OFFLU SWINE INFLUENZA REPORT

JULY 2020 TO DECEMBER 2020

SCOPE

In this document we present a summary of H1 and H3 swine influenza A viruses from sequences deposited July 1 to December 31, 2020.

Swine influenza A viruses

Acknowledgements

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Genetic and Antigenic Analyses - Brief Methods

Analyses were conducted by subtype and lineage or clade. Reference sequences and new data (deposited July 1, 2020 – December 31, 2020) 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 virus events in swine

Epidemiology

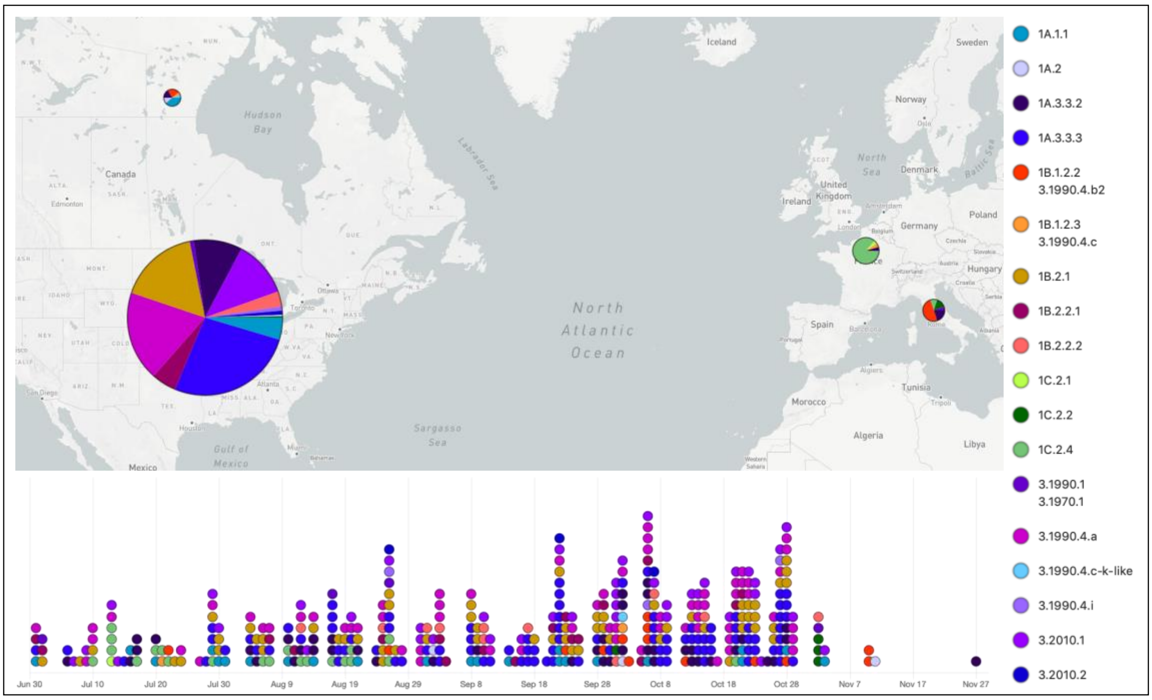


Figure 1. MicroReact map of swine IAV HA genes colored by phylogenetic clade for new sequences collected July 1 - December 31, 2020 (n=416); collection date is represented along bottom timeline. Strains submitted with only partial date were assigned a random date to preserve temporal clusters.

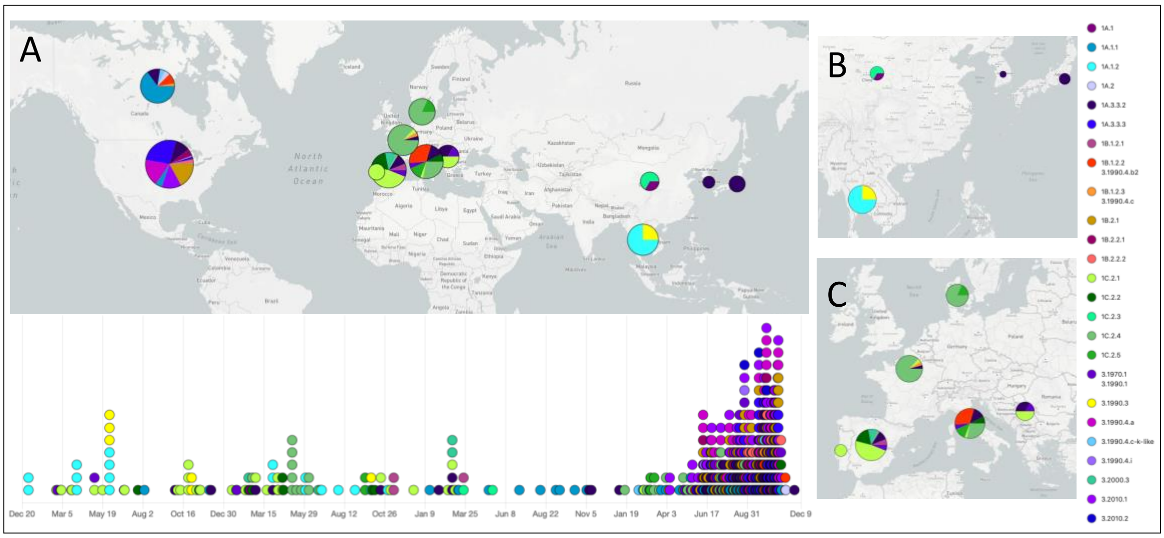


Figure 2. A) MicroReact map of swine IAV HA genes colored by phylogenetic clade for new sequences deposited July 1 - December 31, 2020 (n=662); collection date is represented along bottom timeline and data was truncated to collection dates from 2017-2020 (n=608). Strains submitted with only partial date were assigned a random date within the provided year to preserve temporal clusters. B) Enlarged image of Asia. C) Enlarged image of Europe. Geographic nodes scaled to detection proportions.

