



# OFFLU SWINE INFLUENZA REPORT

JULY 2021 TO DECEMBER 2021

## 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 Jul 1, 2021 – Dec 31, 2021) were 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>). 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

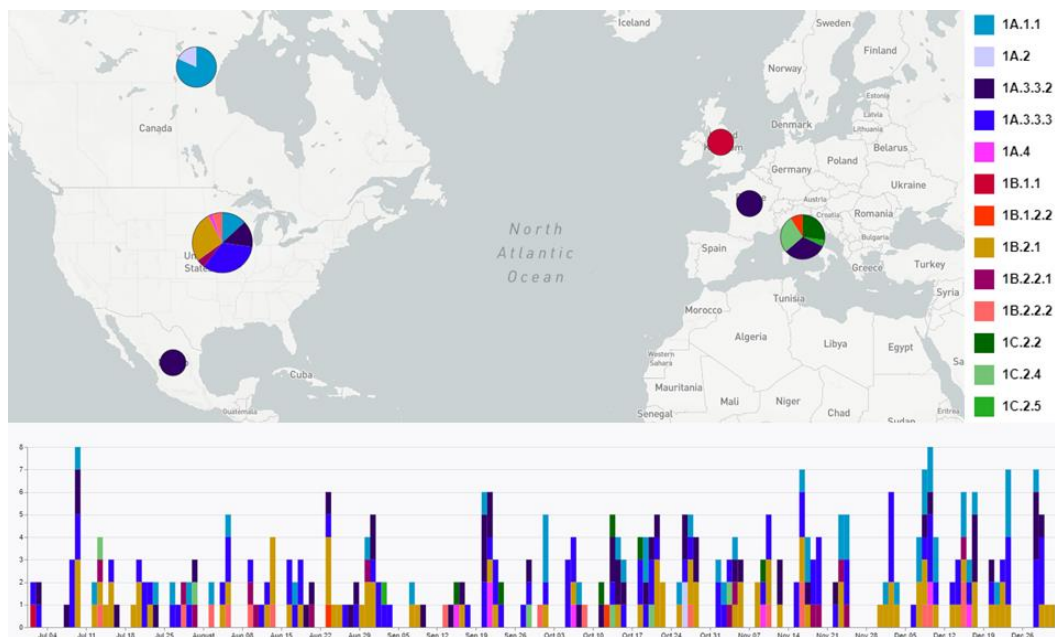


Figure 1. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between July 1 2021 – December 31, 2021 (n=347); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions.

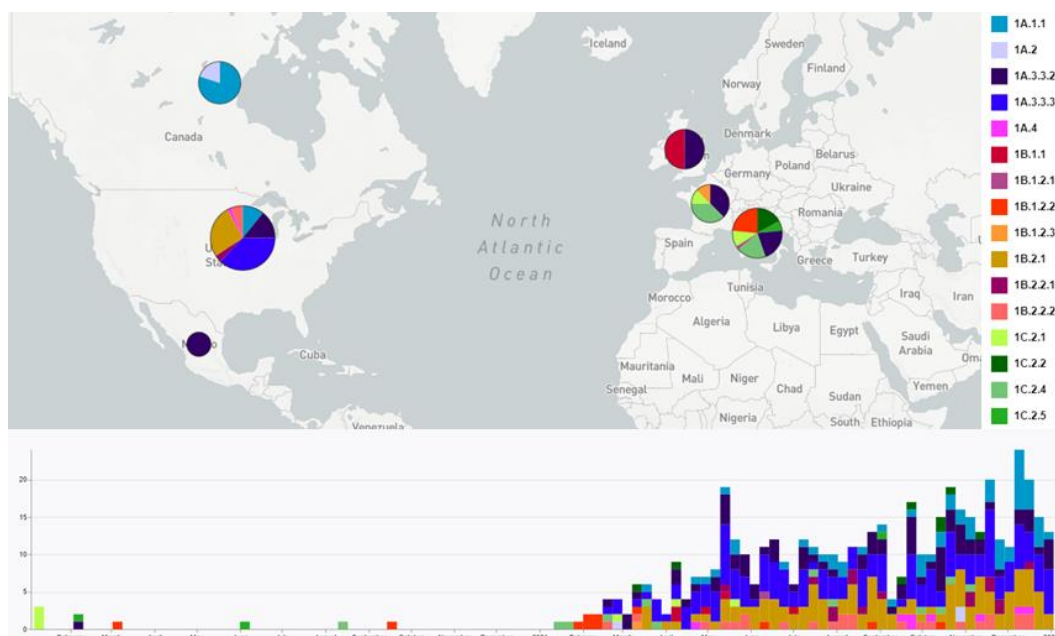


Figure 2. MicroReact map of swine IAV H1 HA genes colored by phylogenetic clade for sequences collected from January 1, 2020 – December 31, 2021 and deposited to GISAID or GenBank between July 1, 2021– December 31, 2021 (n=507); collection date is represented on bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters. Geographic nodes scaled to detection proportions.

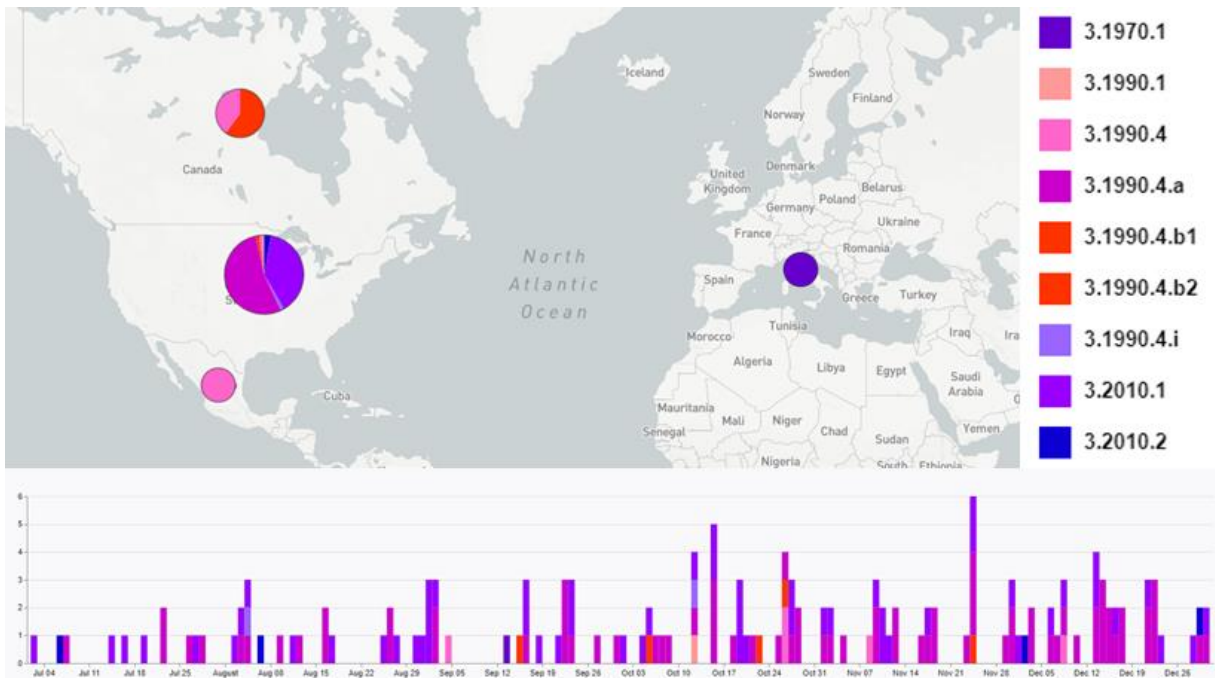


Figure 3. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected and deposited to GISAID or GenBank between July 1, 2021– December 31, 2021 (n=151). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions.

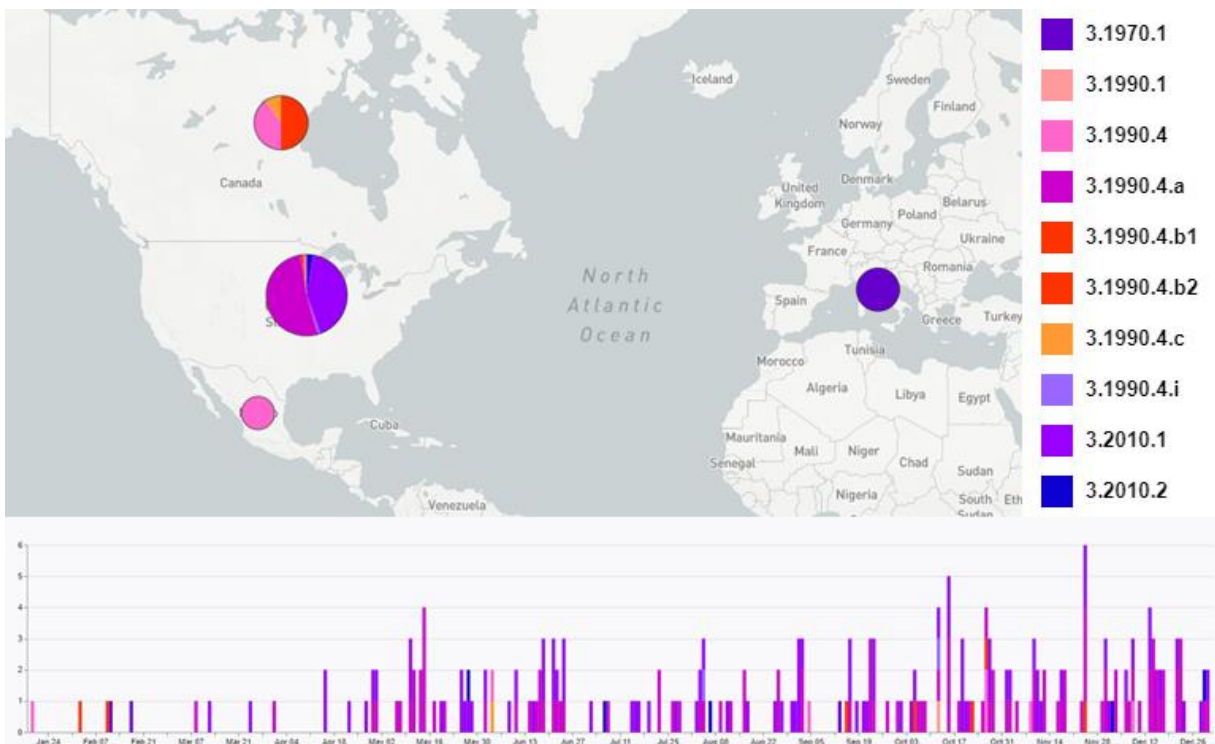


Figure 4. MicroReact map of swine IAV H3 HA genes colored by phylogenetic clade for sequences collected from January 1, 2020 – December 31, 2021 and deposited to GISAID or GenBank between July 1, 2021– December 31, 2021 (n=216). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. Geographic nodes scaled to detection proportions.

## Contemporary Global H1 swine IAV: genetic diversity

### H1 1A swine genetic clades

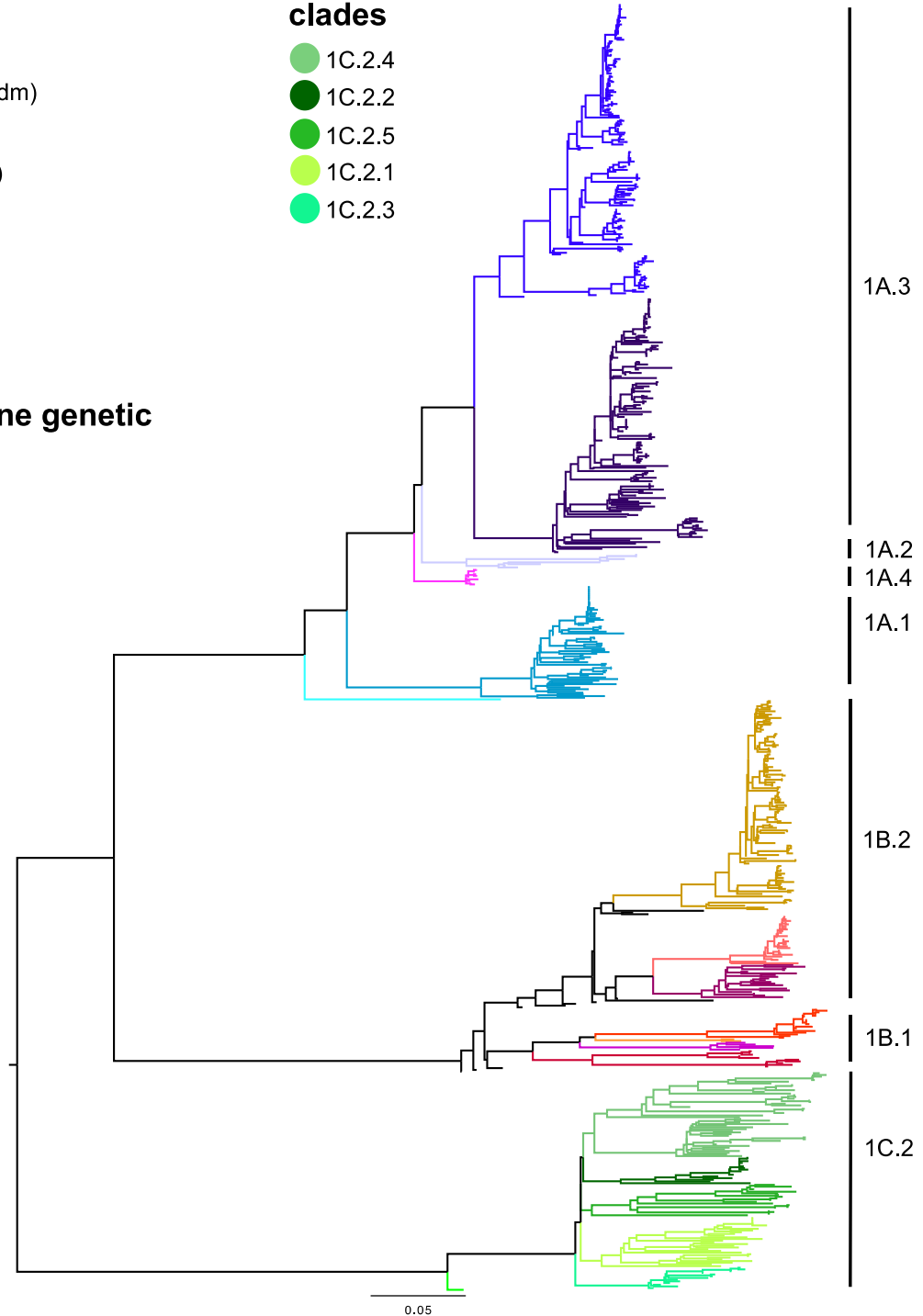
- 1A.3.3.3
- 1A.3.3.2 (pdm)
- 1A.2
- 1A.4 (LAIV)
- 1A.1.1
- 1A.1.4

