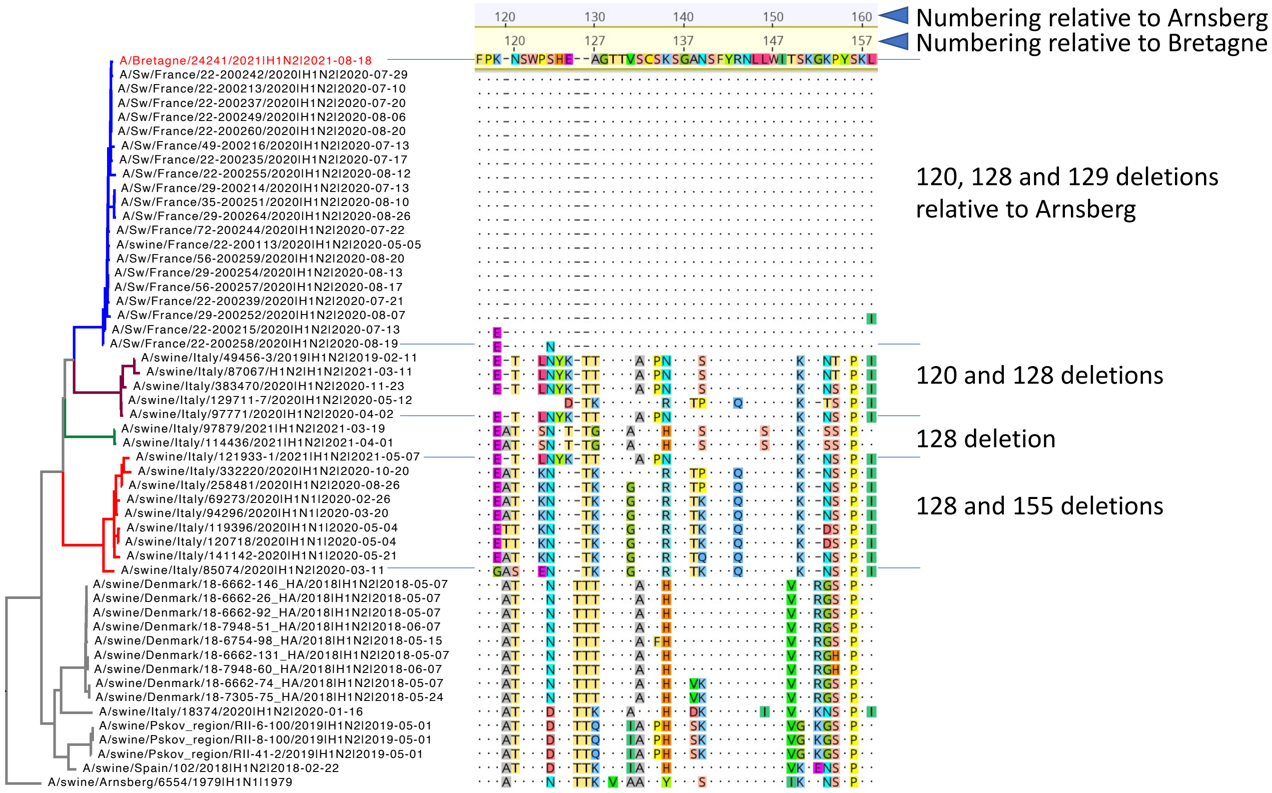
A/Taiwan/1/2021 (H1N1v) in March 2021

This HA gene indicates that a clade with no recent evidence of continued circulation in swine is active and requires an updated swine clade designation, **1A.1.4**.

France

A/Bretagne/24241/2021 (H1N2v) 1C.2.4 in August, 2021.

This 1C.2.4 HA contains 3 amino acid deletions previously described and similar to HI tested strain A/swine/France/29-200254/2020 (Table 17, main report).



1C Eurasian avian lineage

Annex 3. Phylogenies of swine HA lineages with tree backbone annotated by inferred amino acid mutations.