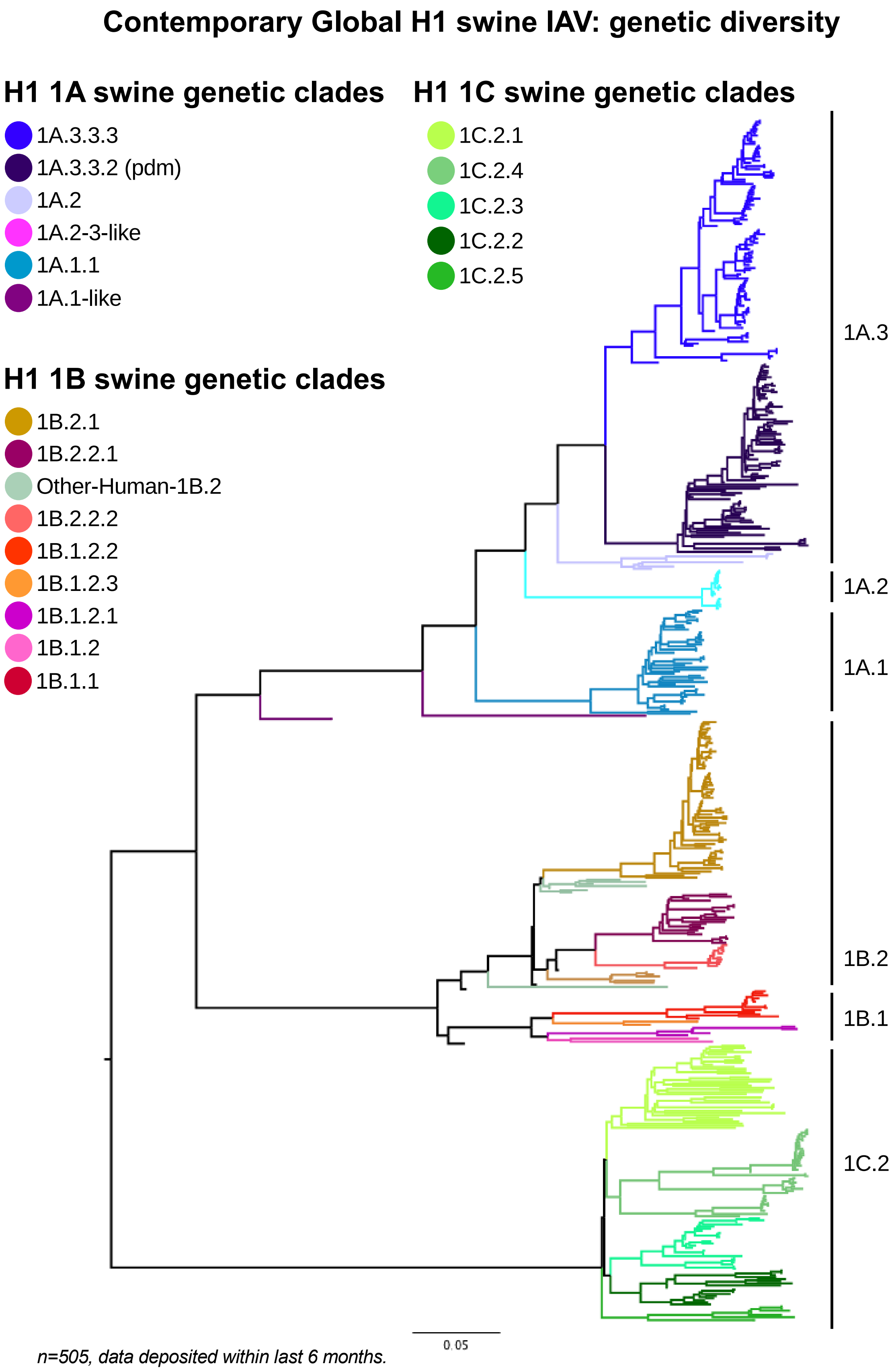


Figure 2. Global swine H1 phylogenetic tree colored by clade and annotated by global H1 lineage nomenclature. Analyses were conducted with reference sequences and new data (504 swine H1 deposited July 1, 2020 – December 31, 2020, 294 of which were also collected in this interval) from Genbank or GISAID or shared via the OFFLU swine IAV working group.

n=202, data deposited within last 6 months, and n=19 reference genes.

Figure 3. 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 (174 new swine H3 deposited July 1, 2020 – December 31, 2020, 122 of which were also collected in this interval) to Genbank or GISAID or shared via the OFFLU swine IAV working group.

Regional geographic summary

32 genetic clades from H1 and H3 IAV in swine were deposited during the reporting period (July 1, 2020 – December 31, 2020). The H1 subtype contained 20 clades detected from each of the lineages: 1A classical swine clades (n=6); 1B human-seasonal clades (n=9); and 1C Eurasian avian clades (n=5). The remaining 12 genetic clades were within 6 distinct lineages that were derived from human seasonal H3 virus spillovers: these lineages are grouped by the decade of introduction into swine: 1970 clades (n=1), 1990 clades (n=3), 2000 clades (n=1) and 2010 clades (n=2). The 1990.4 lineage further diversified and cocirculating genetic clades deposited during this report period (n=5).

- The 1A classical swine lineage viruses were reported globally: 1A.1 in China; 1A.1.1 and 1A.2 in USA and Canada; 1A.1.2 in Thailand; 1A.3.3.3 in USA; and the 1A.3.3.2/pdm detected in 9 reporting countries.
- 1B.1 human seasonal lineage was only in Europe: 1B.1.2 in Spain; 1B.1.2.1 in Spain; 1B.1.2.2 in Italy; and 1B.1.2.3 in France. The 1B.2 human seasonal lineage reported from the Americas with 1B.2.1, 1B.2.2.1, 1B.2.2.2 in the USA and 1B.2.2 in Brazil.
- The 1C Eurasian avian lineage was detected in Europe and Asia but the clades were generally regionally restricted. 1C.2.1 were detected in Spain, France, Italy, Portugal, and Serbia. 1C.2.2 were detected in Spain and Italy. 1C.2.3 were detected in China.
    - **Two new groups of statistically supported were classified as 1C.2.4 in Denmark, Spain, France, and Italy; and 1C.2.5 in Denmark and Italy.**
    - **The 1C.2.4 clade demonstrated novel amino acid deletions (Figure 7) in the HA1 that demonstrated regional circulation patterns. Many of these H1 were now paired with an N2 through a recent reassortment event**.
- The H3 1970.1 clade was restricted to Europe with reports from Spain, Italy, and Serbia. The H3 1990.1, 2010.1, and 2010.2 clades were restricted to the USA, and the 1990.4 lineage clades were detected in the USA and Canada. An H3 1990.3 clade was reported from Thailand and 1990.5 from Brazil.

During this reporting period, variant cases were reported in Canada (H1, 1A.1.1), Brazil (H1, 1A.3.3.2), Netherlands (H1, 1C.2.1), and China (H1, no sequence found).

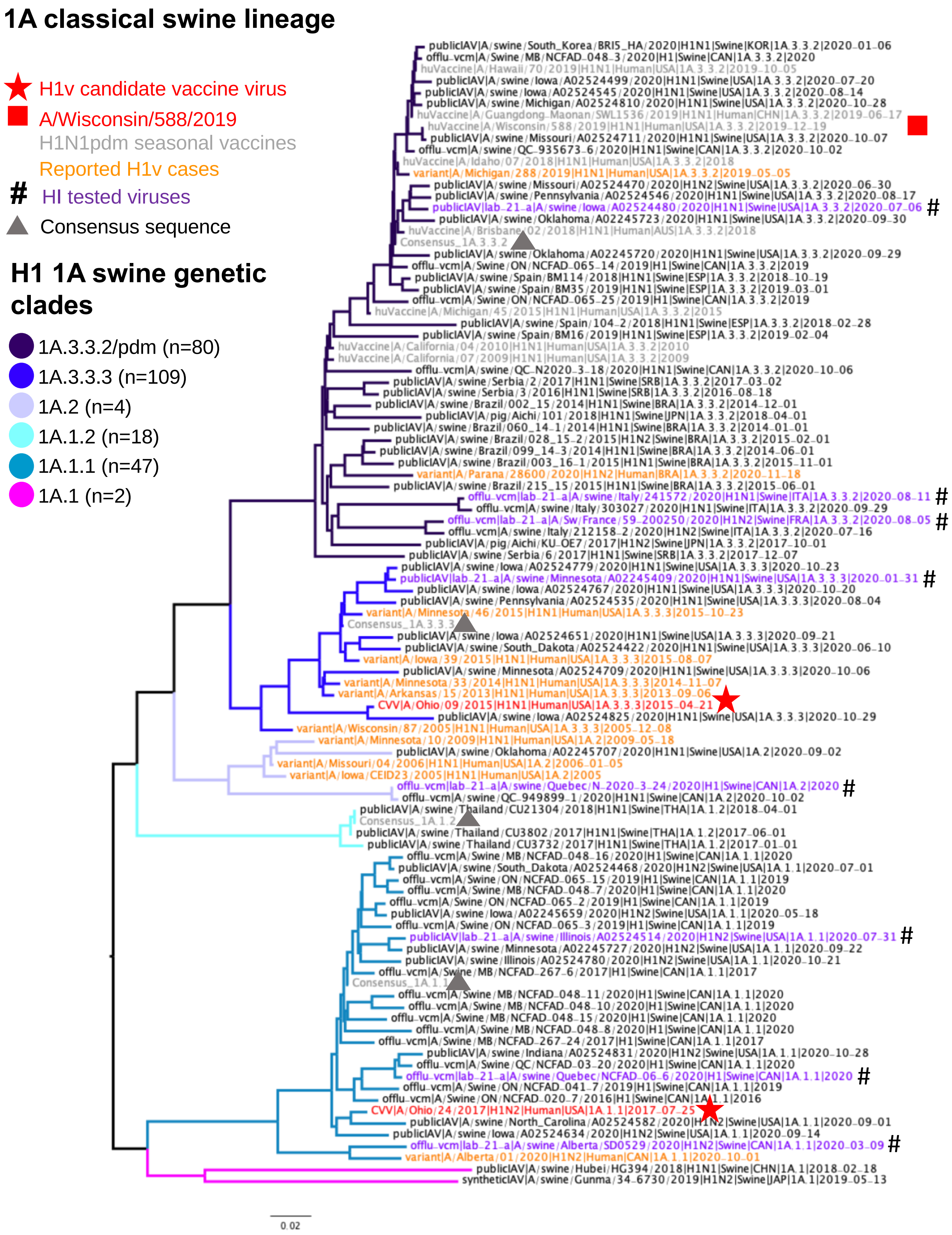


Figure 4. Swine H1 HA genes of the 1A lineage (tree was proportionally down sampled to 74 swine HA genes from 253). Detections of each clade from data deposited between July 1 to December 31, 2020 are presented adjacent to the clade name.