### H1 1C swine genetic clades

- 1C.2.4
- 1C.2.2
- 1C.2.5
- 1C.2.1
- 1C.2.3

### H1 1B swine genetic clades

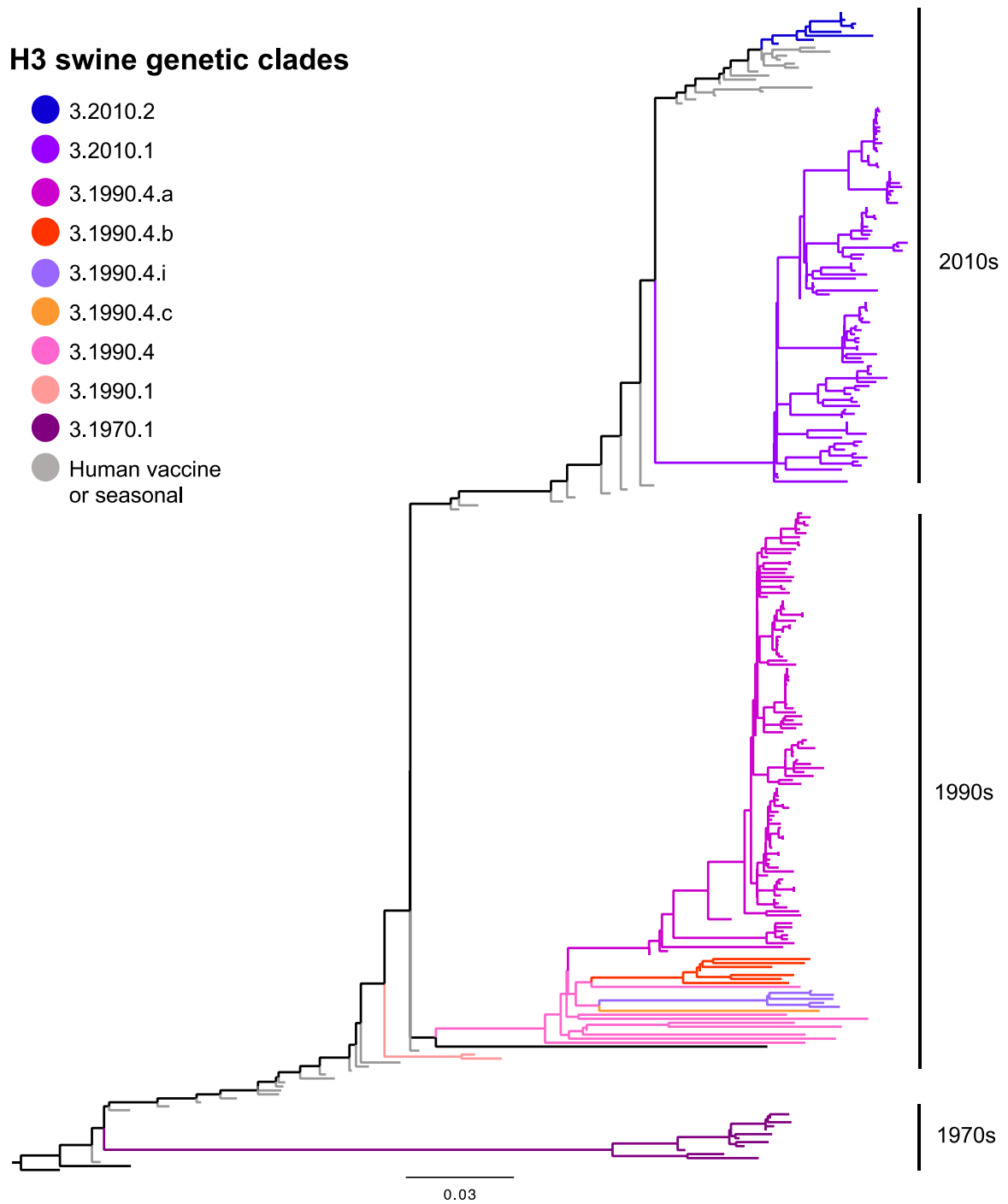
- 1B.2.1
- 1B.2.2.2
- 1B.2.2.1
- 1B.1.2.2
- 1B.1.2.3
- 1B.1.2.1
- 1B.1.1



*n=593, data deposited within last 6 months.*

Figure 5. Global swine H1 phylogenetic tree colored by clade and annotated by global H1 lineage nomenclature. Analyses were conducted with reference sequences and data (all data deposited July 1 2021 – December 31, 2021) from GISAID or shared via the OFFLU swine IAV working group.

## Contemporary Global H3 swine IAV: genetic diversity



*n=219, data deposited within last 6 months, and n=76 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 data (all data deposited July 1, 2021 – December 31, 2021) from GISAID or shared via the OFFLU swine IAV working group.

## Regional geographic summary

Phylogenetic analysis identified nineteen genetic clades from H1 and H3 IAV in swine collected between July 1, 2021 – December 31, 2021.

Twelve clades were H1 subtype, with detections from each of the lineages: 1A classical swine lineage (5 clades); 1B human-seasonal lineage (4 clades); and 1C Eurasian avian lineage (3 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. The 1A.3.3.2/pdm circulated in all countries that collected sequence data during this 6 month 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 detected in Europe. There were no new sequence data reported from the HA clade 1C.2.3 in China.

Seven clades were within five distinct lineages derived from human seasonal H3 virus spillovers grouped by the decade of introduction into swine (1970.1; 1990.1; 1990.4; 2010.1; 2010.2). The 1990.4 lineage diversified into three co-circulating genetic clades.

- The H3 1990.1, 2010.1, and 2010.2 clades only detected in the USA, the H3 1970.1 clade in Europe. The 1990.4 lineage circulates in the USA, Canada, and Mexico.

### Global Variant Cases:

During the reporting period (July 1, 2021 – December 31, 2021), 15 variant cases were reported and included with the swine analyses:

- **USA:** H1N1v (3 1A.3.3.2/pdm, 2 1A.3.3.3); H1N2v (3 1B.2.1); H1v (no sequence); H3N2v (1 2010.1; 1 1990.4a)
- **Canada:** H1N2v (no sequence)
- **Denmark:** H1N1v (1A.3.3.2)
- **France:** H1N2v (1C.2.4, recommended as a CVV A/Bretagne/24241/2021)
- **Austria:** H1N2v (1C.2.4)

An additional 6 variant cases were reported July 1, 2021 – December 31, 2021, but collected outside the report window. The following HA sequences were included in the phylogenetic trees:

- **Taiwan:** H1N2v (1A.1.4, collected 03/12/2021)
- **China:** H1N1v (4 1C.2.3, collected 01/01/2021, 01/27/2021, 02/21/2021, 03/09/2021)
- **Australia:** H3N2v (Other-Human-1990, collected 01/27/2021 see Annex 3)



# 1A classical swine lineage

- ★ H1v candidate vaccine virus
- A/Wisconsin/588/2019
- H1N1pdm seasonal vaccines
- Reported H1v cases
- + New variant(s)
- # HI tested viruses
- Requested/pending HI
- ▲ Consensus sequence

# H1 1A swine genetic clades

- 1A.3.3.3 (n=159)
- 1A.3.3.2/pdm (n=116)
- 1A.2 (n=3)
- 1A.4/LAIV (n=8)
- 1A.1.1 (n=58)
- 1A.1.4 (n=1)

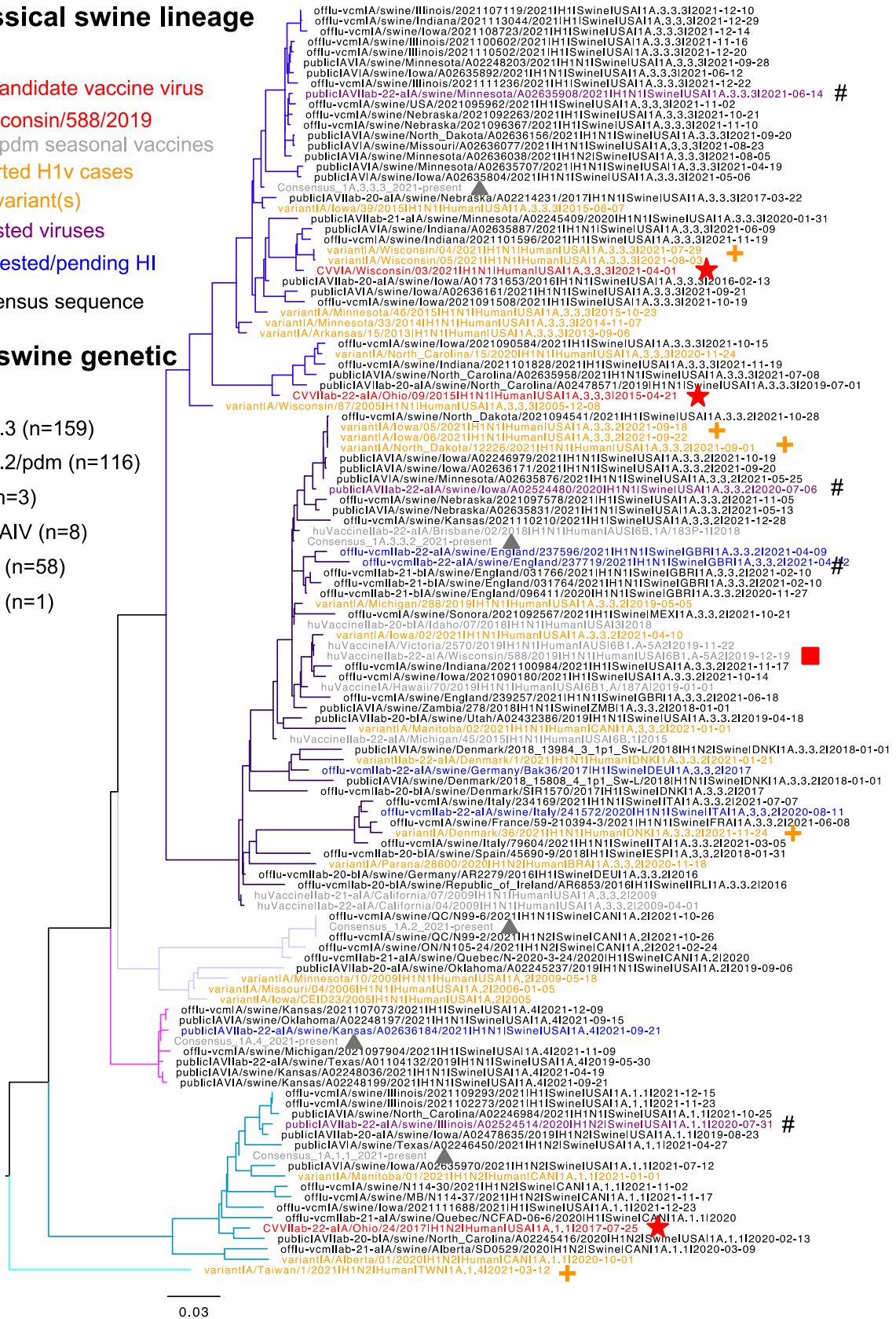


Figure 7. Swine H1 HA genes of the 1A lineage (tree was proportionally downsampled to 65 swine HA genes, excluding references): detections of each clade from data deposited between July 1, 2021 - December 31, 2021 are presented adjacent to the clade name.