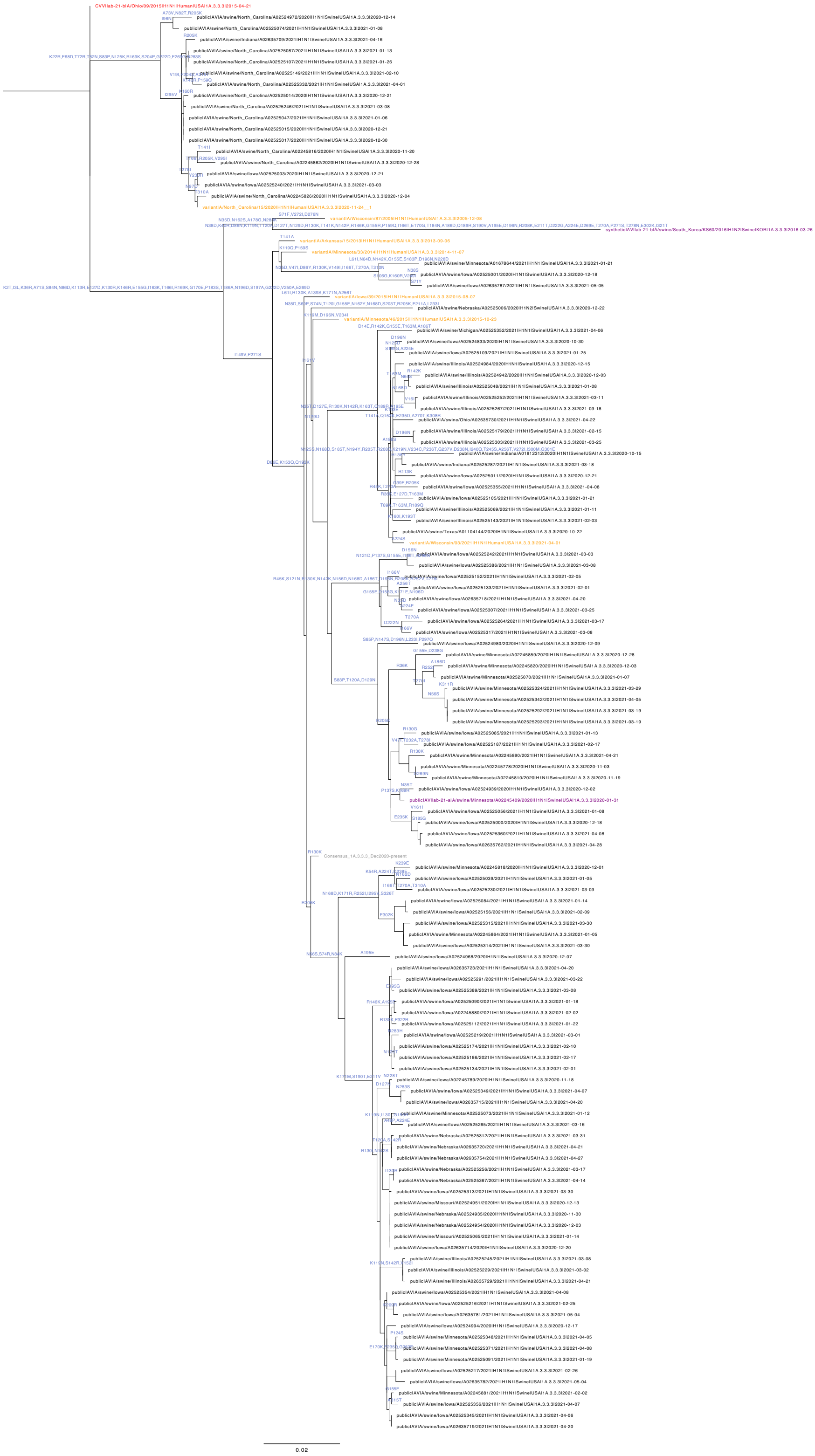


Figure A3. A phylogeny of the **1A.3.3.3** clade displaying sequences deposited January 1, 2021 – June 31, 2021 (n=118) and 10 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Ohio/09/2015) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 7.