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/Ohio/9/2015 CVV RG48A	A/Wisconsin/588/2019
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 H1N2v	<10	<10	<10
A/swine/Alberta/SD0529/2020*	1A.1.1	alpha H1N2	10	<10	20
A/Swine/QC/NCFAD-06-6/2020	1A.1.1	alpha-del H1N2	10	<10	<10
A/swine/Quebec/N-2020-3-24/2020	1A.2	beta H1	20	10	<10
A/Ohio/9/2015 CVV	1A.3.3.3	gamma.1 H1N1v	<10	640	10
A/swine/Minnesota/A02245409/2020	1A.3.3.3	gamma.3 H1N1	<10	40	40
A/Wisconsin/588/2019	1A.3.3.2	H1N1pdm09	<10	40	1280
A/swine/Iowa/A02524480/2020	1A.3.3.2	H1N1pdm09	20	<10	80

Reference CVV in red, seasonal vaccine strains in gray, new swine strains in bold. Homologous titers highlighted in gray. ***A/swine/Alberta/SD0529/2020 has 94% identity to variant strain A/Alberta/01/2020 H1N2v.**

- The contemporary swine 1A.1.1 alpha and alpha-deletion strains demonstrated a significant >8-fold decrease from CVV A/Ohio/24/2017.
- The swine 1A.2 (beta) does not have a within clade CVV and demonstrated a significant >8-fold decrease from all vaccine strain anti-sera.
- The contemporary swine 1A.3.3.3 (gamma) clade 3 demonstrated a significant >8-fold decrease from the 1A.3.3.3 clade 1 CVV A/Ohio/24/2015.
- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine demonstrated a significant >8-fold decrease from the A/Wisconsin/588/2019 vaccine strain.

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	1A 3.3.2	160	80	160
A/Brisbane/02/2018	1A 3.3.2	40	640	160
A/Michigan/45/2015	1A 3.3.2	40	80	640
A/swine/Italy/241572/2020	1A.3.3.2_ITAclade	<20	<20	<20

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

- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine demonstrated no cross-reactivity to three previous human seasonal vaccine strains including A/California/04/2009. See variant section for further assessment of 1A .3.3.2 viruses in European pigs

Table 3. Amino acid substitutions between recent swine 1A.1.1 strains compared to the within-clade 1A.1.1 CVV A/Ohio/24/2017. 1A.1.1 variant case A/Alberta/01/2020 detected during this reporting period is presented in orange.

site	**A/Ohio/24/2017**	**A/swine/Illinois/A02524514/2020**	**A/swine/Quebec/NCFAD-06-6/2020**	**A/swine/Alberta/SD0529/2020**	Consensus_1A.1.1	A/Alberta/01/2020	Annotations
5	I			V		V	
43	K		R				
45	G			R		R	
48	A	S					
69	S					L	Cb
70	I			L		P	Cb
71	L			F			
74	S			N			
84	N		T	D		D	
86	D		G				
94	S			D		D	
106	S			I			
113	K					R	
116	I			M		M	
118	P			S			
119	I			K		K	
127	K		E	E		E	
129	-			D		N	
130	-			K		K	
131	G					S	
132	V	E					RBS
135	S			A		A	RBS
138	D	Y	Y	Y	Y	Y	
141	A	Q	K		K		
149	I	M	M		M		
155	G	D		D	D	D	Sa
156	N	D	D		D	D	Sb
157	S			V			Sa
160	R			M		M	Sa
169	K			R			

170	R	G		G	G	G	Ca2
185	G		D	K		E	
186	N					D	
189	Q					K	Sb, RBS
193	Q		H			K	Sb, RBS
196	N			D		D	
198	S			Y		Y	
209	E	K	K	K	K	K	
211	K			E		E	
216	T		K				
219	K		E			R	RBS
222	G			D		D	RBS
224	T	A			A		RBS
234	V		I				
235	E			K		K	
239	T			K		K	
252	K	R		R	R	R	
261	S			Y			
262	G			E		E	
267	V			I		I	
272	I			V		V	
278	T			A		I	
307	V					I	
310	T	R	K		R		
311	K	R	R		R	R	
321	I			T		T	
aadiff		**13**	**16**	**36**	**11**	**39**	

***Within-clade CVV in red, within-clade variant in orange, swine strains in black.**

Table 4. Amino acid substitutions between recent swine 1A.2 strains compared to the current human seasonal vaccine 1A.3.3.2 A/Wisconsin/588/2019. A direct comparison to the human seasonal vaccine with the highest amino acid similarity A/California/07/2009 is included.

Relative to HI

site	A/Wisconsin/588/2019	**A/swine/Quebec/N-2020-3-24/2020**	**A/swine/Oklahoma/A02245707/2020**	**A/swine/QC-949899-1/2020**	Consensus_1A.2	A/California/07/2009	Annotations
14	D		E				
24	V		I				
35	D	N	N	N	N		
43	K		N				
61	I	L	L	L	L		
71	S	F	F	F	F		
73	A	R		R	R		Cb
74	R		S			S	
83	S	P	P	P	P	P	
84	N					S	
85	S	P		P	P		
86	D		Y	E			
94	D		N				
96	I		N				
97	N					D	
104	Q	H	H	H	H		
109	S	L	Y	L	L		
120	T	A	A	A	A		
121	S	N	N	N	N		
128	S	T	T	T	T		
129	D	N		N	N	N	
130	N	K	K	K	K	K	
137	P	S		S	S		
138	H	Y	Y	Y	Y		
142	K	N	N	N	N		
146	K		R				
156	K	N	N	N	N	N	Sb
161	I	L	L	L	L	L	

Relative to most similar vaccine

site	A/California/07/2009	**A/swine/Quebec/N-2020-3-24/2020**	**A/swine/Oklahoma/A02245707/2020**	**A/swine/QC-949899-1/2020**	Consensus_1A.2	Annotations
14	D		E			
24	V		I			
35	D	N	N	N	N	
43	K		N			
61	I	L	L	L	L	
71	S	F	F	F	F	
73	A	R		R	R	Cb
74	S	R		R	R	
84	S	N	N	N	N	
85	S	P		P	P	
86	D		Y	E		
94	D		N			
96	I		N			
97	D	N	N	N	N	
104	Q	H	H	H	H	
109	S	L	Y	L	L	
120	T	A	A	A	A	
121	S	N	N	N	N	
128	S	T	T	T	T	
129	N		D			
137	P	S		S	S	
138	H	Y	Y	Y	Y	
142	K	N	N	N	N	
146	K		R			
162	S		R			Sa
166	I		S			Ca1
168	D	N	N	N	N	
170	G	K	K	K	K	Ca2

162	N	S	R	S	S	S	Sa
163	Q	K	K	K	K	K	Sa
164	T	S	S	S	S	S	Sa
166	I		S				Ca1
168	D	N	N	N	N		
170	G	K	K	K	K		Ca2
173	V		I				
176	L	I	I	I	I		
183	P		S			S	
185	I	S	D	S	S	S	
186	A	T		T	T		
190	S	T	A	T	T		Sb, RBS
195	A	E		E	E		Sb, RBS
203	T	S	S	S	S	S	
205	R	K	K	K	K		
209	K		R				
216	T	A	A	A	A	I	
224	E	A	A	A	A		RBS
228	N		D				
234	V	I	I	I	I		
235	E	D		D	D		
239	K	T	T	T	T		
240	I		V				
250	A	V	V	V	V	V	
256	T	A	A	A	A	A	
258	E		K				
260	D	G	G	G	G	N	
261	A	S	S	S	S		
267	I		V				
270	T	K	A	K	K		
273	H	Q		Q	Q		
274	D	N		N	N		
276	N	T		T	T		
283	E	K	K	K	K	K	
285	A	S		S	S		
295	V	I		I	I	I	
298	I	V	V	V	V		
302	K	E	E	E	E		
311	K	N		N	N		
314	L	M	M	M	M		
321	V	I	I	I	I	I	
aadiff		**51**	**54**	**52**	**51**	**21**	