## 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 CVV RG48A
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
A/Wisconsin/588/2019 6B1.A-5A2	6B1.A-5A2	H1N1pdm09	<10	1280	20
A/swine/Iowa/A02524480/2020*	1A.3.3.2	H1N1pdm09	20	80	<10
A/Ohio/9/2015 CVV RG48A	1A.3.3.3	gamma.1 H1N1v	<10	10	320
A/swine/Minnesota/A02635908/2021	1A.3.3.3	gamma.3	<10	40	20
A/swine/Kansas/A02636184/2021	1A.4	gamma-2- beta-like	<10	20	40

Reference CVV in red, seasonal vaccine strains in dark red, new swine strains in bold. Homologous titers highlighted in grey. \*Previously tested in report 2021a; \*\*Previously tested in report 2020b

- 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 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 (gamma) clade 3 virus had a 16-fold decrease with the 1A.3.3.3-clade 1 CVV A/Ohio/09/2015.
- The contemporary swine 1A.4 (gamma-2-beta-like) virus had a significant 8-fold decrease 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/California/7/2009	A/Brisbane/02/2018	A/Michigan/45/2015
A/California/4/2009	H1N1pdm09	1280	640	320
A/Brisbane/02/2018	6B.1A/183-P1 H1N1pdm09	1280	1280	640
A/Michigan/45/2015	6B.1 H1N1pdm09	320	320	320
A/swine/Italy/241572/2020	1A.3.3.2 H1N1pdm09	160	80	80
A/Denmark/1/2021	1A 3.3.2 H1N1v	640	320	320
A/swine/Germany/Bak36/2017	1A.3.3.2 H1N1pdm09	160	320	80

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

- The swine 1A.3.3.2 clade diversity reflects multiple human-to-swine transmission events. A/swine/Germany/Bak36/2017 was included as it is genetically similar to the H1N1v A/Denmark/1/2021 (Jan 2021).
- A/swine/Italy/241572/2020 clusters genetically with A/Denmark/36/2021 – a recently reported variant for which we did not have contemporaneous swine viruses or the human variant isolate.
- The contemporary swine 1A.3.3.2 (pdm09) viruses in Europe had decreased cross-reactivity to human seasonal vaccine strains and different patterns of cross-reactivity within the swine endemic strains.
- The variant A/Denmark/1/2021 cross reacted with the H1N1pdm09 CVV A/California/7/2009.

Table 3. Amino acid substitutions between representative swine 1A.1.1 clade strain compared to the within-clade CVV (**IDCDC-RG59 A/Ohio/24/2017-like CVV**).

Site	<b>IDCDC-RG59 A/Ohio/24/2017-like CVV</b>	A/swine/Illinois/A02524514/2020	Annotations
48	A	S	
132	V	E	
138	D	Y	
141	A	Q	
149	I	M	
155	G	D	Sa
156	N	D	Sb
170	R	G	Ca1
209	E	K	
224	T	A	
252	K	R	
310	T	R	
311	K	R	
<b>aadiff</b>		<b>13</b>	

Reference CVV in red, swine strains in black.

Table 4 USA: Amino acid substitutions between a representative swine 1A.3.3.2 clade strains compared to a human seasonal H1 vaccine strain (A/Wisconsin/588/2019|6B1.A-5A2) used in HI assays. Within-clade H1N1v strains reported this period are colored orange.

Site	A/Wisconsin/588/2019 6B1.A-5A2	A/swine/Iowa/A02524480/2020	A/North_Dakota/12226/2021	A/Iowa/06/2021	A/Iowa/05/2021	Annotations
47	V		I	I	I	
51	H		N	N	N	
85	S		P	P	P	
113	R	K				
129	D	N	N	N	N	
130	N	K	K	K	K	
137	P	S	S	S	S	Ca2
156	K	N	N	N	N	Sb
161	I	L	L	L	L	
173	V	I	I	I	I	
185	I	T	T	T	T	
205	R		K	K	K	
250	A	V	V	V	V	
260	D	N	N	N	N	
<b>aadiff</b>		<b>10</b>	<b>13</b>	<b>13</b>	<b>13</b>	

Reference vaccine strain in gray, swine strains in black, variant detections in orange.

Table 5. EU: Amino acid substitutions between representative swine 1A.3.3.2 clade strains compared to a human seasonal H1 vaccine strain (A/Michigan/45/2015|6B.1) used in HI assays. The within-clade H1N1v strain reported this period is colored orange.

Site	A/Michigan/45/2015 6B.1	A/swine/Italy/241572/2020	A/swine/Germany/Bak36/2017	A/swine/England/237596/2021	A/Brisbane/02/2018 6B.1A/183P-1	A/California/04/2009	A/Denmark/36/2021	Annotations
3	L			X				
19	V	I		I			I	
43	K	Q					Q	
45	R				G			
54	K			N				
69	S	P					T	
70	L	I					I	Cb
74	S	G		R	R		G	Cb
82	T	A					A	
83	S					P		
84	N	S	S			S	S	
85	S	P					P	
97	N	D				D	D	
104	Q	H					H	
119	K	N					N	
120	T	A						
123	W		G					
124	P		T					Sa
125	N		D					Sa
126	H		N					
127	D		N					
128	S		L					
129	N	S	K				S	
130	K		E					
132	V		A					
135	A		S					
139	A	N					N	
142	K	N	S				S	Ca2

149	I		M					
152	V	I					I	
155	G	E	T				E	Sa
156	N		D					Sb
162	N	S				S		Sa
163	Q	I	I			K	I	Sa
164	S			T	T			Sa
166	I	V					V	Ca1
168	D		N					
176	L	V					V	
183	S	P	P	P	P		P	
185	T	N	A			S	S	
186	A	D	V				D	
187	D	S					S	
190	S	W	R				W	Sb
191	L					I		
194	N	D					D	
196	D	N					N	
197	A					T		
202	G	S					S	
203	T					S		
205	R	K					K	
210	F	Y					Y	
216	T	R	I			I	R	
222	D		N					Ca2
223	R	Q	Q	Q		Q	Q	
228	N		D					
233	L			I				
250	V	A					A	
256	T	A	A			A	A	
259	R	K						
261	A	T					T	
270	T	A					A	
272	V	I					I	
274	D	N	E				N	
282	P				A			
283	E	K	K			K	K	
295	I		V	V	V			
298	I				V			
302	K		Q					
308	K	R					R	
311	K	R					R	

313	R			K				
321	V	T	D			I	T	
<b>aadiff</b>		<b>44</b>	<b>31</b>	<b>10</b>	<b>7</b>	<b>14</b>	<b>41</b>	

Reference human seasonal vaccine in gray, swine strains in black, variant detection in orange.



Table 6. Amino acid substitutions between representative swine 1A.3.3.3 clade strain compared to the within-clade CVV (A/Ohio/09/2015 RG48A) used in the HI assay, and the most similar within-clade CVV (A/Wisconsin/03/2021). Within-clade H1N1v detected within this reporting period are presented in orange.

Relative to HI						
Site	A/Ohio/09/2015 RG48A	A/swine/Minnesota/A02635908/2021	A/Wisconsin/03/2021	A/Wisconsin/04/2021	A/Wisconsin/05/2021	Annotations
2	K	T	T	T	T	
3	I	L	L	L	L	
35	N		T	T	T	
36	K	R	R	R	R	
45	R		K	K	K	
48	X	A	A	A	A	
56	N	S				
71	A	S	S	S	S	Cb
74	S	R				Cb
84	S	K	N	N	N	
86	N	E	E	E	E	
113	K	R	R	R	R	
127	E	D				
129	N		D	D	D	
142	N	S	R	R	R	Ca2
146	K	R	R	R	R	
149	I	V	V	V	V	
153	K	Q	Q	Q	Q	Sb
155	E	G	G	G	G	Sa
161	I		V	V	V	
163	I	K	T	T	T	Sa
166	T	I	I	I	I	Ca1
169	R	K	K	K	K	
170	G	E	E			Ca1
171	K	M				

Relative to most similar CVV						
Site	A/Wisconsin/03/2021	A/swine/Minnesota/A02635908/2021	A/Ohio/09/2015	A/Wisconsin/04/2021	A/Wisconsin/05/2021	Annotations
2	T		K			
3	L		I			
35	T	N	N			
36	R		K			
45	K	R	R			
48	A		X			
56	N	S				
71	S		A			Cb
74	S	R				Cb
84	N	K	S			
86	E		N			
113	R		K			
127	E	D				
129	D	N	N			
142	R	S	N			Ca2
146	R		K			
149	V		I			
153	Q		K			Sb
155	G		E			Sa
161	V	I	I			
163	T	K	I			Sa
166	I		T			Ca1
169	K		R			
170	E		G	G	G	Ca1
171	K	M				

183	P	S	S	S	S	
186	T	A	A	S	S	
189	Q		R			Sb
190	S	T				Sb
193	Q	K	K	K	K	Sb
195	A		E	E	E	Sb
196	N	D	D	D	D	
197	S	A	A	A	A	
205	R	K				
211	E	V				
222	G	D	D	D	D	Ca2
224	A		S			
250	V	A	A	A	A	
269	E	D	D	D	D	
270	T		A	A	A	
271	P	S	S	S	S	
323	S	X				
<b>aadiff</b>		<b>34</b>	<b>34</b>	<b>31</b>	<b>31</b>	

183	S		P			
186	A		T	S	S	
189	R	Q	Q	Q	Q	Sb
190	S	T				Sb
193	K		Q			Sb
195	E	A	A			Sb
196	D		N			
197	A		S			
205	R	K				
211	E	V				
222	D		G			Ca2
224	S	A	A	A	A	
250	A		V			
269	D		E			
270	A	T	T			
271	S		P			
323	S	X				
<b>aadiff</b>		<b>19</b>	<b>34</b>	<b>4</b>	<b>4</b>	

Reference CVV in red, swine strains in black, variant detections in orange.

Table 7. Amino acid substitutions between 1A.4 compared to the human H1 vaccine (A/Wisconsin/588/2019|6B1.A-5A2) used in HI assays and the most similar vaccine (A/California/07/2009).

Relative to HI

Site	A/Wisconsin/588/2019 6B1.A-5A2	A/swine/Texas/A01104132/2019	Annotations
35	D	F	
40	K	R	
71	S	F	Cb
73	A	T	Cb
74	R	S	Cb
119	K	N	
120	T	A	
126	H	Y	
127	D	E	
128	S	T	
129	D	S	
130	N	R	
138	H	Y	
142	K	N	Ca2
146	K	R	
149	I	L	
156	K	N	Sb
161	I	L	
162	N	S	Sa
163	Q	K	Sa
164	T	S	Sa
168	D	N	
170	G	E	Ca1
183	P	S	
185	I	S	
186	A	T	
203	T	S	

Relative to most similar vaccine

Site	A/California/07/2009	A/swine/Texas/A01104132/2019	Annotations
35	D	F	
40	K	R	
71	S	F	Cb
73	A	T	Cb
83	P	S	
84	S	N	
97	D	N	
119	K	N	
120	T	A	
126	H	Y	
127	D	E	
128	S	T	
129	N	S	
130	K	R	
138	H	Y	
142	K	N	Ca2
146	K	R	
149	I	L	
168	D	N	
170	G	E	Ca1
186	A	T	
205	R	K	
216	I	T	
224	E	A	RBS
239	K	T	
258	E	K	
260	N	G	

205	R	K	
224	E	A	
239	K	T	
250	A	V	
256	T	A	
258	E	K	
260	D	G	
261	A	S	
271	P	S	
278	T	N	
283	E	K	
295	V	I	
298	I	V	
302	K	E	
310	T	K	
314	L	M	
321	V	I	
<b>aadiff</b>		<b>44</b>	

261	A	S	
271	P	S	
278	T	N	
298	I	V	
302	K	E	
310	T	K	
314	L	M	
<b>aadiff</b>		<b>34</b>	

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

# 1B human-like lineage

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

# H1 1B swine genetic clades

- 1B.2.1 (n=113)
- 1B.2.3 (n=1)
- 1B.2.2.2 (n=25)
- 1B.2.2.1 (n=15)
- 1B.2.4 (n=1)
- 1B.1.2.2 (n=11)
- 1B.1.2.3 (n=1)
- 1B.1.2.1 (n=1)
- 1B.1.1 (n=5)

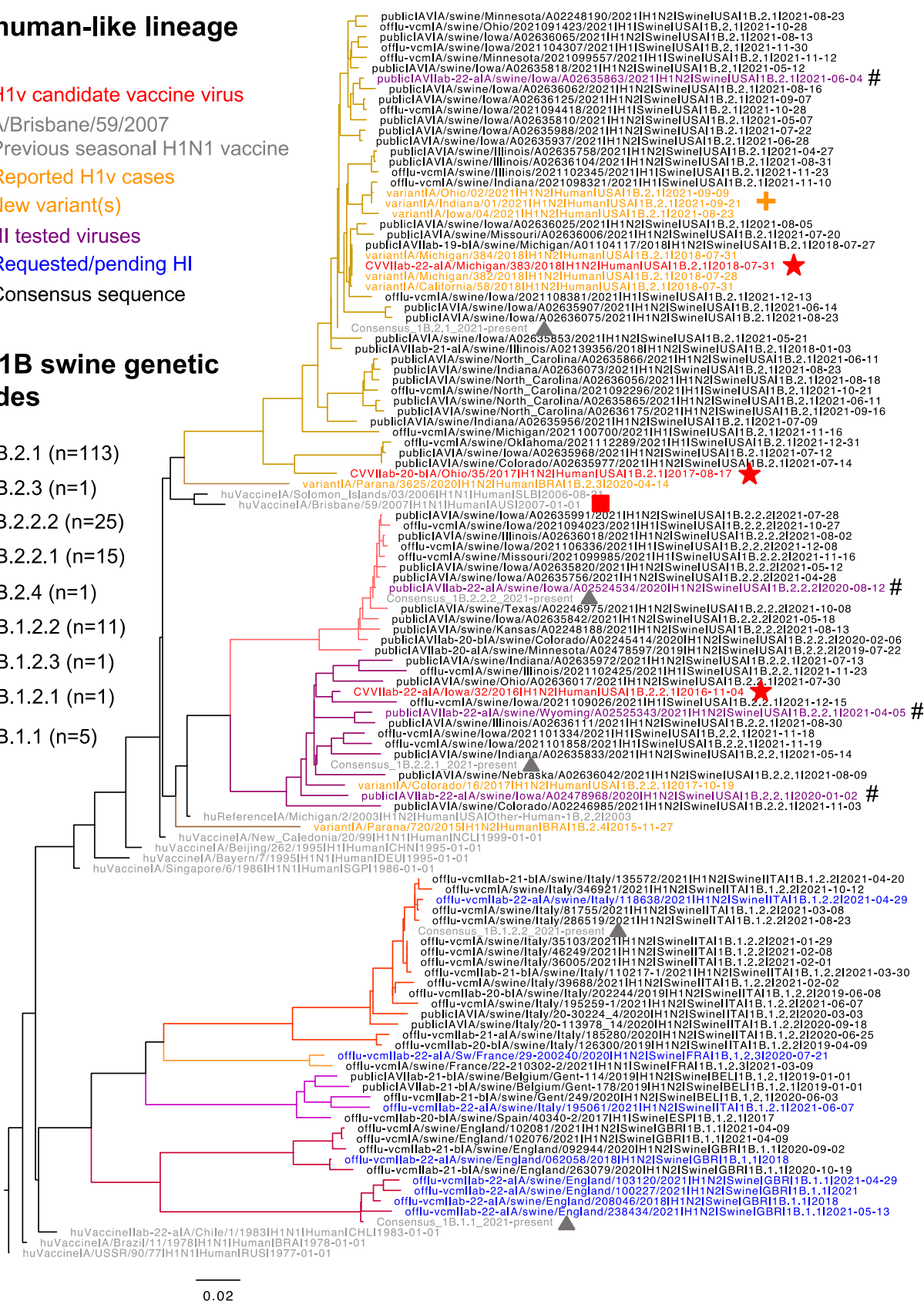


Figure 8. Swine H1 HA genes of the 1B lineage (tree was proportionally down sampled to 78 swine HA genes, excluding references): detections of each clade from data deposited between July 1, 2021 - December 31, 2021 are presented adjacent to the clade name.