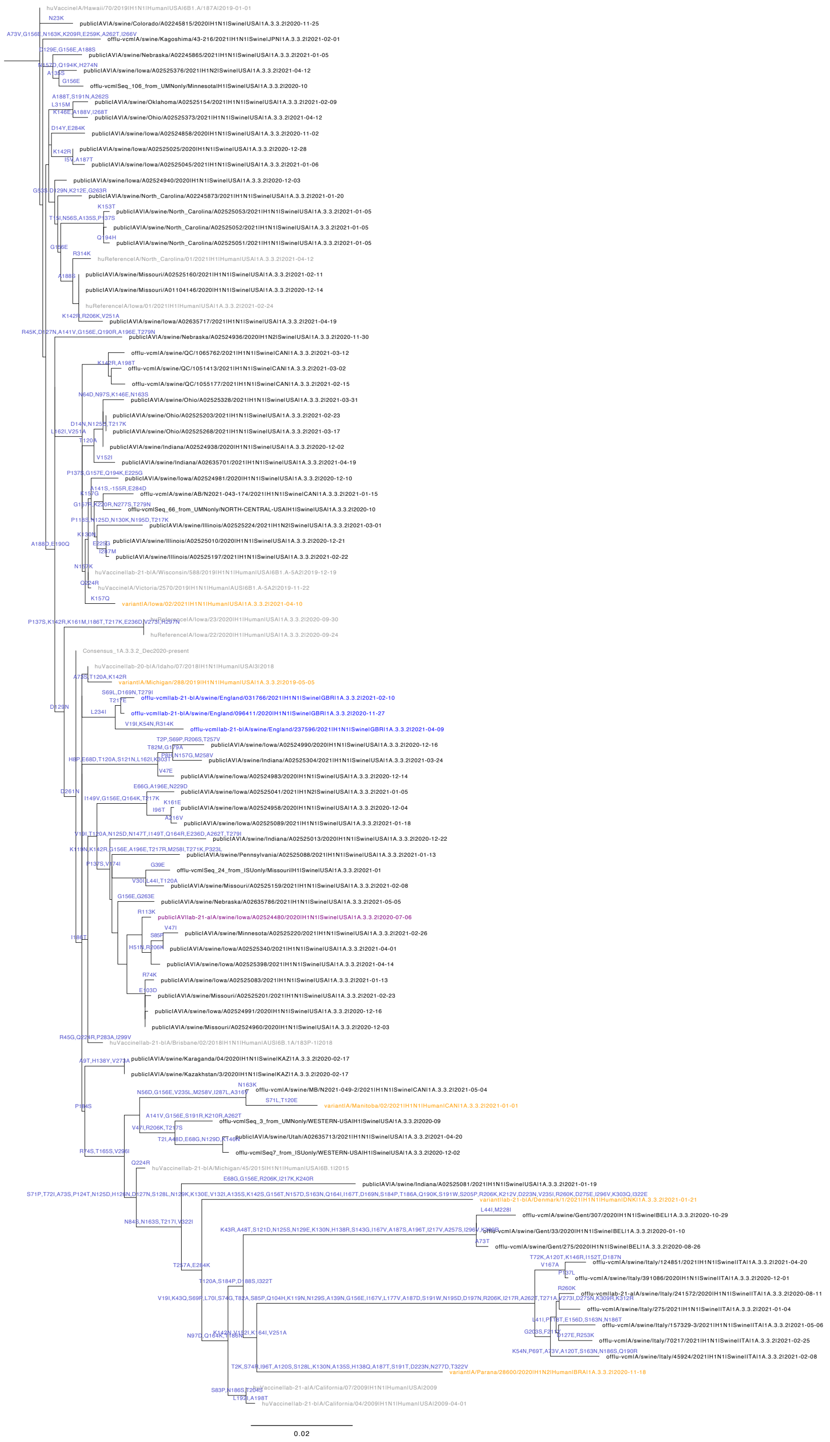


Figure A4. A phylogeny of the **1A.3.3.2 (pdm)** clade displaying n=70 sequences deposited January 1, 2021 – June 30, 2021 and n=20 reference genes. The clade is rooted relative to the most recent human vaccine strain (A/Hawaii/70/2019) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 7.