173	V		I			
176	L	I	I	I	I	
183	S	P		P	P	
185	S		D			
186	A	T		T	T	
190	S	T	A	T	T	Sb, RBS
195	A	E		E	E	Sb, RBS
205	R	K	K	K	K	
209	K		R			
216	I	A	A	A	A	
224	E	A	A	A	A	RBS
228	N		D			
234	V	I	I	I	I	
235	E	D		D	D	
239	K	T	T	T	T	
240	I		V			
258	E		K			
260	N	G	G	G	G	
261	A	S	S	S	S	
267	I		V			
270	T	K	A	K	K	
273	H	Q		Q	Q	
274	D	N		N	N	
276	N	T		T	T	
285	A	S		S	S	
295	I		V			
298	I	V	V	V	V	
302	K	E	E	E	E	
311	K	N		N	N	
314	L	M	M	M	M	
aadiff		**40**	**45**	**41**	**40**	

Table 5. Amino acid substitutions between recent swine 1A.3.3.2 strains compared to the human seasonal H1 vaccine strain A/Wisconsin/588/2019. Recent human seasonal vaccines are also presented for context. 1A.3.3.2 variant case A/Parana/28600/2020 detected during this reporting period, and within-clade variant A/Michigan/288/2019 are presented in orange. Three additional swine strains are included that reflect regional diversity of the 1A.3.3.2 clade: A/swine/Serbia/2/2017, A/swine/France/59-200250/2020, and A/swine/Brazil/099_14-3/2014.

site	A/Wisconsin/588/2019	**A/swine/Iowa/A02524480/2020**	**A/swine/Italy/241572/2020**	Consensus_1A.3.3.2	A/swine/Serbia/2/2017	A/Sw/France/59-200250/2020	A/swine/Brazil/099_14-3/2014	A/Parana/28600/2020	A/Michigan/288/2019	A/Idaho/07/2018	A/Brisbane/02/2018	A/Michigan/45/2015	A/California/04/2010	A/California/07/2009	Annotations
2	T							K							
19	V		I												
43	K		Q			R									
45	R										G				
48	A					T									
54	K				R										
69	S		P												Cb
70	L		I												Cb
73	A						V		S						Cb
74	R		G		S	S						S	S	S	
77	S						P								
82	T		A												
83	S													P	
84	N		S		S	S	S	S					S	S	
85	S		P												
96	I							T							
97	N		D		D	D	D	D					D	D	
104	Q		H												
113	R	K													
119	K		N												
120	T		A			A	S	S	A						
121	S					D									
125	N					S									Sa
127	D				E										
128	S							L							
129	D	N	S	N	N	E	N	N	N	N	N	N	N	N	
130	N	K	K	K	K				K	K	K	K	K	K	
134	A					S									RBS
135	A						S	S							RBS
137	P	S													
138	H					R		Q							

139	A		N												
142	K		N				N	N	R						
143	S				G	G									
152	V		I		T		I	I							
155	G		E		V										Sa
156	K	N	N	N	N	N	N	N	N	N	N	N	N	N	Sb
161	I	L	L	L		L	L	L	L	L	L	L	L	L	
162	N		S		S	S	S	S					S	S	Sa
163	Q		I		K	K	K	I					K	K	Sa
164	T		S		S	S	S	S				S	S	S	Sa
166	I		V			V									Ca1
173	V	I													
176	L		V												
183	P				S							S	S	S	
185	I	T	N	T	S	N	N	N	T	T	T	T	S	S	
186	A		D			S	T	T							
187	D		S			S	S	S							RBS
190	S		W					T							Sb, RBS
194	N		D												RBS
195	A					T	E								Sb, RBS
196	D		N												
202	G		S												
203	T													S	
205	R		K												
210	F		Y												
211	K				T										
216	T		R			I	I	I					I	I	
222	D				N		N	N							RBS
223	Q										R	R			RBS
250	A	V		V	V	V	V		V	V	V	V	V	V	
256	T		A		A	S	A	A					A	A	
259	R		K												
260	D	N	N	N	N	N	N	N	N	N	N	N	N	N	
261	A		T		T										
270	T		A												
272	V		I												
274	D		N												
276	N							D							
282	P										A				
283	E		K		K	K	K	K					K	K	
295	V		I		I		I	I				I	I	I	
298	I										V				
308	K		R			R	R								
311	K		R												
321	V		T		I	T								I	
aadiff		**10**	**47**	**7**	**25**	**29**	**26**	**27**	**10**	**7**	**11**	**12**	**18**	**21**	

***Reference human seasonal vaccines in gray, within-clade variants in orange, swine strains in black.**

Table 5a. Amino acid substitutions between recent swine 1A.3.3.2 strains compared to the human seasonal H1 vaccine strain with the highest amino acid similarity – A/California/04/2010 or A/Idaho/07/2018. A/Wisconsin/588/2019. The 1A.3.3.2 variant case A/Parana/28600/2020 detected during this reporting period, and within-clade variant A/Michigan/288/2019 are presented in orange. Three swine strains are included that reflect regional diversity of the 1A.3.3.2 clade: A/swine/Serbia/2/2017, A/swine/France/59-200250/2020, and A/swine/Brazil/099_14-3/2014.

Relative to most similar vaccine

site	A/California/04/2010	**A/swine/Italy/241572/2020**	A/swine/Serbia/2/2017	A/Sw/France/59-200250/2020	A/swine/Brazil/099_14-3/2014	A/Parana/28600/2020	A/Michigan/45/2015	A/California/07/2009	Annotations
2	T					K			
19	V	I							
43	K	Q		R					
48	A			T					
54	K		R						
69	S	P							Cb
70	L	I							Cb
73	A				V				Cb
74	S	G			R	R			
77	S				P				
82	T	A							
83	S							P	
84	S						N		
85	S	P							
96	I					T			
97	D						N		
104	Q	H							
119	K	N							
120	T	A		A	S	S			
121	S			D					
125	N			S					Sa
127	D		E						
128	S					L			
129	N	S		E					
130	K			N	N	N			
134	A			S					RBS
135	A				S	S			RBS
138	H			R		Q			
139	A	N							
142	K	N			N	N			
143	S		G	G					

Relative to most similar vaccine

site	A/Idaho/07/2018	**A/swine/Iowa/A02524480/2020**	Consensus_1A.3.3.2	A/Michigan/288/2019	Annotations
73	A			S	Cb
113	R	K			
120	T			A	
137	P	S			
142	K			R	
173	V	I			
aadiff		**3**	**0**	**3**	

152	V	I	T		I	I			
155	G	E	V						Sa
161	L		I						
162	S						N		Sa
163	K	I				I	Q		Sa
166	I	V		V					Ca1
176	L	V							
183	S	P		P	P	P			
185	S	N		N	N	N	T		
186	A	D		S	T	T			
187	D	S		S	S	S			RBS
190	S	W				T			Sb, RBS
194	N	D							RBS
195	A			T	E				Sb, RBS
196	D	N							
202	G	S							
203	T							S	
205	R	K							
210	F	Y							
211	K		T						
216	I	R	T				T		
222	D		N		N	N			RBS
223	Q						R		RBS
250	V	A				A			
256	A			S			T		
259	R	K							
261	A	T	T						
270	T	A							
272	V	I							
274	D	N							
276	N					D			
283	K						E		
295	I			V					
308	K	R		R	R				
311	K	R							
321	V	T	I	T				I	
aadiff		**38**	**11**	**20**	**15**	**19**	**9**	**3**	

***Reference human seasonal vaccine in gray, within-clade variants in orange, swine strains in black.**

Table 6. Amino acid substitutions between recent swine 1A.3.3.3 strains compared to the within-clade 1A.3.3.3 CVV A/Ohio/09/2015.