## Antigenic analysis: Swine 1B Lineage

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

	Global Clade	US Clade	A/Michigan/383/2018 RG58A	A/Iowa/32/2016
A/Michigan/383/2018 RG58A	1B.2.1	d2 H1N2v	80	<10
<b>A/swine/Iowa/A02635863/2021</b>	1B.2.1	d2 H1N2	80	<10
A/Iowa/32/2016	1B.2.2.1	d1a H1N2v	<10	320
A/swine/Iowa/A02478968/2020*	1B.2.2.1	d1a H1N2	20	160
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

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

Reference CVV in red, new swine strains in bold. Homologous titers highlighted grey. \*Previously tested in report 2021a; \*\*Previously tested in report 2021b

- The contemporary swine 1B.2.1 (delta-2) was cross-reactive to CVV A/Michigan/383/2018.
- Two contemporary swine 1B.2.2.1 (delta-1a) representing diverging clade were tested. A/swine/Iowa/A02478968/2020 had a 2-fold decrease and A/swine/Wyoming/A02525343/2021 had a 4-fold reduction 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.

Table 9. UK & EU Data: Hemagglutination inhibition of CVV vaccine ferret antisera against contemporary swine 1B lineage strains selected to represent clade consensus.

	Global Clade	A/Michigan/383/2018 RG58A	A/Chile/1/1983
A/Michigan/383/2018 RG58A	1B.2.1 d2 H1N2v	1280	160
A/Chile/1/1983	H1 seasonal	160	1280
A/swine/England/062058/2018	1B.1.1	80	640
A/Sw/France/29-200240/2020	1B.1.2.3	80	160

- > 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.

- The swine 1B.1.1 was cross-reactive to CVV A/Chile/1/1983.
- The contemporary swine 1B.2.3 representing strains circulating in France had a significant decrease in cross reactivity to the 1B.2.1. CVV and the human seasonal strain reference.

Table 10. Amino acid substitutions between representative swine 1B.2.1 strain compared to the within-clade CVV (A/Michigan/383/2018 RG58A). Within-clade H1N2v detected within this reporting period are presented in orange.

Site	A/Michigan/383/2018 RG58A	A/swine/Iowa/A02635863/2021	A/Indiana/01/2021	A/Ohio/02/2021	A/Iowa/04/2021	Annotations
71	N	T	T	T	-	Cb
130	T				P	
149	L	V				
166	T				I	Ca1
170	G	E				Ca1
173	V	I				
189	R		K	K	K	Sb, RBS
256	E		K	K	K	
260	S	G				
261	F	S				
289	S		N	N	N	
<b>aadiff</b>		<b>6</b>	<b>4</b>	<b>4</b>	<b>6</b>	

Reference CVV in red, swine strains in black, variant detections in orange.



Table 11. Amino acid substitutions between representative swine 1B.2.2.1 and 1B.2.2.2 strains compared to the most similar CVV ([A/Iowa/32/2016](#) 1B.2.2.1).

Site	<a href="#">A/Iowa/32/2016</a>	1B.2.2.1 A/swine/Iowa/A02478968/2020	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	V	
50	L			I	
72	S		P		
74	K	E	E		Cb
82	A			T	
84	N	D			
85	S			P	
86	D			E	
94	Q			D	
96	T	A	A	A	
106	S		N		
119	R			K	
129	V			T	
130	T		D		
132	K	V	T		RBS
141	E	K			
149	I			V	
153	V			E	Sb
157	L		R		Sa
166	K	E			Ca1
168	E	D	D	N	
170	G			E	Ca1
175	I		V		
176	L			I	
183	S			P	

184	N			D	
186	G			E	
187	D			N	RBS
193	H			R	Sb, RBS
194	T			K	RBS
202	M			A	
208	R		K	E	
215	T		A		
222	D		N	N	Ca2, RBS
228	N			K	
236	P			A	
237	G	R			Ca1
241	I		V		
244	T			A	
249	I			V	
256	A			T	
269	K			N	
271	P			S	
273	D	N		N	
274	E			K	
277	A	T		T	
283	K			Q	
287	N		T		
289	N			S	
310	T		R	A	
314	M			L	
<b>aadiff</b>		<b>11</b>	<b>16</b>	<b>38</b>	

Reference CVV in red, swine strains in black.

Table 12. Amino acid substitutions between representative swine 1B.1.X strains compared to the most similar human seasonal vaccine **A/Chile/1/1983** used in HI assays.

Site	<b>A/Chile/1/1983</b>	1B.1.1 A/swine/England/062058/2018	1B.1.1 A/swine/England/208046/2018	1B.1.1 A/swine/England/103120/2021	1B.1.1 A/swine/England/100227/2021	1B.1.2.1 A/swine/Italy/195061/2021	1B.1.2.2 A/swine/Italy/118638/2021	1B.1.2.3 A/Sw/France/29-200240/2020	Annotations
14	D			-				F	
19	V		I	L	I				
35	D		N	N	N	N			
36	N	S	S	S	S				
43	K	R				R			
44	L		M	M	M				
47	I						V	T	
48	A							T	
54	K						R		
57	I	V							
68	E	T							
70	L		S	S	S				Cb
71	F						L		Cb
73	K	R	R	R	R				Cb
82	T				A		A		
83	P					T			
84	N					T		S	
85	S	P	P	P	P	P	A	P	
88	G	D							
89	T	V	I	I	I	I	L	I	
94	Y	H					H		
96	A	S	S	S	S	S	S	S	
106	S	N						G	
111	F		V	I	I		I	L	
116	I		M	M	M				
124	P					S			Sa
125	K	Q				N			Sa

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

209	R						K		
211	T	I				I			
215	A	T	E	E	E				
216	K					R		R	
220	V		I	I	I	I	I	I	RBS
222	N					D	D	D	Ca2, RBS
232	T	I							
235	E							G	
237	G			S	S	K	S	K	Ca1
238	D		E	E	E				
241	I	M							
248	L		F	F	F	F			
252	W	R				L			
256	A	S							
258	S						N		
260	G	S							
261	F		L	L	L				
262	G	E	R	R	K	E	E	K	
267	T	V	I	I	I	I	V	I	
268	S		T						
270	A						V		
272	M	L		L		V			
276	D	N					N		
277	A	T	T	T	T	T		T	
278	K		E	E	E		T		
283	Q						R		
288	S		N	N	N		N		
290	L	R							
295	V						I		
298	V						I		
299	T		A	A	A				
305	K			R	R				
307	V		I	I	I				
308	R		K	K	K	K	K		
310	T					K			
311	K		R	R	R				
313	R						K		
315	V						A		
321	I	V							
<b>aadiff</b>		<b>42</b>	<b>44</b>	<b>46</b>	<b>45</b>	<b>35</b>	<b>49</b>	<b>32</b>	

Reference vaccine strain in gray, swine strains in black.

# 1C Eurasian avian lineage

- ★ H1v candidate vaccine virus
- Reported H1v cases
- + New variant(s)
- # HI tested viruses
- Requested/pending HI
- ▲ Consensus sequences

## H1 1C swine genetic clades

- 1C.2.4 (n=41)
- 1C.2 (n=1)
- 1C.2.2 (n=9)
- 1C.2.1 (n=12)
- 1C.2.5 (n=15)
- 1C.2.3 (n=4v)



Figure 9. Swine H1 HA genes of the 1C lineage (tree was proportionally down sampled to 63 swine HA genes, excluding references): detections of each clade from data deposited between July 1, 2021 - December 31, 2021 are presented adjacent to the clade name.

## Antigenic analysis: Swine 1C Lineage

Table 13. 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/Pavia/65/2016*	A/Netherlands/10370-1b/2020 CVV	A/Hessen/47/2020-like CVV	A/Hunan/42443/2015 CNIC-1601-CVV
A/Netherlands/3315/2016 CVV (Crick)	1C.2.1 H1N1v	160	40	40	160	160
A/Pavia/65/2016*	1C.2.1 H1v	1280	320	320	320	1280
A/Netherlands/10370-1b/2020 CVV	1C.2.1 H1N1v	640	1280	1280	640	640
A/Hessen/47/2020-like CVV	1C.2.2 H1N2v	80	320	2560	2560	80
A/Hunan/42443/2015 CNIC-1601 CVV	1C.2.3 H1N1v	320	160	160	1280	2560
<b>A/swine/Italy/358072/2021</b>	1C.2.4 H1N2	40	40	40	80	40
<b>A/Sw/France/56-180424/2018</b>	1C.2.4 H1N2	320	320	160	320	320
<b>A/Sw/France/72-210055-1/2021</b>	1C.2.4 H1N2	160	80	40	80	80
A/swine/Italy/127069/2020	1C.2.5	40	40	80	80	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 A/swine/Italy/358072/2021 and A/swine/Italy/127069/2020 represented the diverse 1C.2.4 - with multiple deletion patterns and 1C.2.5 groups. They showed cross reactivity to CVV A/Netherlands/3315/2016 – CVV but not to the updated 1C.2.1 CVV A/Netherlands/10370-1b/2020.

The contemporary swine A/Sw/France/56-180424/2018 represents a second genetic group within 1C.2.4 viruses in the submitted data and this shows good cross reactivity with 1C CVV A/Netherlands/3315/2016 and with A/Pavia/65/2016\* and poor cross reactivity to other 1C CVVs.

Table 14. Amino acid substitutions between representative swine 1C.2.1 strains compared to the within-clade CVV (A/Netherlands/3315/2016 CVV); the additional within-clade CVV (A/Netherlands/10370-1b/2020 CVV) is included.

Site	A/Netherlands/3315/2016 CVV	A/swine/France/22-210309-2/2021	A/swine/Italy/175739-1/2021	A/Netherlands/10370-1b/2020 CVV	Annotations
20	M	L	L		
31	N			D	
44	L			M	
48	A				
51	Q	H			
56	N				
66	E				
71	I	L		L	Cb
74	N			K	Cb
80	I	V			
83	S			P	
84	N	K			
89	A		T		
96	A	T			
97	D			N	
102	R				
120	E	A	A	A	
127	E				
130	K				
132	S			T	
137	S	T		P	Ca2
141	I	V		A	
142	K	N	N	N	Ca2
155	E	A	G	G	Sa
161	I			L	
162	S	N			Sa
163	T	E		K	Sa
169	K	R			



175	I	V		V	
199	V			I	
202	G	E		E	
208	K			Q	
215	V			A	
232	T		I		
236	Q	K			
253	Y			H	
258	N		D	D	
262	N	S			
266	M	V			
267	M			I	
271	N	S		H	
272	V	I			
288	S				
298	I	L			
311	Q			R	
321	T	I	I	I	
324	V	I		I	
<b>aadiff</b>		<b>24</b>	<b>8</b>	<b>26</b>	

Reference CVV in red, swine strains in black.

Table 15. Amino acid substitutions between a representative swine 1C.2.2 strain compared to the within-clade CVV (**A/Hessen/47/2020 CVV**).

Site	<b>A/Hessen/47/2020 CVV</b>	A/swine/Italy/348919/2021	Annotations
3	I	L	
53	K	R	
66	E	D	
84	D	N	
97	D	N	
118	P	S	
120	A	S	
125	N	D	Sa
161	L	I	
163	N	K	Sa
175	V	I	
185	D	E	
214	T	I	
235	D	N	
253	Y	H	
267	M	I	
269	N	D	
302	E	K	
<b>aadiff</b>		<b>18</b>	

Reference CVV in red, swine strain in black.

Table 16. Amino acid substitutions between representative swine 1C.2.4 strains compared to the within-clade CVV ([A/Bretagne/24241/2021 CVV](#)). Within-clade H1N2v detected within this reporting period is presented in orange.