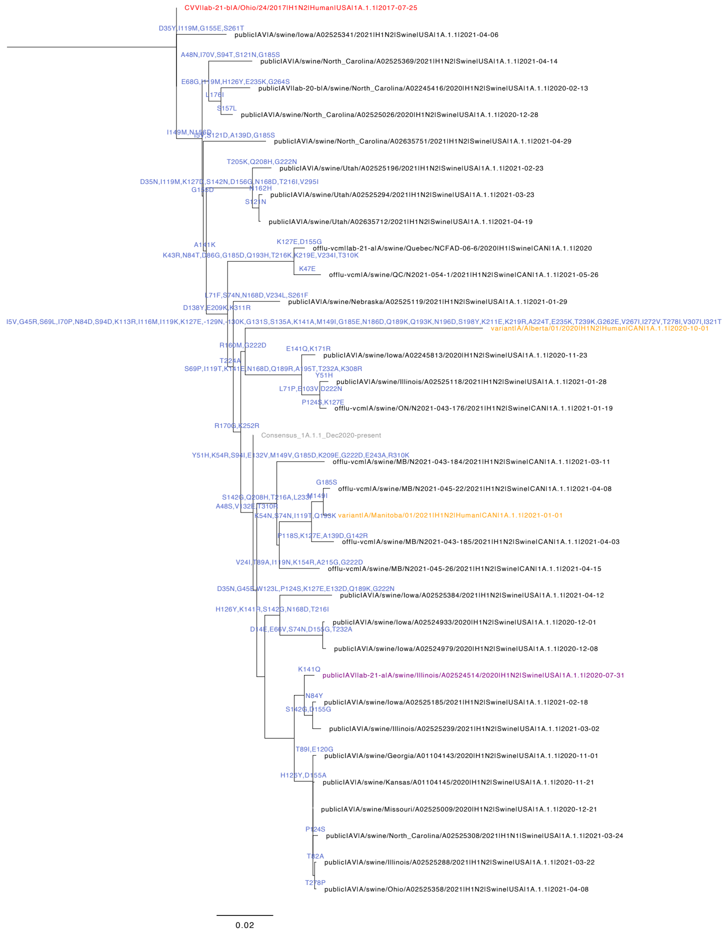


Figure A5. A phylogeny of the **1A.1.1** clade displaying n=27 sequences deposited January 1, 2021 – June 30, 2021 and n=7 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Ohio/24/2017) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 7.