site	A/Ohio/09/2015	**A/swine/Minnesota/A02245409/2020**	Consensus_1A.3.3.3	Annotations
2	K	T	T	
3	I	L	L	
36	K	R	R	
71	A	S	S	
83	S	P		
84	S	N	N	
86	N	E	E	
113	K	R	R	
120	T	A		
127	E	D	D	
130	K	R	R	
137	P	S		
146	K	R	R	
149	I	V	V	
153	K	Q	Q	Sb
155	E	G	G	Sa
161	I	V		
163	I	K	K	Sa
166	T	I	I	Ca1
169	R		K	
170	G	E	E	Ca2
183	P	S	S	
186	T	A	A	
193	Q	K	K	Sb, RBS
196	N	D	D	
197	S	A	A	
205	R	K	K	
222	G	D	D	RBS
250	V	A	A	

269	E	D	D	
271	P	S	S	
aadiff		**30**	**27**	

***Within-clade CVV in red, swine strains in black.**

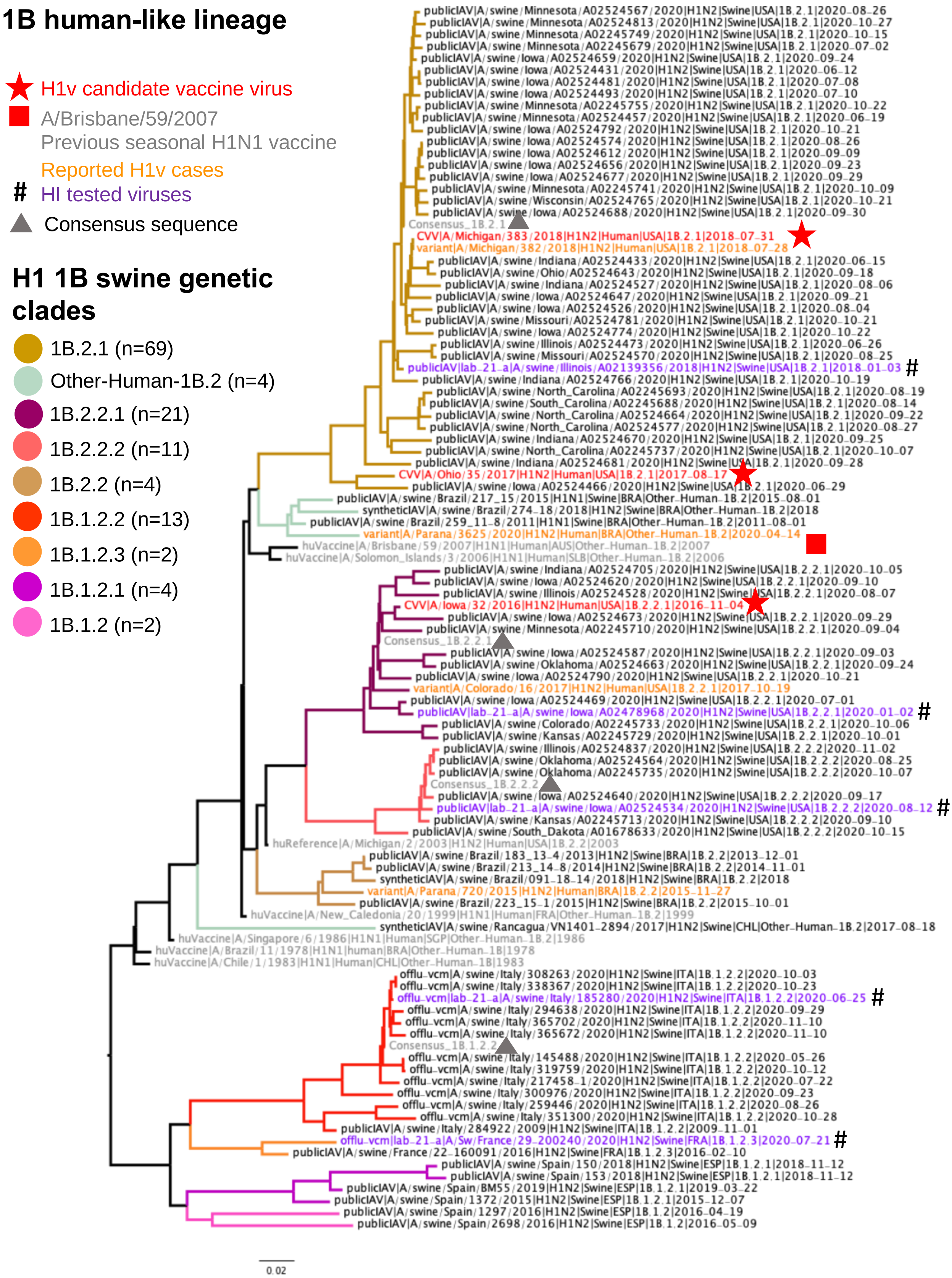


Figure 5. Swine H1 HA genes of the 1B lineage (tree was proportionally down sampled to 85 swine HA genes from 130). Detections of each clade deposited between July 1 to December 31, 2020 are presented adjacent to the clade name.

Antigenic analysis: Swine 1B Lineage

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

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

	Global Clade	**US Clade**	**A/Iowa/32/2016**	**A/Michigan/383/2018 RG58A**
A/Iowa/32/2016	1B.2.2.1	Delta 1a H1N2v	320	10
A/swine/Iowa/A02478968/2020	1B.2.2.1	Delta 1a H1N2	160	20
A/swine/Iowa/A02524534/2020	1B.2.2.2	Delta 1b H1N2	40	10
A/Michigan/383/2018 RG58A	1B.2.1	Delta 2 H1N2v	20	160
A/swine/Illinois/A02139356/2018	1B.2.1	Delta 2 H1N2	10	160

Reference CVV in red, new swine strains in bold. Homologous titers highlighted grey.

- The contemporary swine 1B.2.2.1 (delta-1a) demonstrated cross-reactivity to CVV A/Iowa/32/2016.
- The contemporary swine 1B.2.2.2 (delta-1b) does a within clade CVV and demonstrated a significant 8-fold decrease from CVV A/Iowa/32/2016.
- The contemporary swine 1B.2.1 (delta-2) remained cross-reactive to CVV A/Michigan/383/2018.

Table 8. 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/BRAZIL/11/1978
A/Michigan/383/2018	1B.2.1	160	<20
A/BRAZIL/11/1978	H1 Seasonal	<20	1280
A/swine/Italy/185280/2020	1B.1.2.2	40	80
A/Sw/France/29-200240/2020	1B.1.2.3	640	640

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

- The contemporary swine 1B.1.2.2. strain demonstrated reduced cross-reactivity to A/Michigan/383/2018 and poor cross-reactivity to A/Brazil/11/1978.
- The contemporary swine 1B.1.2.3 strain demonstrated good cross-reactivity to both A/Michigan/383/2018 and to A/Brazil/11/1978.

Table 9. Amino acid substitutions between recent swine 1B.2.2.1 compared to the within-clade 1B.2.2.1 CVV A/Iowa/32/2016.

site	A/Iowa/32/2016	**A/swine/Iowa/A02478968/2020**	Consensus_1B.2.2.1	Annotations
19	L	V	V	
74	K	E	E	
84	N	D		
96	T	A	A	
132	K	V	V	RBS
141	E	K		
166	K	E		Ca1
168	E	D	D	
237	G	R		Ca1
273	D	N		
277	A	T		
aadiff		**11**	**5**	

***Within-clade CVV in red, swine strains in black.**

Table 10. Amino acid substitutions between recent swine 1B.2.2.2 compared to the CVV with the highest amino acid similarity, 1B.2.2.1 A/Iowa/32/2016. A direct comparison to the human seasonal H1 vaccine with the highest amino acid similarity A/New_Caledonia/20/1999 is included.