Site	<a href="#">A/Bretagne/24241/2021</a>	A/swine/France/72-210055-1/2021	A/swine/France/53-210091-2/2021	A/swine/France/35-210140-1/2021	A/swine/France/56-180424/2018	A/swine/Italy/358072/2021	<a href="#">A/Austria/1445532/2021</a>	Annotations
2	T						I	
5	I				V	V	V	
20	M				L	L	L	
31	N				D			
36	N				S	S	S	
39	G					E		
53	G			R		R		
54	N						S	
56	D	N	N	N	N	N	N	
71	V				L	L	L	Cb
74	N					S		Cb
82	T					A		
83	S				A	P	P	
86	K				T	E	E	
89	T				I			
96	A				S	S		
104	L					R	Q	
107	T						A	
119	K			N	G			
120	-				A	A	A	
121	N				T	T	T	
124	P				E			Sa
125	S				N	N	G	Sa
127	E					D		
128	A				T	T	T	
129	-					T	T	
130	-				K	R	K	
134	V				G		T	

135	S					A		
137	S					P		Ca2
138	K				R	H	H	
141	A				T		S	
142	N				K		K	Ca2
146	R				Q			
149	L						I	
152	T					V	V	
153	S				K			Sb
155	G				N	R		Sa
156	K				S	N	N	Sb
157	P				-	S	S	Sa
159	S				P	P	P	Sa
161	L				I		I	
162	S					N		Sa
163	K					R		Sa
166	K		R		T	T	T	Ca1
168	N				D			
169	K				R			
172	E				K			
175	V						I	
186	S					R	G	
190	T				A			Sb
194	N				S	H		
202	V					E	E	
203	S				T			
208	Q					R	R	
216	P				D	T	T	
218	P					Q		
219	E				K	K	K	
222	E				K		D	Ca2
224	R				A	A	A	
237	E				G	G	G	Ca1
239	T				N			
242	F						I	
252	R					W	W	
258	E	D			D		N	
259	K				M			
260	G				S			
261	S					L	I	
262	N				S	S		
266	M					V	V	

267	K		E			I	M	
269	D					E	E	
270	A				V			
278	K				T			
283	H						L	
288	S					G	G	
289	D				N	N	N	
290	R					L	L	
298	I						T	
<b>aadiff</b>		<b>2</b>	<b>3</b>	<b>3</b>	<b>48</b>	<b>49</b>	<b>49</b>	

Reference CVV in red, swine strains in black, variant detection in orange.

Table 17. Amino acid substitutions between a representative swine 1C.2.5 strain compared to the most similar CVV (A/Hunan/42443/2015 CNIC-1601 1C.2.3).

Site	A/Hunan/42443/2015 CNIC-1601	A/swine/Italy/127069/2020	Annotations
30	V	I	
44	L	M	
47	K	N	
48	I	A	
56	S	D	
66	K	E	
69	L	F	
82	T	A	
85	S	P	
102	K	R	
111	F	L	
120	A	R	
125	N	D	Sa
127	D	E	
130	R	K	
134	V	S	
135	A	S	
162	S	N	Sa
163	K	N	Sa
166	T	I	Ca1
169	K	R	
190	T	A	Sb
208	K	R	
215	V	I	
222	E	N	Ca2
227	M	I	
245	T	N	
253	H	Y	
267	R	I	

271	Q	H	
283	H	N	
287	K	N	
288	G	N	
289	N	S	
298	V	I	
310	T	K	
<b>aadiff</b>		<b>36</b>	

Reference CVV in red, swine strain in black.

### H3 swine lineage

- ★ H3v candidate vaccine virus
- A/Darwin/6/2021
- Previous H3N2 seasonal vaccines
- Reported H3N2v cases
- + New variant(s)
- # HI tested viruses
- Requested/pending HI
- ▲ Consensus sequences

### H3 swine genetic clades

- 3.2010.2 (n=5)
- 3.2010.1 (n=85)
- 3.1990.4.a (n=106)
- 3.1990.4.b (n=6)
- 3.1990.4 (n=8)
- 3.1990.4.i (n=2)
- 3.1990.4.c (n=1)
- 3.1990.1 (n=1)
- 3.1970.1 (n=3)
- Human vaccine or seasonal

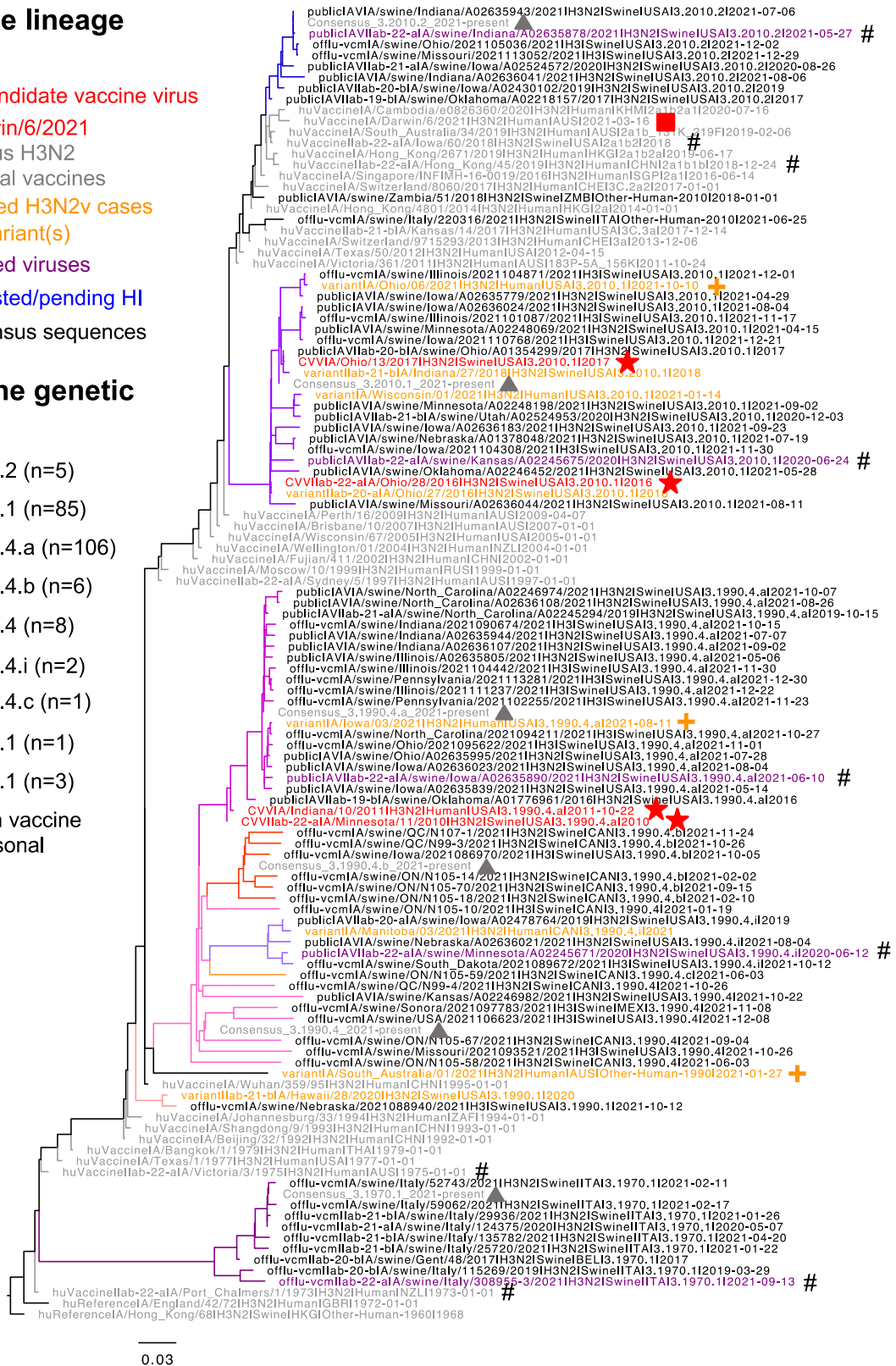


Figure 10. Swine H3 HA genes: detections of each clade from data deposited between July 1, 2021 - December 31, 2021 are presented adjacent to the clade name. Clades within the tree were proportionally down sampled to 58 swine HA genes (excluding references); tree includes clade consensus sequences, and human vaccine strains.



## Antigenic analysis: Swine H3

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

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

	Global Clade	A/Minnesota/11/2010 x 203	IDCDC-RG55C A/Ohio/28/2016-like CVV	A/Iowa/60/2018	A/Hong Kong/45/2019
A/Minnesota/11/2010 x 203	1990.4.a H3N2v	640	20	20	20
<b>A/swine/Iowa/A02635890/2021</b>	1990.4.a H3N2	80	10	20	20
A/swine/Minnesota/A02245671/2020*	1990.4.i H3N2	20	20	20	<10
IDCDC-RG55C A/Ohio/28/2016-like CVV	2010.1 H3N2v	10	2560	10	10
A/swine/Kansas/A02245675/2020*	2010.1 H3N2	10	40	20	<10
<b>A/swine/Indiana/A02635878/2021</b>	2010.2 H3N2	10	10	10	20
A/Iowa/60/2018	C.2a1b2 H3N2	<10	<10	160	40
A/Hong Kong/45/2019	C.2a1b H3N2	<10	<10	80	160

Reference CVV in red, seasonal vaccine strains in gray, new swine strains in bold, variant in orange. Homologous titers highlighted in gray. \*Previously tested in report 2021a

- The contemporary swine 1990.4.a demonstrated a significant 8-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 had a significant 64-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/Iowa/60/2018 and an 8-fold decrease to A/Hong Kong/45/2019.

Table 19. 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	A/Sydney/5/97
A/Victoria/3/1975	Human seasonal	1280	40	160
IDCDC-RG55C A/Ohio/28/2016-like CVV	2010.1 H3N2v	160	1280	160
A/Sydney/5/97	Human seasonal	320	80	2560
<b>A/swine/Italy/308955-3/2021</b>	1970.1 H3N2	640	320	320

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 A/swine/Italy/308955-3/2021 demonstrated good reactivity with A/Victoria/3/1975 and poor cross reactivity to the 2010.1 CVV and another antiserum raised to a human seasonal strain.

Table 20. 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); the most similar human seasonal vaccine by amino acid similarity (A/Victoria/3/1975) is included.

Site	A/Port_Chalmers/1/1973	A/swine/Italy/308955-3/2021	A/Victoria/3/1975	Annotations
3	F		L	
6	N	K		
7	D	G		
8	-		N	
9	S	N		
31	N	D		
45	S	N		
46	S	F		
48	T	M		
53	N	K		Site C
62	I	A		
67	I	V		
69	A	S		
83	T	K	K	
88	V	I		
92	K	R		
104	D	E		
106	A	T		
112	V	I		
117	T	S		
121	I	T		
124	G	N		
126	T	N	N	
137	N	S		Site A
144	D	N		Site A
145	S	N		Site A
146	G	S		Site A
159	S	N		
160	A	T	T	
163	V	M		

164	L		Q	
171	N	S		
173	N	D		
174	F		S	
189	Q	R	K	Site B
196	V	I		
198	A	T		
201	R	K	K	Site D
202	V	I		
203	T	I		
217	I		V	Site D
242	I	T		
244	V	I		
260	M	I		
261	R	Q		
262	T	I		
267	I	V		
269	R	K		
278	I	D	S	Site C
299	K	R		
307	K	H		
309	V	I		
323	V	I		
<b>aadiff</b>		<b>48</b>	<b>11</b>	

Reference vaccine in gray, swine strain in black.

Table 21. Amino acid substitutions between a representative swine 1990.4.a strain compared to the within-clade CVV (A/Minnesota/11/2010 x 203) used in the HI assay. A secondary within-clade CVV (A/Indiana/10/2011), and the within-clade H3N2v (A/Iowa/03/2021) detected during this reporting period are presented.

Site	A/Minnesota/11/2010 x 203	A/swine/Iowa/A02635890/2021	A/Indiana/10/2011	A/Iowa/03/2021	Annotations
79	F		L	L	
96	N	S		S	
109	R	K			
119	E	K			
124	S	I		I	
138	A	S		S	
156	N	H		H	
172	D			E	
207	K	R		R	Site D
289	P	S		S	
323	V	I		I	
<b>aadiff</b>		<b>9</b>	<b>1</b>	<b>9</b>	

Reference CVV in red, swine strains in black, variant detection in orange.

Table 22. Amino acid substitutions between a representative swine 1990.4.i strain compared to the most similar CVV (A/Minnesota/11/2010 x 203 1990.4.a).

Site	A/Minnesota/11/2010 x 203	A/swine/Minnesota/A02245671/2020	Annotations
8	N	K	
9	S	N	
10	M	T	
12	T	M	
45	S	X	
49	G	D	
50	R	K	
57	Q	K	
58	I	V	
62	K	G	
80	Q	E	
96	N	D	
107	T	S	
117	N	T	
121	T	N	
122	Q	L	Site A
124	S	N	
131	A	D	
137	Y	F	Site A
138	A	S	
140	R	K	
142	G	N	
155	Y	H	Site B
156	N	Q	
159	Y	H	
163	E	A	
164	Q	L	
189	K	M	Site B

193	N	S	Site B
198	A	E	
203	I	T	
260	I	M	
261	Q	R	
262	S	I	
269	R	K	
273	H	L	
275	D	G	Site C
276	E	N	
299	K	R	
<b>aadiff</b>		<b>39</b>	

Reference CVV in red, swine strain in black.

Table 23. Amino acid substitutions between a representative swine 2010.1 strain compared to the within-clade CVV (A/Ohio/28/2016 IDCDC-RG55C) used in the HI assay. A second within-clade CVV (A/Ohio/13/2017), and the within-clade detected H3N2v (A/Ohio/06/2021) are presented.

Site	A/Ohio/28/2016 IDCDC-RG55C	A/swine/Kansas/A02245675/2020	A/Ohio/13/2017	A/Ohio/06/2021	Annotations
56	H		Y	Y	
62	E			G	
85	D			E	
106	A			V	
138	A	S	S	S	
145	K	N	N	N	Site A
159	F			Y	
188	D	E			Site B
193	S			A	Site B
198	A	T			
209	S		N	N	
212	A			T	
238	R			K	
264	K			R	
280	A	E			
<b>aadiff</b>		<b>5</b>	<b>4</b>	<b>12</b>	

Reference CVV in red, swine strains in black, variant detection in orange.