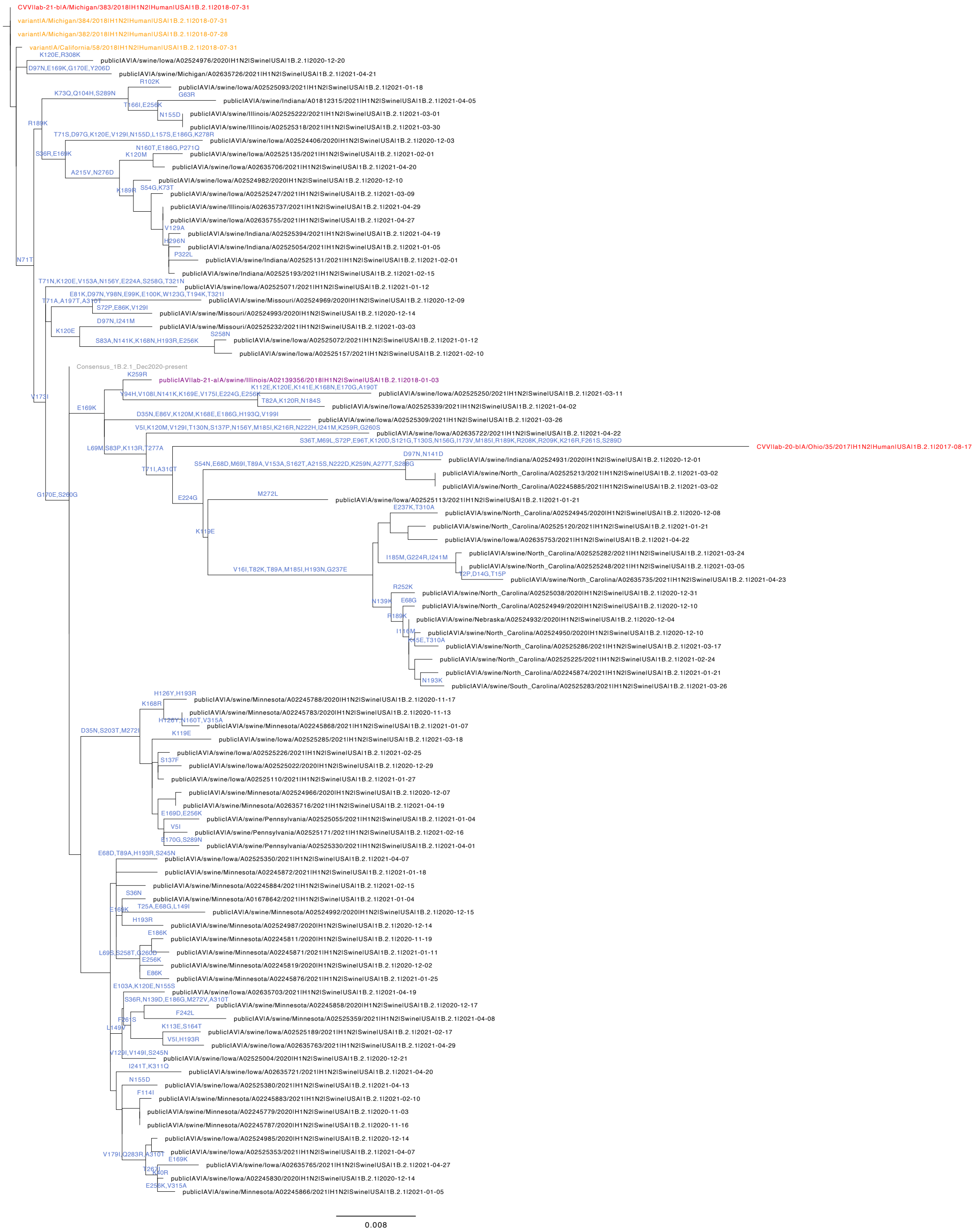


Figure A6. A phylogeny of the **1B.2.1** clade displaying n=83 sequences deposited January 1, 2021 – June 30, 2021 and n=7 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Michigan/383/2018) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 8.