Relative to HI

site	A/Iowa/32/2016	**A/swine/Iowa/A02524534/2020**	Consensus_1B.2.2.2	A/New_Caledonia/20/1999	Annotations
2	T	M			
19	L	V	V	V	
50	L	I	I		
69	S			L	Cb
74	K			E	
82	A	T	T	T	
85	S	P	P	P	
86	D	E	E	E	
89	A			T	
94	Q	D	D	Y	
96	T	A	A	A	
119	R	K	K	K	
125	N			T	Sa
129	V	T	T		
132	K			V	RBS
141	E		K	K	
142	G			S	
149	I	V	V	L	
153	V	E	E	G	Sb
166	K			V	Ca1
168	E	N	N	N	
170	G	E	E	E	Ca2
175	I			V	
176	L	I	I		
183	S	P	P	P	
184	N	D	D		
186	G	E	E		
187	D	N	N		RBS
190	T			A	Sb, RBS

Relative to most similar vaccine

site	A/New_Caledonia/20/1999	**A/swine/Iowa/A02524534/2020**	Consensus_1B.2.2.2	Annotations
2	T	M		
50	L	I	I	
69	L	S	S	Cb
74	E	K	K	
89	T	A	A	
94	Y	D	D	
125	T	N	N	Sa
129	V	T	T	
132	V	K	K	RBS
141	K	E		
142	S	G	G	
149	L	V	V	
153	G	E	E	Sb
166	V	K	K	Ca1
175	V	I	I	
176	L	I	I	
184	N	D	D	
186	G	E	E	
187	D	N	N	RBS
190	A	T	T	Sb, RBS
193	H	R	R	Sb, RBS
194	T	K	K	RBS
202	V	A	A	
208	R	E	E	
215	A	T	T	
222	D	N	N	RBS
228	N	K	K	
236	P	A	A	
249	I	V	V	

193	H	R	R		Sb, RBS
194	T	K	K		RBS
202	M	A	A	V	
208	R	E	E		
215	T			A	
222	D	N	N		RBS
228	N	K	K		
236	P	A	A		
244	T	A	A	A	
249	I	V	V		
256	A	T	T		
261	L			F	
269	K	N	N	N	
271	P	S	S		
273	D	N			
274	E	K	K		
277	A	T	T		
283	K	Q	Q	Q	
289	N	S	S	S	
310	T	A	A	A	
311	K		E		
314	M	L			
aadiff		**38**	**37**	**30**	

256	A	T	T	
261	F	L	L	
271	P	S	S	
273	D	N		
274	E	K	K	
277	A	T	T	
311	K		E	
314	M	L		
aadiff		**36**	**33**	

***Reference human seasonal vaccines in gray, within-lineage CVV in red, swine strains in black.**

Table 11. Amino acid substitutions between recent swine 1B.2.1 strains compared to the within-clade 1B.2.1 CVV A/Michigan/383/2018.

site	**A/Michigan/383/2018**	**A/swine/Illinois/A02139356/2018**	Consensus_1B.2.1	Annotations
71	N	T	T	
169	E	K		
170	G	E	E	Ca2
173	V	I	I	
259	K	R		
260	S	G	G	
aadiff		**6**	**4**	

***Within-clade CVV in red, swine strains in black.**

Table 12. Amino acid substitutions between recent swine 1B.1.2 strains compared to the human seasonal H1 vaccine with the highest amino acid similarity, A/Chile/1/1983. An additional human seasonal H1 vaccine A/Brazil/11/1978 is included. This swine clade has fewer than five detections; a consensus sequence comparison was not conducted.

site	A/Chile/1/1983	**A/swine/Spain/1297/2016**	**A/swine/Spain/2698/2016**	A/Brazil/11/1978	Annotations
19	V	I	I		
36	N	S	S	S	
43	K		R	R	
45	K	R			
47	I	V	V		
69	S	P			Cb
80	A	V	T		
85	S	P	P		
89	T	I	I		
96	A	S	S		
121	S	N	G	R	
125	K		E		Sa
127	N		S		
128	V	A		I	
129	T	N	N		
130	K	N		R	
134	A	K	K		RBS
135	A	S	S	S	RBS
139	K		W		
141	K	Q	E		
149	L	I	I		
151	L	I			
157	S		A		Sa
163	K		R		Sa
166	V	M			Ca1
168	N	D			
170	E	K	R		Ca2
181	H	N			
189	K	M	M		Sb, RBS
190	T	A	A		Sb, RBS

196	N	T	T		
198	Y	H			
199	V		I		
205	H			N	
210	F	Y			
215	A	T	T		
216	K	R	R		
217	R		K		
218	P	T	T		RBS
222	N	D	D	G	RBS
237	G	E	E		Ca1
241	I	T			
258	S	N			
261	F	S	S		
262	G	R	R		
267	T	V	V		
272	M		T		
276	D		N		
277	A	T	S	T	
298	V	F			
299	T	A			
300	I	L			
aadif		**41**	**36**	**9**	

***Reference human seasonal vaccines in gray, swine strains in black.**

Table 13. Amino acid substitutions between recent swine 1B.1.2.1 strains compared to the human seasonal H1 vaccine with the highest amino acid similarity, A/Chile/1/1983. An additional human seasonal H1 vaccine A/Brazil/11/1978 is included.

site	A/Chile/1/1983	**A/swine/Spain/BM55/2019**	Consensus_1B.1.2.1	A/Brazil/11/1978	Annotations
36	N	S	S	S	
43	K	R	R	R	
73	K	Q	Q		Cb
83	P	T	T		
85	S	P	P		
89	T	I	I		
96	A	S	S		
108	V	I	I		
121	S			R	
124	P	S	S		Sa
125	K	N	N		Sa
127	N	S	S		
128	V			I	
129	T	N	N		
130	K			R	
135	A	S	S	S	RBS
138	H	Q	Q		
166	V	M	M		Ca1
167	N	Y	Y		
169	K	N	N		
170	E	R	R		Ca2
185	I	M	M		
189	K	M	M		Sb, RBS
190	T	A	A		Sb, RBS
196	N	T	T		
205	H			N	
208	R	K	K		
211	T	I	I		
215	A	S	S		
216	K	R	R		

217	R	K	K		
218	P	T	T		RBS
220	V	I	I		RBS
222	N	D	D	G	RBS
237	G	K	K		Ca1
241	I	M			
248	L	F	F		
252	W	L	L		
258	S	N	N		
261	F	S			
262	G		R		
267	T	V	V		
272	M	V	V		
277	A	T	T	T	
310	T	R	K		
aadiff		**40**	**39**	**9**	

***Reference human seasonal vaccines in gray, swine strains in black.**

Table 14. Amino acid substitutions between recent swine 1B.1.2.2 strains compared to the human seasonal H1 vaccine with the highest amino acid similarity, A/Chile/1/1983. An additional human seasonal H1 vaccine A/Brazil/11/1978 is included.

site	A/Chile/1/1983	**A/swine/Italy/185280/2020**	Consensus_1B.1.2.2	A/Brazil/11/1978	Annotations
35	D	N	N		
36	N	S	S	S	
43	K			R	
47	I	V	V		
82	T	P	P		
85	S	A	A		
89	T	L	L		
96	A	S	S		
109	S	A	A		
121	S			R	
124	P	S			Sa
127	N	S	S		
128	V			I	
129	T	-	-		
130	K	-	-	R	
132	V	S	S		RBS
135	A	S	S	S	RBS
137	S	P	P		
138	H	K	K		
139	K	Q	Q		
146	R	T	T		
162	S	T	T		Sa
163	K	M	M		Sa
166	V	M	M		Ca1
168	N	D	D		
171	K	R	R		
185	I	M	M		
189	K	R	R		Sb, RBS
190	T	A	A		Sb, RBS
196	N	T	T		

202	V	M	M		
205	H	Q	Q	N	
211	T	A	A		
220	V	I	I		RBS
222	N			G	RBS
235	E	D	D		
237	G	R	R		Ca1
241	I	T	T		
249	I	V	V		
258	S	K	K		
262	G	E	E		
267	T	V	V		
277	A			T	
288	S	N	N		
308	R	K	K		
310	T	A	A		
313	R	K	K		
315	V	A	A		
aadif		**43**	**42**	**9**	