Table 24. Amino acid substitutions between a representative 2010.2 strain compared to the human seasonal vaccine (A/Iowa/60/2018 C.2a1b2), and a second human seasonal vaccine (A/Hong Kong/45/2019 C.2a1b).

Site	A/Iowa/60/2018 C.2a1b2	A/swine/Indiana/A02635878/2021	A/Hong Kong/45/2019 C.2a1b	Annotations
9	S	G		
31	N	D		
58	I	V		
62	G	E		
83	K	Q		
106	A	I		
128	T		A	
131	K	T	T	
135	T	I	K	
137	S		F	Site A
138	A	S	S	
144	S	N		Site A
160	T	K		
167	T	I		
171	K	N		
189	K	N		Site B
192	I	T		
193	F	S	S	Site B
197	Q	R		
203	T	I		
291	D	N		
312	S		N	
<b>aadiff</b>		<b>19</b>	<b>7</b>	

Reference vaccine strain in gray, swine strain in black.

## Summary and Risk Assessment

### Global Variant Cases:

During this reporting period (July 1, 2021 – December 31, 2021), 15 variant cases were reported and included with the swine analyses:

- USA: H1N1v (3 1A.3.3.2/pdm, 2 1A.3.3.3); H1N2v (3 1B.2.1); H1v (no sequence); H3N2v (1 2010.1; 1 1990.4a)
- Canada: H1N2v (no sequence)
- Denmark: H1N1v (1A.3.3.2)
- France: H1N2v (1C.2.4, recommended as a CVV A/Bretagne/24241/2021)
- Austria: H1N2v (1C.2.4)

An additional 6 variant cases were reported July 1, 2021 – December 31, 2021, but collected outside the report window. The following HA sequences were included in the phylogenetic trees:

- Taiwan: H1N2v (1A.1.4, collected 03/12/2021)
- China: H1N1v (4 1C.2.3, collected 01/01/2021, 01/27/2021, 02/21/2021, 03/09/2021)
- Australia: H3N2v (Other-Human-1990, collected 01/27/2021)

### Europe Swine:

- 1A 3.3.2 viruses representing contemporary circulating and variant strains showed variable reactivity to CVVs. Good cross reactivity to seasonal vaccine strain A/Michigan/45/2015 was seen. A/Swine/Italy/241572/2020 strain was representative of an European variant case and showed variable moderate to reduced cross reactivity to seasonal vaccine strains.
- 1B 1.2.3 viruses representing a significant proportion of viruses currently detected in Europe showed no cross reactivity to human seasonal virus and CVV.
- 1C: 1C2.4 and 1C2.5 viruses which represent a genetically diverse and significant proportion of 1C lineage viruses detected in Europe showed cross reactivity with CVV strain A/Netherlands/3315/2016 and variable cross reactivity against other CVV and variant strains.
- H3 1970.1 lineage viruses demonstrated cross reactivity against all human seasonal and CVV antisera tested.

### North America Swine:

- 1A.1.1 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).
- 1A.3.3.2 swine strains exhibit loss in cross-reactivity with current human seasonal vaccine strains.
- 1A.3.3.3 clade-3 is a substantial proportion of 1A lineage viruses detected in U.S. pigs and a representative strain demonstrated a 16-fold decrease against the tested 1A.3.3.3-clade1 CVV A/Ohio/24/2015 (Table 1). A variant case within the 1A.3.3.3-clade3, A/Wisconsin/03/2021 H1N1v was recently recommended for development as a CVV. An additional two variant cases were reported from 1A.3.3.3-clade3.
- 1B.2.1 cross-reacts with the CVV A/Michigan/383/2018 RG58A (Table 8). This clade contained three reported H1N2v during this reporting period.
- 1B.2.2.1 ranged from 2- to 4-fold reduction from the within clade CVV A/Iowa/32/2016. 1B.2.2.2 (delta-1b) demonstrated a significant loss in cross-reactivity against the CVV A/Iowa/32/2016 (Table 8). Both clades represent a consistent proportion of 1B lineage viruses detected in U.S. pigs (Figure 8 and A1).

- H3.1990.4.a lineage viruses demonstrated a significant loss of cross-reactivity against the CVV A/Minnesota/ 11/2010 (Table 18) and represent a substantial proportion of H3 viruses detected in U.S. pigs (Figure 10 and A1).
- H3.1990.4i lineage viruses demonstrated significant loss of cross-reactivity against all CVV and human seasonal vaccine strains (Table 18). These swine viruses are less frequent but consistently detected (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 strains. H3 2010.1 lineage viruses are a substantial proportion of H3 viruses detected in U.S. pigs (Figure 10 and A1).

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

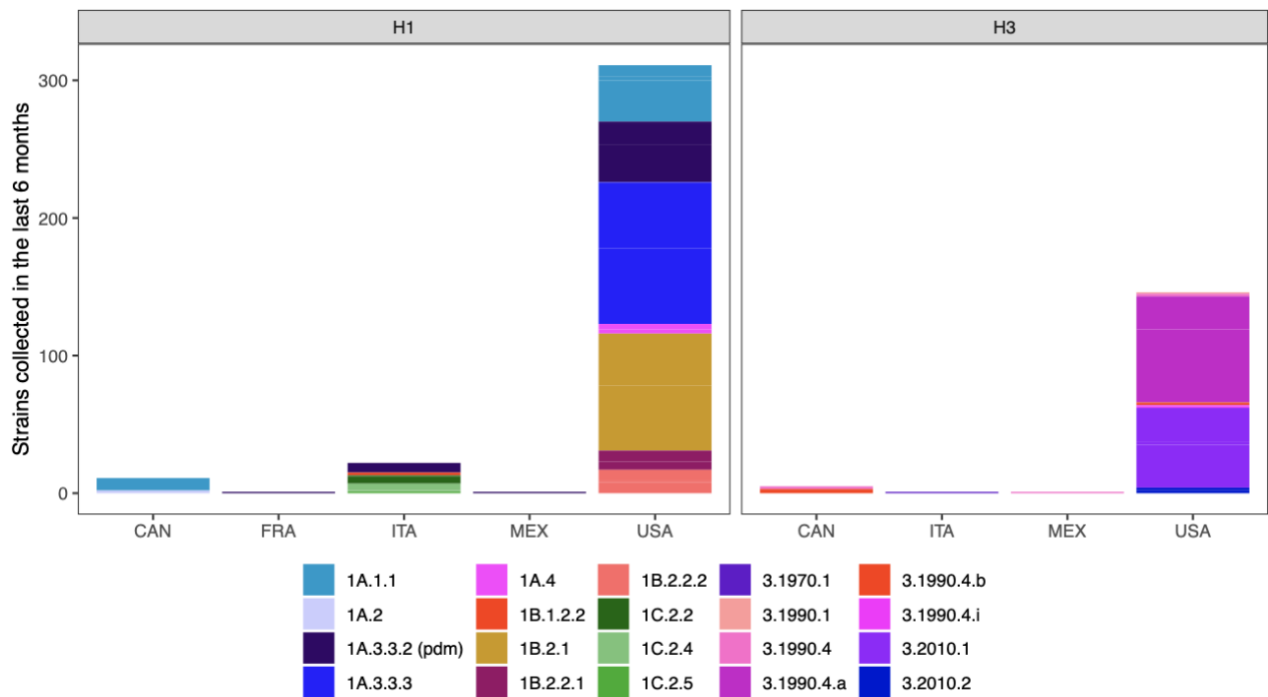


Figure A1. Summary of swine HA clades by country, colored by phylogenetic clade for sequences collected July 1, 2021 - December 31, 2021 (n=499).

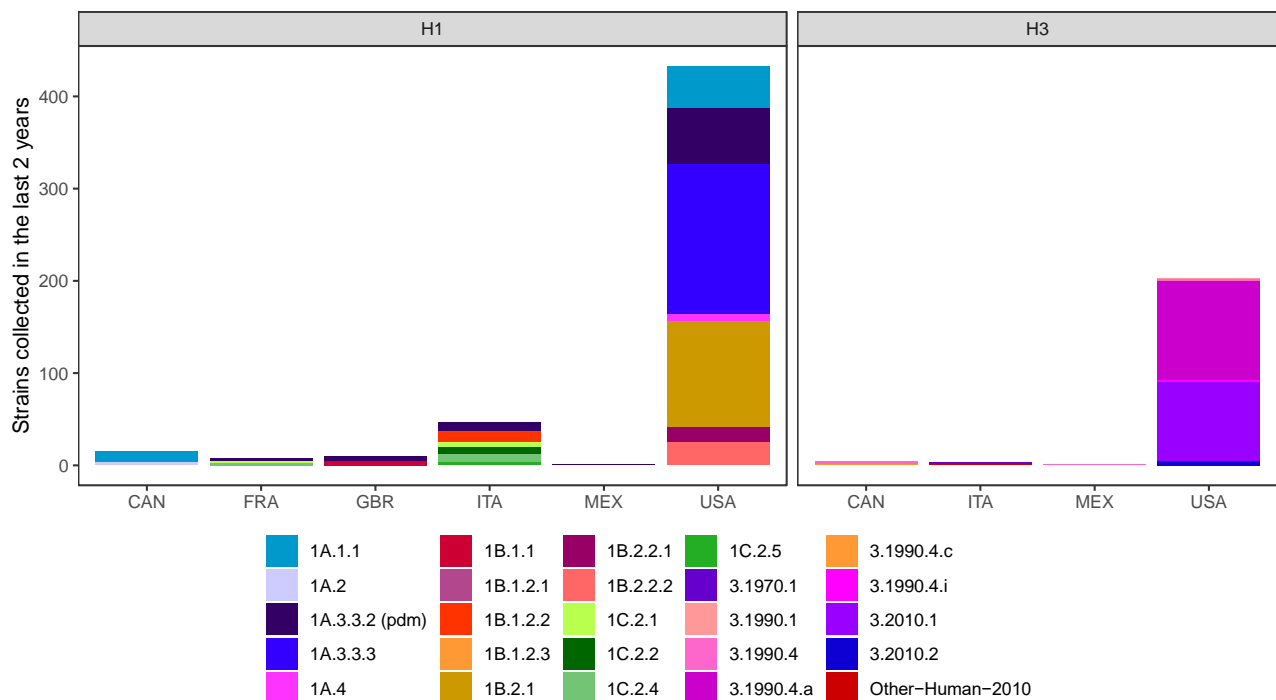


Figure A2. Summary of swine HA genes colored by phylogenetic clade for sequences deposited January 1, 2020 - December 31, 2021 and truncated to those collected within the last 24 months (n=724).

Table A1a. Summary of swine H1 clades by country collected in the past 6 months and deposited July 2021 – December 2021.

<b>clade</b>	<b>country</b>	<b>count</b>
1A.1.1	CAN	9
1A.2	CAN	2
1A.3.3.2	FRA	1
1A.3.3.2	ITA	7
1B.1.2.2	ITA	2
1C.2.2	ITA	6
1C.2.4	ITA	6
1C.2.5	ITA	1
1A.3.3.2	MEX	1
1A.1.1	USA	41
1A.3.3.2	USA	44
1A.3.3.3	USA	103
1A.4	USA	7
1B.2.1	USA	85
1B.2.2.1	USA	14
1B.2.2.2	USA	17

Table A1b. Summary of swine H1 clades by country collected in the past 24 months and deposited January 1, 2020 - December 31, 2021.

<b>clade</b>	<b>country</b>	<b>count</b>
1A.1.1	CAN	12
1A.2	CAN	3
1A.3.3.2	FRA	3
1B.1.2.3	FRA	1
1C.2.1	FRA	1
1C.2.4	FRA	3
1A.3.3.2	GBR	5
1B.1.1	GBR	4
1A.3.3.2	ITA	10
1B.1.2.1	ITA	1
1B.1.2.2	ITA	11
1C.2.1	ITA	5
1C.2.2	ITA	8
1C.2.4	ITA	9
1C.2.5	ITA	3
1A.3.3.2	MEX	1
1A.1.1	USA	46
1A.3.3.2	USA	60
1A.3.3.3	USA	159

1A.4	USA	8
1B.2.1	USA	113
1B.2.2.1	USA	15
1B.2.2.2	USA	25

Table A2a. Summary of swine H3 clades by country collected in the past 6 months and deposited July 2021 – December 2021.

clade	country	count
3.1990.4	CAN	2
3.1990.4.b	CAN	3
3.1970.1	ITA	1
3.1990.4	MEX	1
3.1990.1	USA	1
3.1990.4	USA	2
3.1990.4.a	USA	77
3.1990.4.b	USA	2
3.1990.4.i	USA	2
3.2010.1	USA	58
3.2010.2	USA	4

Table A2b. Summary of swine H3 clades by country collected in the past 24 months and deposited January 2020 – December 2021.

clade	country	count
3.1990.4	CAN	4
3.1990.4.b	CAN	5
3.1990.4.c	CAN	1
3.1970.1	ITA	3
Other-Human-2010	ITA	1
3.1990.4	MEX	1
3.1990.1	USA	1
3.1990.4	USA	2
3.1990.4.a	USA	105
3.1990.4.b	USA	2
3.1990.4.i	USA	2
3.2010.1	USA	85
3.2010.2	USA	5