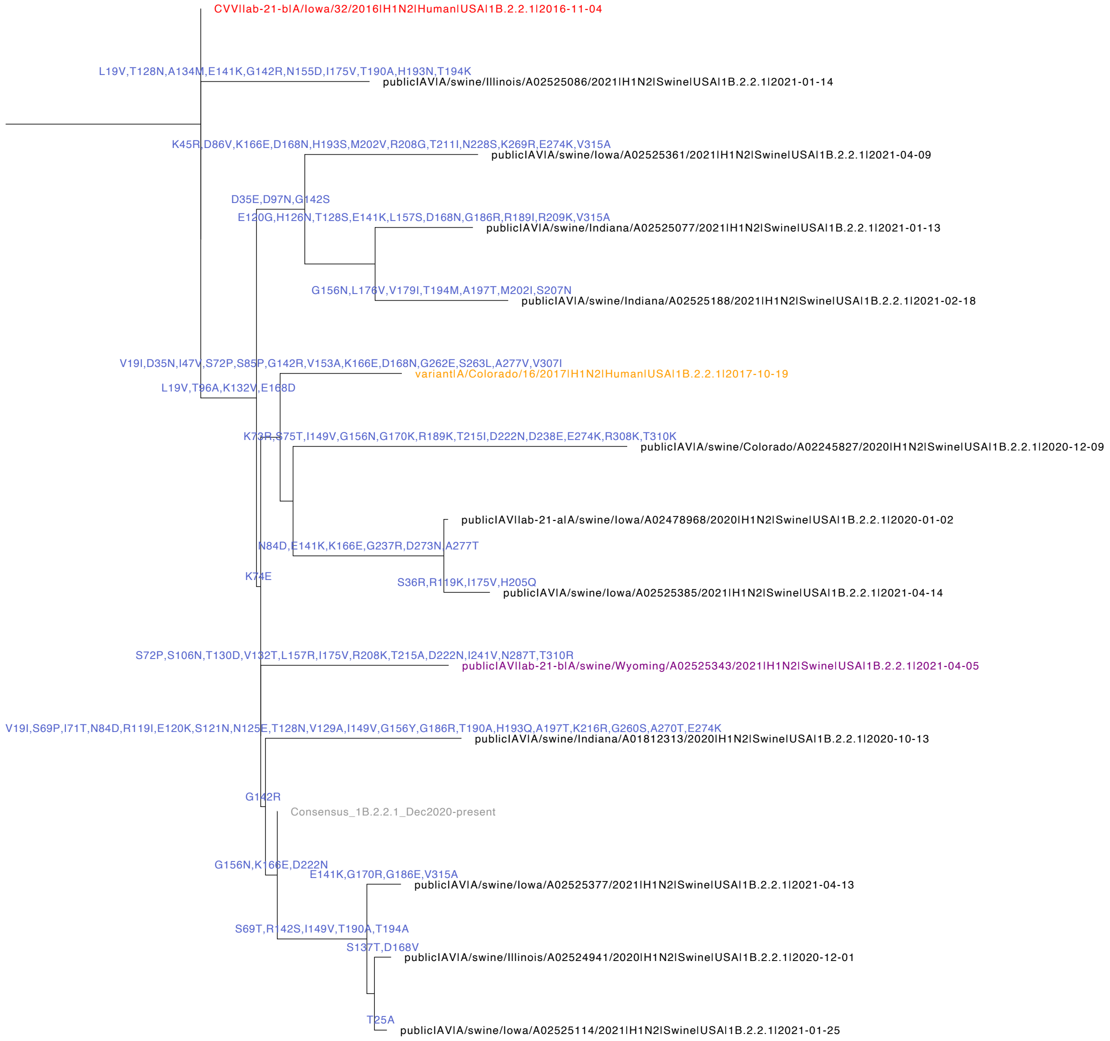


Figure A7. A phylogeny of the **1B.2.2.1** clade displaying n=10 sequences deposited January 1, 2021 – June 30, 2021 and n=5 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Iowa/32/2016) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 8.

Figure A8. A phylogeny of the **1C.2.1** clade displaying n=22 sequences deposited January 1, 2021 – June 30, 2021 and n=15 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Netherlands/3315/2016) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 9.

Figure A9. A phylogeny of the **1C.2.2** clade displaying n=12 sequences deposited January 1, 2021 – June 30, 2021 and n=3 reference genes. The clade is rooted relative to the most recent 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 in consistence with Figure 9.