***Reference human seasonal vaccines in gray, swine strains in black.**

Table 15. Amino acid substitutions between recent swine 1B.1.2.3 strains compared to the human seasonal H1 vaccine with the highest amino acid similarity, A/Chile/1/1983. An additional human seasonal H1 vaccine A/Brazil/11/1978 is included. This swine clade has fewer than five detections; a consensus sequence comparison was not conducted.

site	A/Chile/1/1983	**A/Sw/France/29-200240/2020**	A/Brazil/11/1978	Annotations
14	D	E		
36	N		S	
43	K		R	
47	I	T		
48	A	T		
84	N	S		
85	S	P		
89	T	I		
96	A	S		
106	S	G		
111	F	L		
121	S		R	
127	N	S		
128	V	I	I	
129	T	N		
130	K		R	
135	A	S	S	RBS
142	S	R		
143	S	N		
161	L	I		
168	N	D		
179	V	I		
189	K	R		Sb, RBS
190	T	A		Sb, RBS
196	N	T		
203	S	A		
205	H		N	
207	N	S		
216	K	R		
220	V	I		RBS

222	N	D	G	RBS
235	E	G		
237	G	K		Ca1
262	G	K		
267	T	I		
277	A	T	T	
aadiff		**31**	**9**	

***Reference human seasonal vaccines in gray, swine strains in black.**

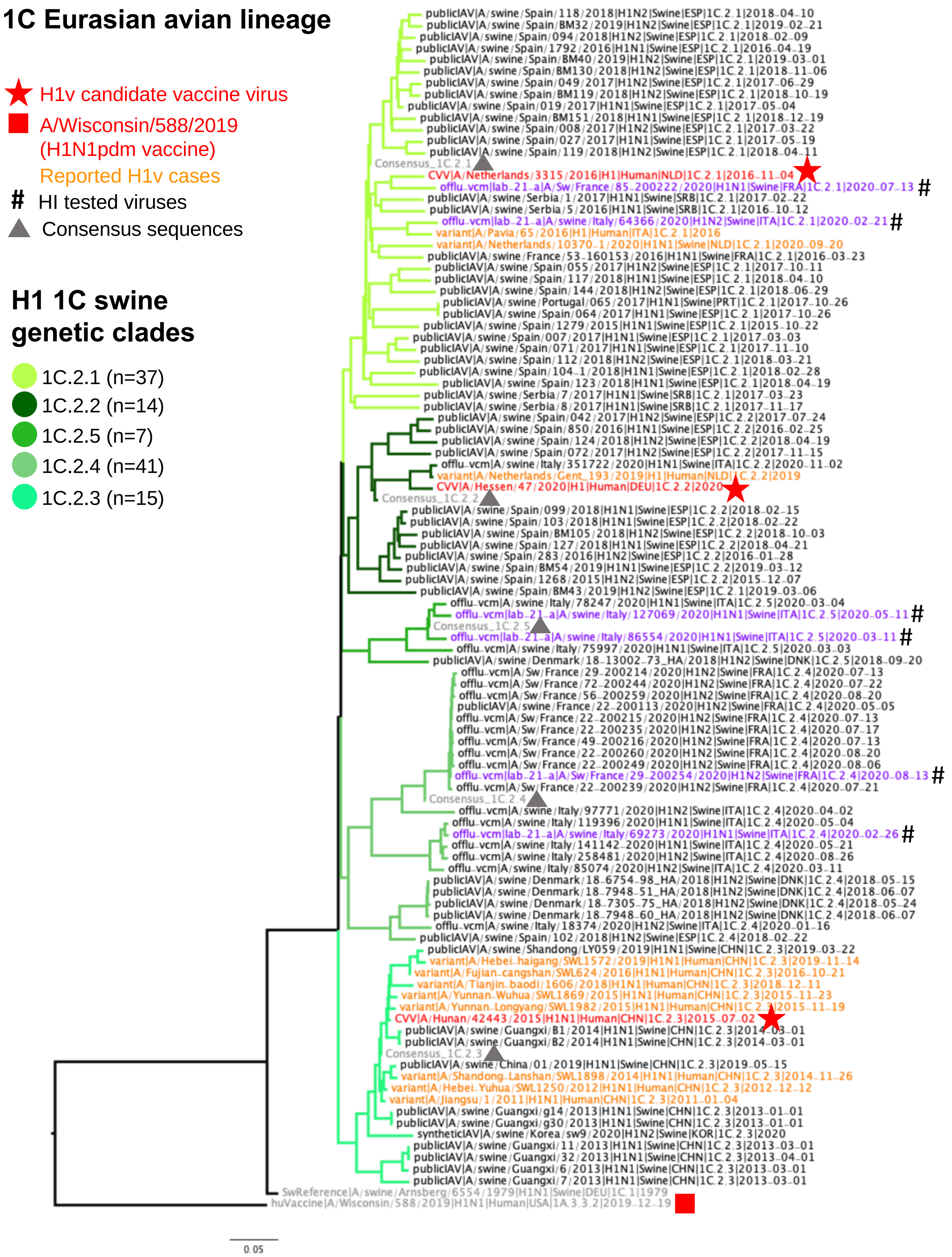


Figure 6. Swine H1 HA genes of the 1C lineage (tree was down sampled to 86 swine HA genes from 115). Detections of each clade from data deposited between July 1 to December 31, 2020 are presented adjacent to the clade name.

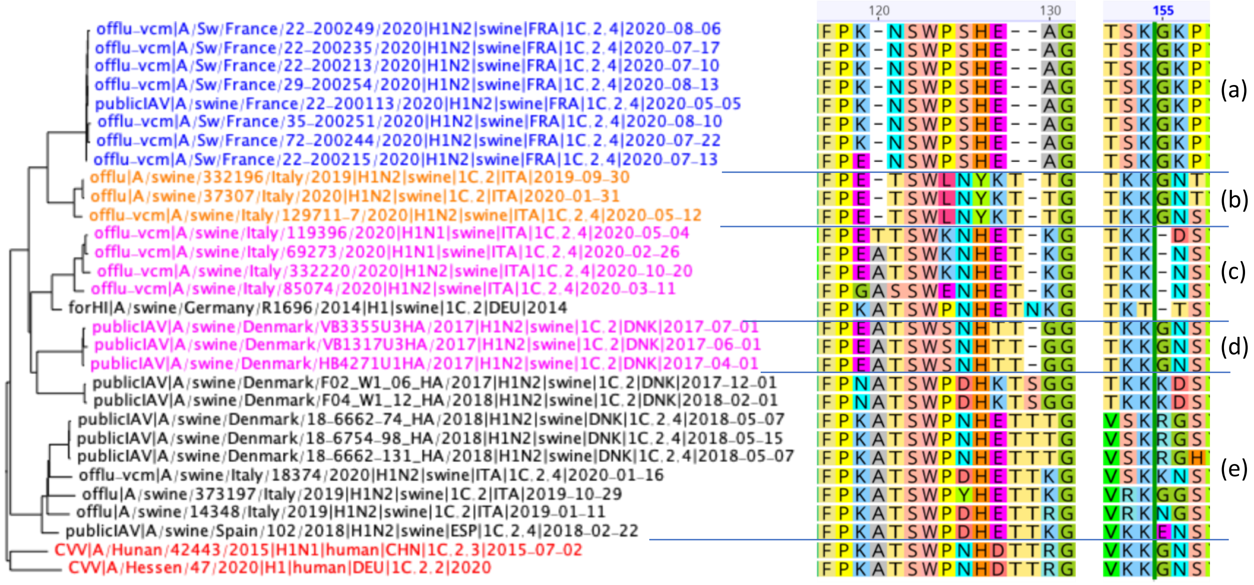


Figure 7. Deletion patterns identified in 1C.2.4 strains. This tree was subsampled from available 1C.2.4 strains, however deletion patterns in the full tree are consistent with those of the subsampled tree. Signal peptides were trimmed with the indices of [Burke 2014]. The alignment was made with MAFFT and visualized in Geneious. Group (a) has 3 deletions at 120, 128, and 129; Group (b) has 2 deletions at 120 and 129; Group (c) has deletions at 129 and 155 (except for A/swine/Germany/R1696/2014, with only a deletion at 155); Group (d) has a single 129 deletion; and Group (e) has no deletions. Many of the 1C.2.4 strains with mutations were observed to contain N2, a subtype switch from the previous predominance of Eurasian avian-lineage N1. The CVV A/Hunan/42443/2015 and A/Hessen/47/2020 were included for reference.