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

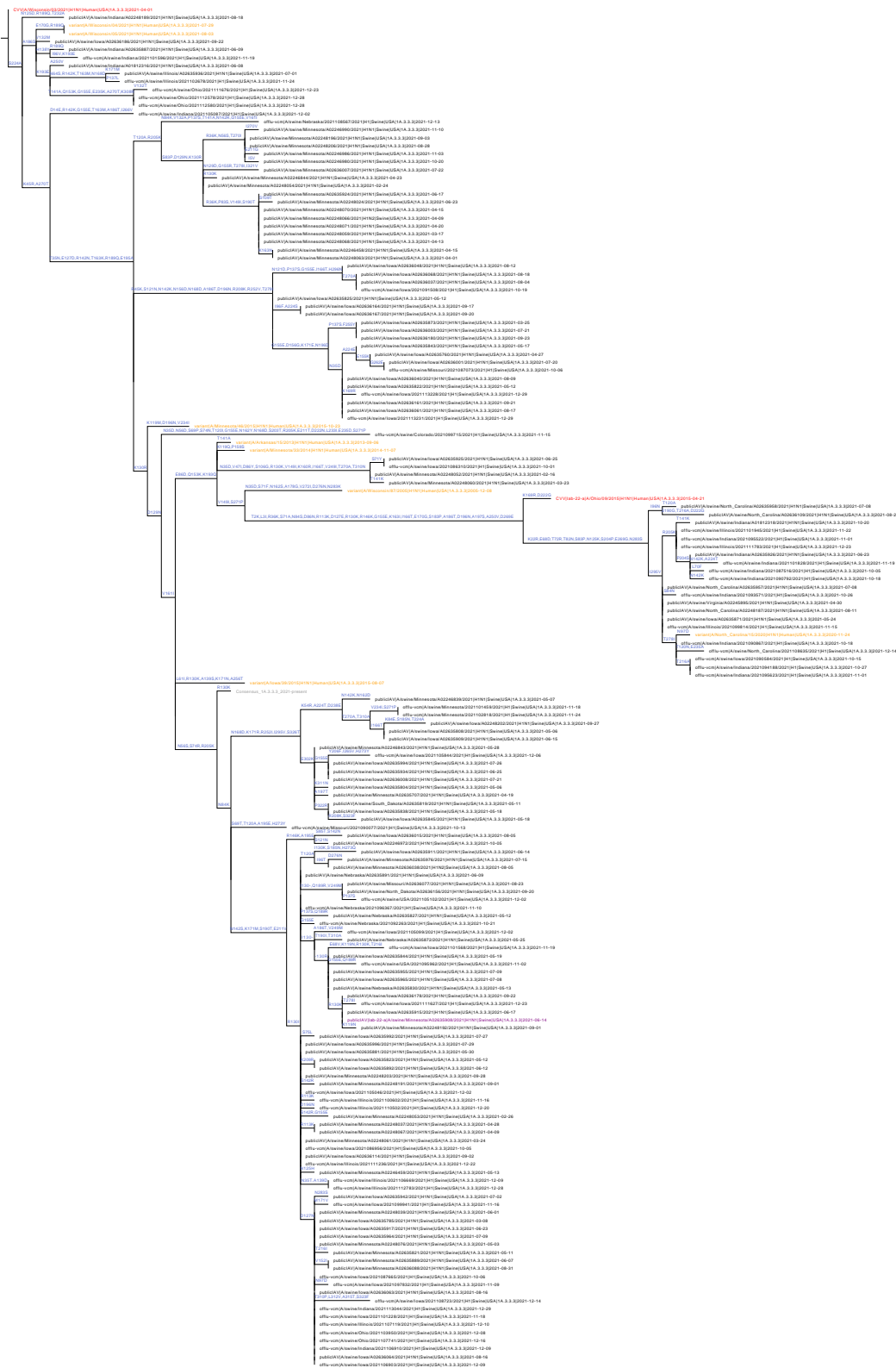


Figure A3. A phylogeny of the 1A.3.3 clade displaying sequences deposited July 1, 2021 - December 31, 2021 and collected in 2021 (n=159) and 11 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Wisconsin/03/2021) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 7.

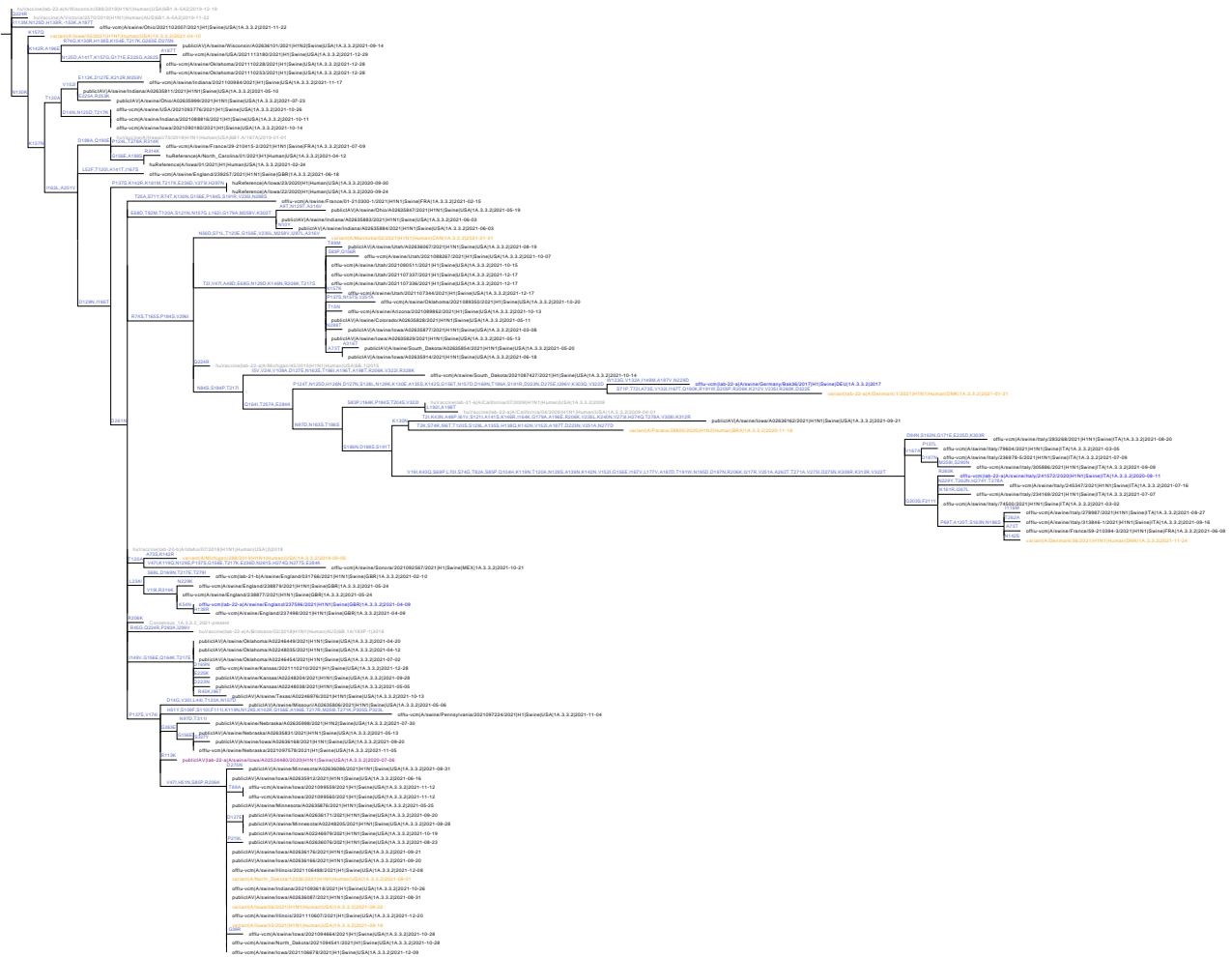


Figure A4. A phylogeny of the **1A.3.3.2 (pdm)** clade displaying n=81 sequences deposited July 1, 2021 - December 31, 2021 and collected in 2021, and n=23 reference genes. The clade is rooted relative to the most recent human vaccine strain (A/Wisconsin/588/2019) and all branches are annotated by the 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=59 sequences deposited July 1, 2021 - December 31, 2021 and collected in 2021, 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 by the respective amino acid mutations in the HA1 region. The strains are colored in consistency with Figure 7.

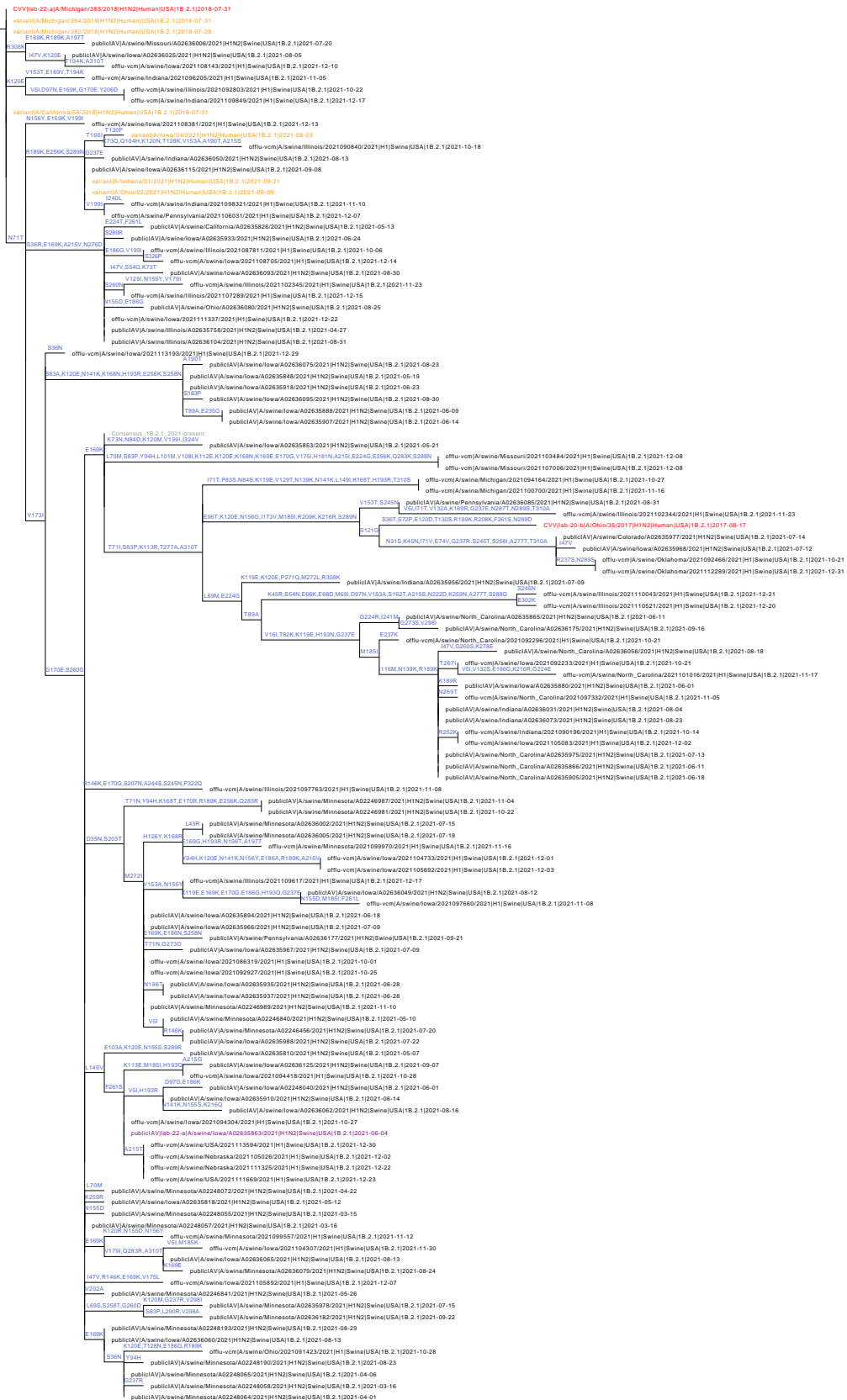


Figure A6. A phylogeny of the **1B.2.1** clade displaying n=113 sequences deposited July 1, 2021 - December 31, 2021 and collected in 2021, and n=9 reference genes. The clade is rooted relative to the most recent in-clade CVV (A/Michigan/383/2018) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 8.