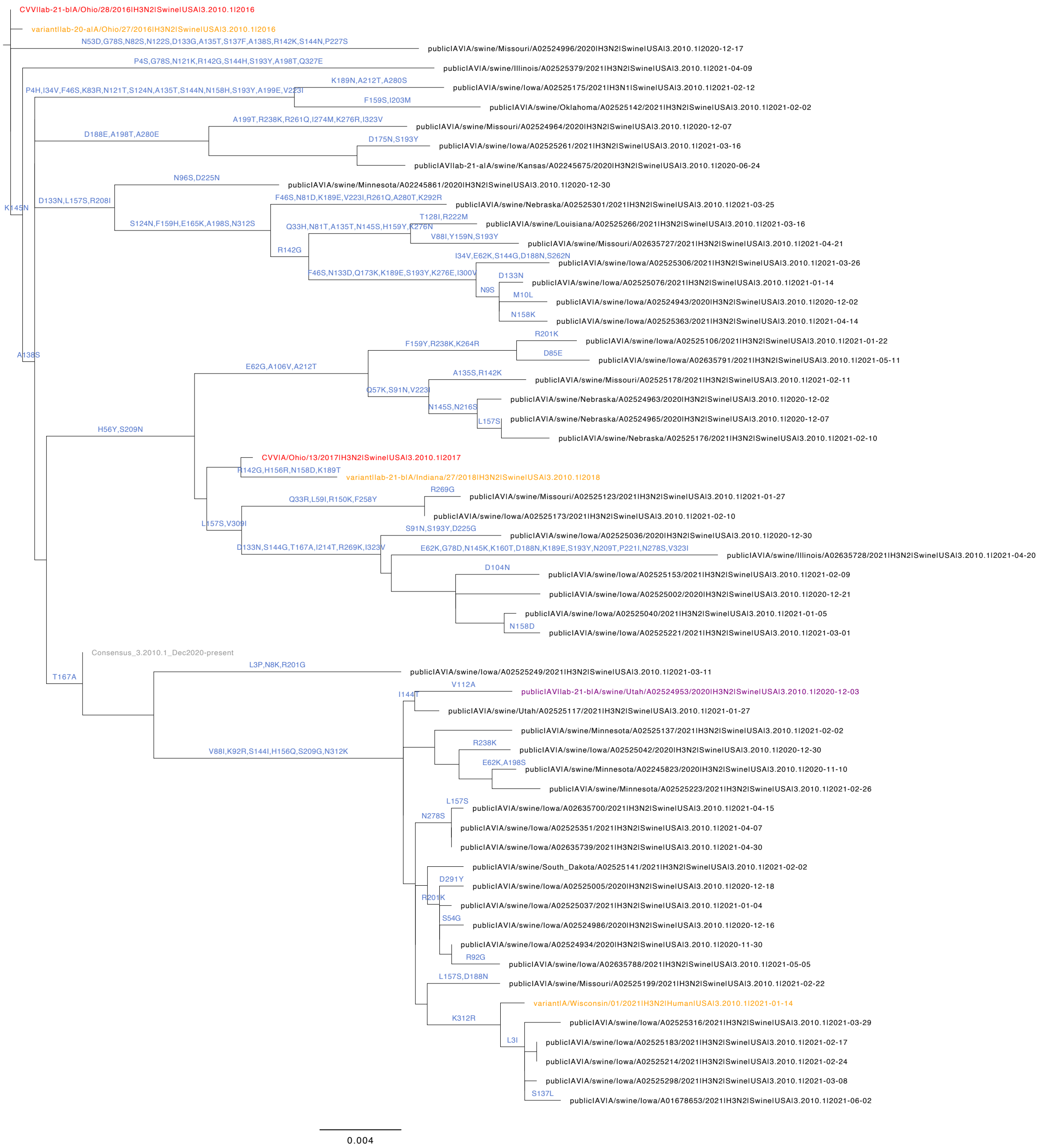


Figure A10. A phylogeny of the **3.2010.1** clade displaying n=50 sequences deposited January 1, 2021 – June 30, 2021 and n=7 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Ohio/28/2016) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 10.

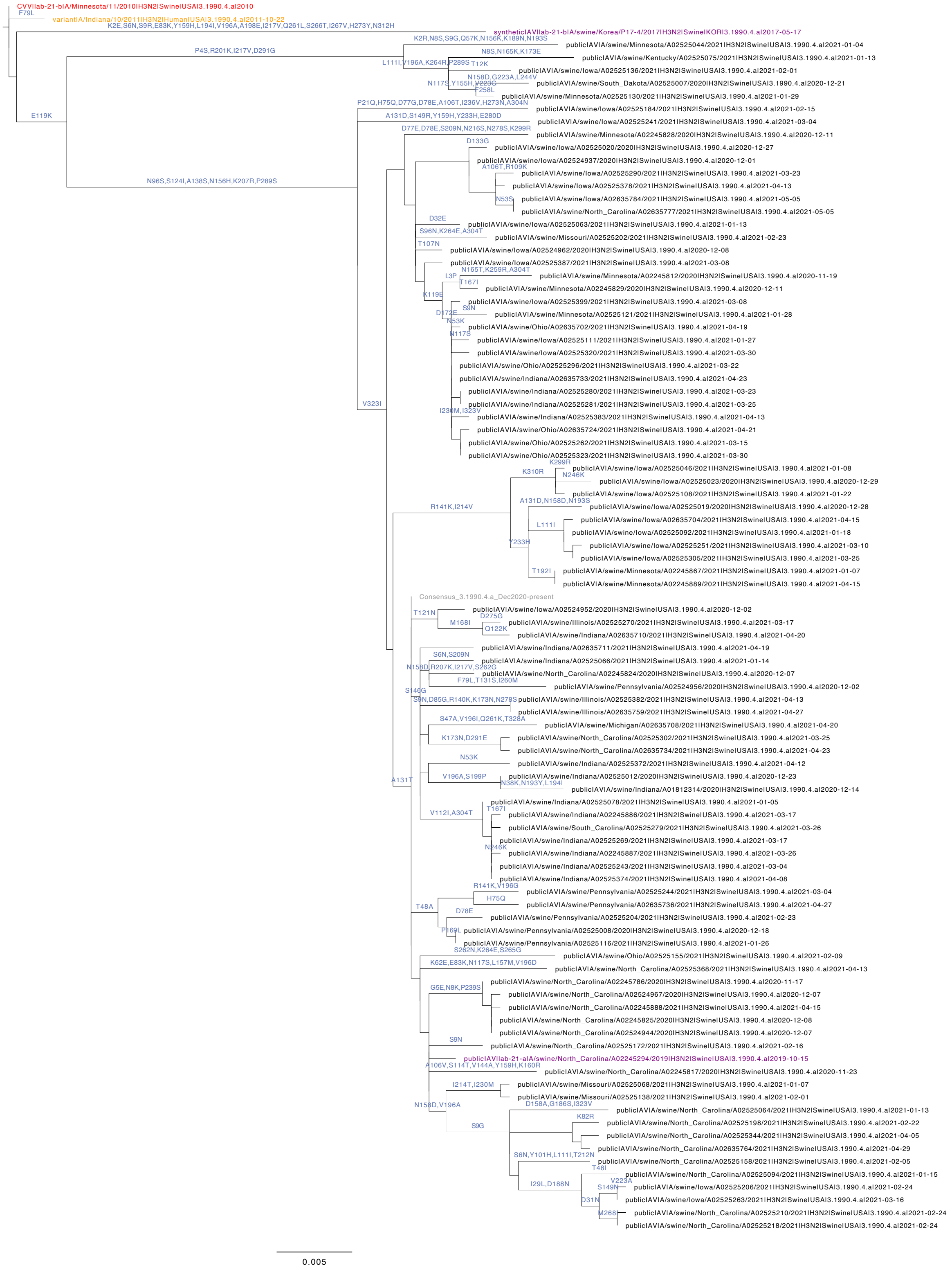


Figure A11. A phylogeny of the **3.1990.4.a** clade displaying n=91 swine sequences deposited January 1, 2021 – June 30, 2021 and n=5 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Minnesota/11/2010) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 10.