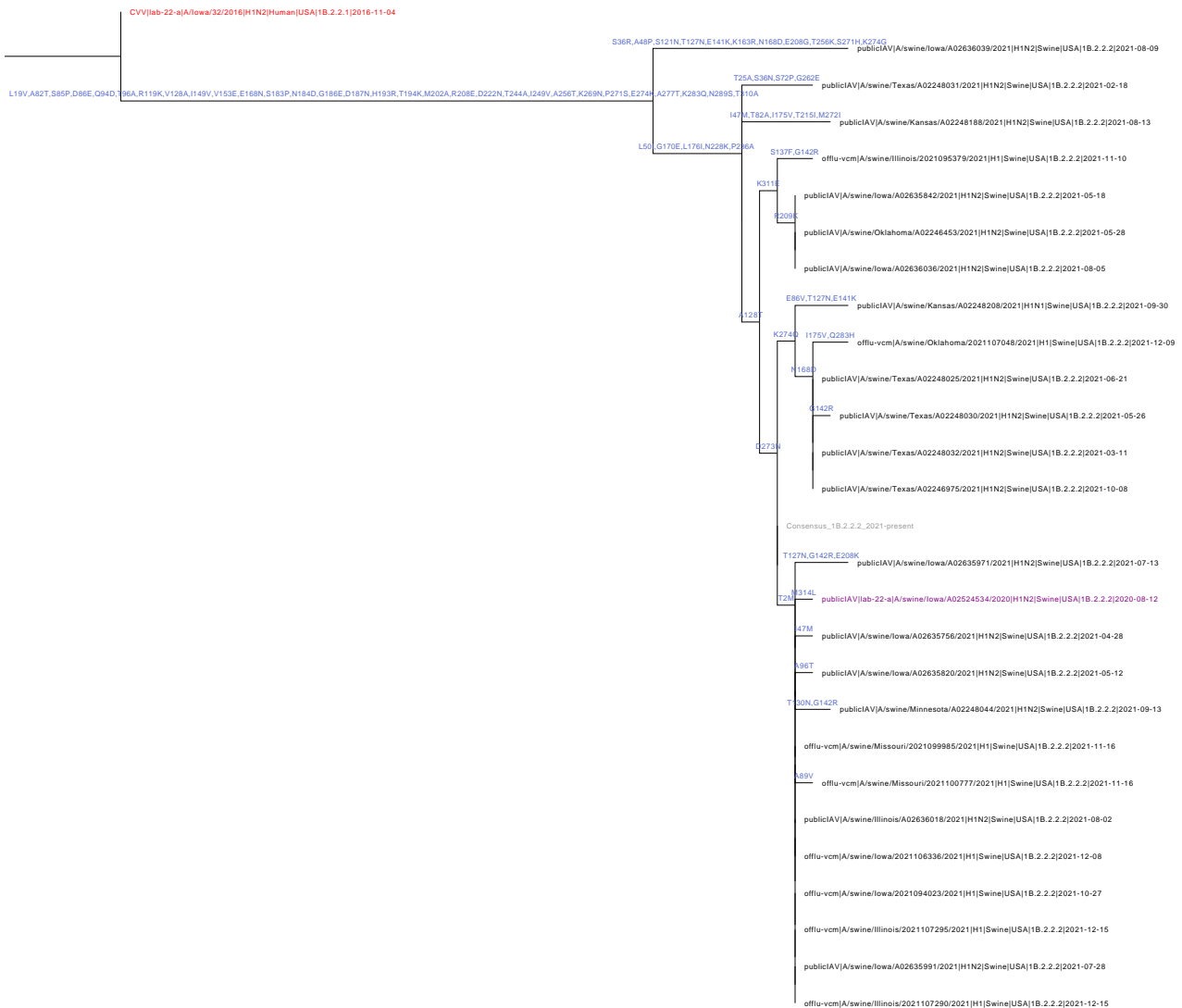


Figure A7. A phylogeny of the **1B.2.2.2** clade displaying n=26 sequences deposited July 1, 2021 - December 31, 2021 and collected in 2021, and n=2 reference genes. The clade is rooted relative to the closest CVV (A/Iowa/32/2016) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 8.

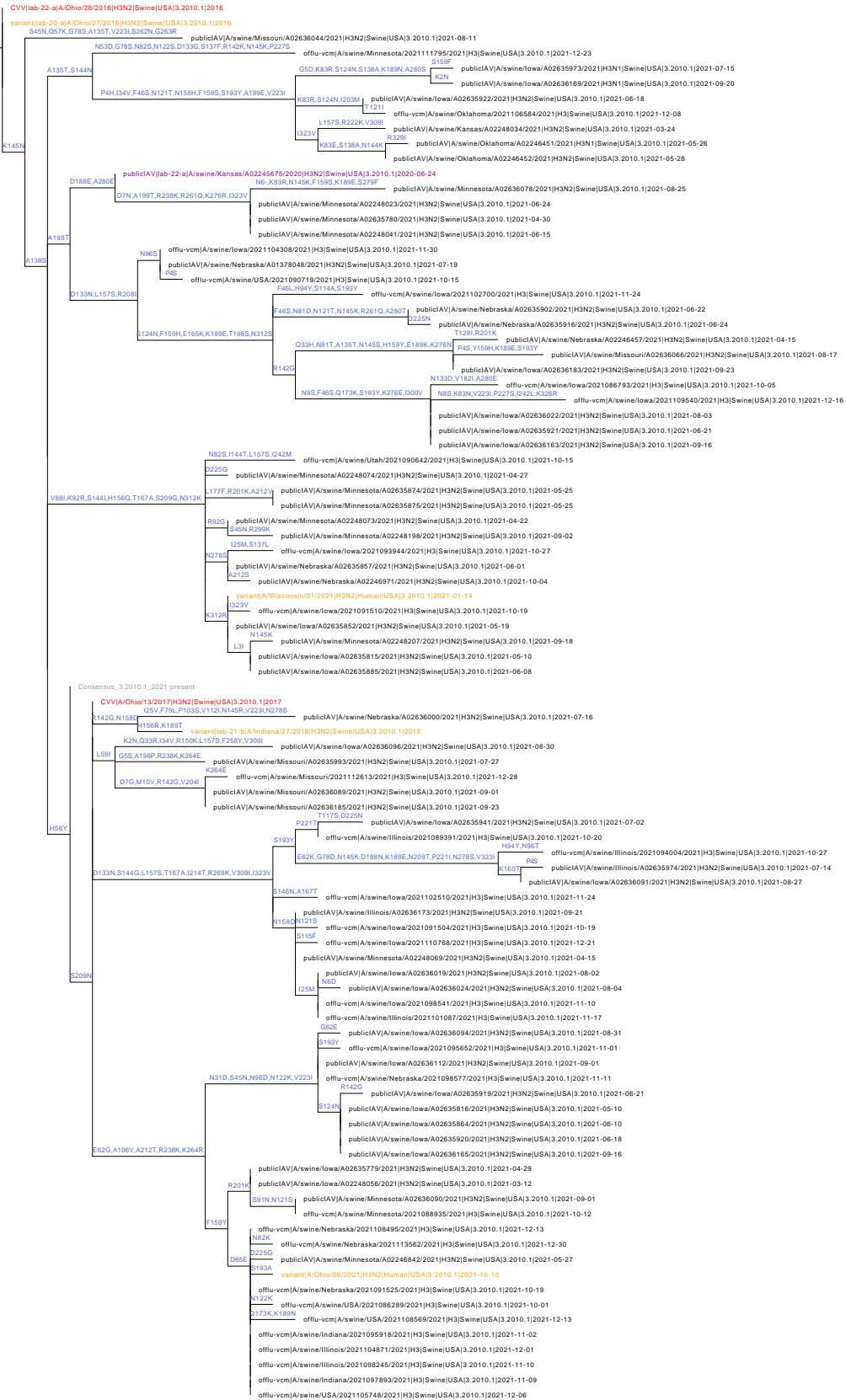


Figure A8. A phylogeny of the **3.2010.1** clade displaying n=86 sequences deposited July 1, 2021 - December 31, 2021 and collected in 2021, and n=7 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Ohio/28/2016) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistence with Figure 10.

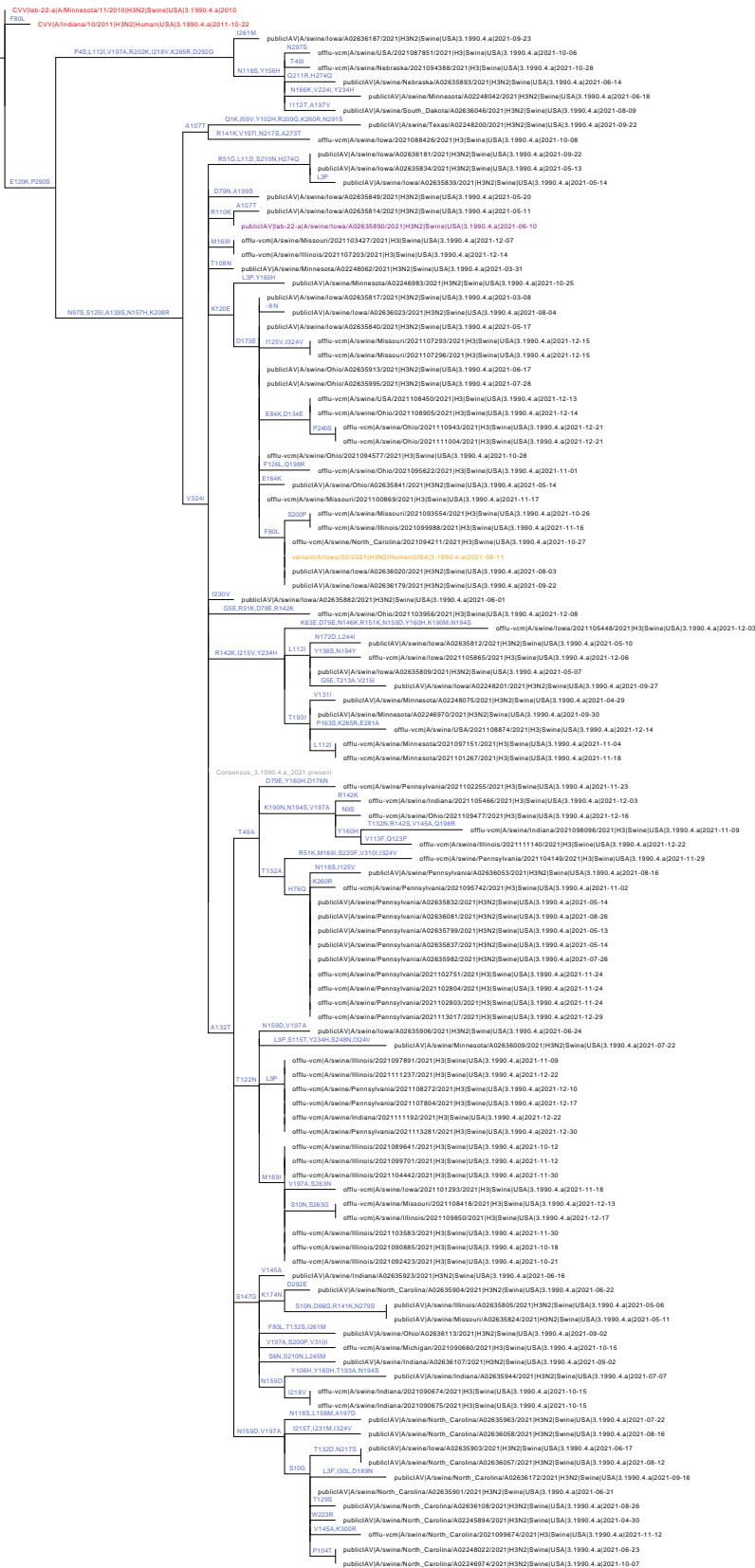


Figure A9. A phylogeny of the **3.1990.4.a** clade displaying n=105 swine sequences deposited July 1, 2021 - December 31, 2021 and collected in 2021, and n=4 reference genes. The clade is rooted relative to the most representative in-clade CVV (A/Minnesota/11/2010) and all branches are annotated by the respective amino acid mutations in the HA1 region. The strains are colored in consistency with Figure 10.

## ANNEX 3. Supplemental Information on Variant Detections

Australia: A/South\_Australia/01/2021 H3N2v (Other-Human-1990, collected 01/27/2021)

Appendix Table 1. Hemagglutinin inhibition assay using ferret antisera against human and swine H3N2 viruses isolated during 1993–2018.

Antigen	Antisera <sup>1</sup>												
	A/Shangdong/ 9/93	A/Johannesburg/ 33/94	A/Wuhan/ 359/95	A/Tasmania/ 1/97	A/Sydney/ 5/97	A/Moscow/ 10/99	A/Minnesota/ 11/2010 (H3N2v)	A/swine/WA/ 2577766G /2012	A/Texas/ 50/ 2012	A/Hong Kong/ 4801/ 2014	A/Singapore/ INFIMH- 16- 0019/2016	A/South Australia/ 85/2018	
A/Shangdong/9/93	1280	160	40	<20	<20	<20	20	320	<20	<20	<40	<40	
A/Johannesburg/33/94	160	640	40	<20	20	<20	<20	160	<20	<20	<40	<40	
A/Wuhan/359/95	40	20	640	80	40	20	<20	80	<20	<20	<40	<40	
A/Tasmania/1/97	20	<20	640	>2560	20	40	<20	40	<20	<20	<40	<40	
A/Sydney/5/97	<20	<20	80	<20	>2560	320	20	40	<20	<20	<40	<40	
A/Moscow/10/99	<20	<20	<20	<20	>2560	>2560	<20	40	<20	<20	<40	40	
A/Minnesota/11/2010 (H3N2v)	20	<20	<20	<20	<20	<20	320	<20	<20	<20	<40	<40	
A/swine/WA/2577766G/2012	40	20	<20	<20	<20	<20	<20	1280	<20	<20	<40	80	
A/Texas/50/2012	<20	<20	<20	<20	<20	<20	<20	<20	80	40	<40	<40	
A/Hong Kong/4801/2014	<20	<20	<20	<20	<20	20	<20	<20	80	320	40	80	
A/Singapore/INFIMH-16-0019/2016	<20	<20	<20	<20	<20	<20	<20	<20	<20	80	80	<40	
A/South Australia/85/2018	40	<20	<20	<20	<20	<20	<20	40	<20	<20	<40	1280	

- i. The A/South\_Australia/01/2021 H3N2v collected 01/27/2021 retains high genetic similarity and is within the same phylogenetic clade as a previously detected variant, A/South\_Australia/85/2018 H3N2v
- ii. The A/South\_Australia/85/2018 H3N2v was characterized in Deng et al. 2020. Locally acquired human infection with swine-origin influenza A (H3N2) variant virus, Australia, 2018. *Emerging infectious diseases*, 26(1), p.143.
- iii. These data demonstrate a lack of cross-reactivity between human seasonal vaccine strains (H3 vaccines from 1993 to 2016) and the variant strain. (Appendix table 1: Deng et al. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6924914/>)

## **ANNEX 4. Late breaking information 1A.1.1 (alpha lineage) variant**

H1N2v California –

- Adult ≥ 18 years of age
- Patient worked directly with swine
- Specimen was collected 12/27/2021
- Patient was not hospitalized & has fully recovered
- Unknown antiviral and vaccination status