Antigenic analysis: Swine 1C Lineage

Table 16. 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/Hunan/42443/2015 CNIC-1601–CVV
A/Netherlands/3315/2016 CVV	1C.2.1	80	<20	20
A/PAVIA/65/2016	1C.2.1	40	640	<20
A/Hunan/42443/2015 CNIC-1601 CVV	1C.2.3	40	40	320
A/Netherlands/10370-1/2020	1C.2.1	80	40	80
A/swine/Italy/64366/2020	1C.2.1	40	40	40
A/Sw/France/85-200222/2020	1C.2.1	160	160	80
A/Sw/France/29-200254/2020	1C.2	80	80	80
A/swine/Italy/69273/2020	1C.2.4	80	80	40
A/swine/Italy/127069/2020	1C.2.5	40	40	<20
A/swine/Italy/86554/2020	1C.2.5	80	<20	<20

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

- The contemporary swine clades 1C.2.1, and 1C.2 demonstrate good cross-reactivity to CVV A/Netherlands/3115/2016, variable cross reactivity to CVV A/Hunan/42443/2015 CNIC-1601 and poor cross-reactivity to A/PAVIA/65/2016.
- The contemporary swine clade 1C.2.5 demonstrated cross-reactivity to CVV A/Netherlands/3115/2016 and poor cross reactivity to other strains.

Table 17. Amino acid substitutions between recent swine 1C.2.1 strains compared to the within-clade 1C.2.1 CVV A/Netherlands/3315/2016. A 1C.2.1 variant case A/Netherlands/10370-1/2020 detected during this reporting period is presented in orange, along with a prior 1C.2.1 variant A/Pavia/65/2016. Additional 1C lineage CVV strains 1C.2.1 A/Netherlands/3315/2016 and 1C.2.2 A/Hessen/47/2020 are included. A direct comparison to the CVV with the highest amino acid similarity 1C.2.3 A/Hunan/42443/2015 is included.

Relative to HI

site	**A/Netherlands/3315/2016**	**A/swine/Italy/64366/2020**	**A/swine/Spain/007/2017**	**A/Sw/France/85-200222/2020**	Consensus_1C.2.1	A/Pavia/65/2016	A/Netherlands/10370-1/2020	**A/Hunan/42443/2015**	**A/Hessen/47/2020**	Annotations
20	M	L	L	L	L	L		L	L	
31	N						D			
35	N		S	T					T	
44	L						M			
48	A							I		
51	Q			H						
53	G					E			K	
56	N							S		
57	V								L	
66	E							K		
68	D			E						
69	L	F				F				Cb
71	I	L	L	L	L	L	L	L	L	
74	N					Y	K			
80	I	V							V	
82	T	N		N						
83	S			P			P		P	
84	N			K					D	
89	A	T		T		T			T	
95	F			L						
96	A	T		T		N				
97	D			N		N	N			
102	R							K		
111	F					L				
120	E		A	A	A	A	A	A	A	
127	E					D		D	D	

Relative to most similar CVV

site	**A/Hunan/42443/2015**	**A/swine/Italy/64366/2020**	**A/swine/Spain/007/2017**	**A/Sw/France/85-200222/2020**	Consensus_1C.2.1	A/Pavia/65/2016	A/Netherlands/10370-1/2020	Annotations
20	L						M	
31	N						D	
35	N		S	T				
44	L						M	
48	I	A	A	A	A	A	A	
51	Q			H				
53	G					E		
56	S	N	N	N	N	N	N	
66	K	E	E	E	E	E	E	
68	D			E				
69	L	F				F		Cb
74	N					Y	K	
80	I	V						
82	T	N		N				
83	S			P			P	
84	N			K				
89	A	T		T		T		
95	F			L				
96	A	T		T		N		
97	D			N		N	N	
102	K	R	R	R	R	R	R	
111	F					L		
120	A	E						
127	D	E	E	E	E		E	
130	R	K	K	K	K	K	K	
132	T			S				RBS

130	K						R	R	
132	S	T	T		T	T	T	T	T	RBS
134	V		I		I				I	RBS
137	S			T			P			
141	I	V	A	V	A	A	A	A	A	
142	K	N		N	N	N	N	N	N	
155	E	G	G	A	G		G	G	G	Sa
161	I	L	L		L	L	L	L	L	
162	S			N					R	Sa
163	T	K	N	K	K	K	K	K	N	Sa
168	N			D						
175	I	V	V	V	V	V	V	V	V	
190	T	A								Sb, RBS
196	H					N				
199	V	I					I		I	
202	G			E		E	E			
208	K	Q	R		Q	Q	Q		Q	
214	I								T	
215	V						A			
220	V	I							I	RBS
222	E					K				RBS
227	M								I	
230	Y			H						
232	T	V				I				
253	Y	H	H		H	X	H	H		
258	N	E				D	D	K		
259	K			E						
261	S			P						
262	N	S		S	S	S		S	S	
266	M			L					I	
267	M		I	V			I	R		
269	D								N	
271	N	Q	H		H	H	H	Q	R	
272	V	L								
273	H	Q				Q				
274	N	D								
278	K								M	
288	S							G		
298	I							V		
311	Q						R		H	
321	T	I	I	I	I	I	I	I	I	

134	V		I		I			RBS
137	S			T			P	
141	A	V		V				
142	N		K					
155	G			A		E		Sa
161	L			I				
162	S			N				Sa
163	K		N					Sa
168	N			D				
190	T	A						Sb, RBS
196	H					N		
199	V	I					I	
202	G			E		E	E	
208	K	Q	R		Q	Q	Q	
215	V						A	
220	V	I						RBS
222	E					K		RBS
230	Y			H				
232	T	V				I		
253	H			Y		X		
258	K	E	N	N	N	D	D	
259	K			E				
261	S			P				
262	S		N				N	
266	M			L				
267	R	M	I	V	M	M	I	
271	Q		H	N	H	H	H	
272	V	L						
273	H	Q				Q		
274	N	D						
288	G	S	S	S	S	S	S	
298	V	I	I	I	I	I	I	
311	Q						R	
diff		**25**	**17**	**34**	**13**	**25**	**24**	

324	V	I	I	I	I	I	I	I	I	
diff		**28**	**17**	**30**	**17**	**29**	**26**	**25**	**34**	

***Within-lineage CVV in red, within-lineage variants in orange, swine strains in black.**

Table 18. Amino acid substitutions between recent swine 1C.2.2 strains compared to within-clade 1C.2.2 CVV A/Hessen/47/2020. Additional 1C lineage CVV strains 1C.2.1 A/Netherlands/3315/2016 and 1C.2.2 A/Hessen/47/2020 are included, along with the within-clade 1C.2.2 variant A/Netherlands/Gent_193/2019 and 1C.2.1 variant A/Pavia/65/2016.

site	**A/Hessen/47/2020**	**A/swine/Spain/BM54/2019**	Consensus_1C.2.2	1C.2.2|A/Netherlands/Gent_193/2019	1C.2.1|A/Pavia/65/2016	**1C.2.1|A/Netherlands/3315/2016**	**1C.2.3|A/Hunan/42443/2015**	Annotations
3	I			L				
20	L					M		
35	T	N	N		N	N	N	
36	S	N						
47	K	R						
48	A						I	
51	Q	H	H					
53	K	R	R	R	E	G	G	
54	N	T						
56	N						S	
57	L	V	V		V	V	V	
66	E						K	
69	L				F			Cb
71	L					I		
72	T	K	K					Cb
74	N				Y			
80	V				I	I	I	
83	P				S	S	S	
84	D	N	N	N	N	N	N	
89	T	I				A	A	
96	A				N			
97	D			N	N			
102	R						K	
111	F				L			
120	A	E				E		
125	N		D	D				Sa
127	D					E		
130	R				K	K		

132	T					S		RBS
134	I				V	V	V	RBS
141	A	T	T			I		
142	N					K		
155	G				E	E		Sa
161	L			I		I		
162	R	S	S		S	S	S	Sa
163	N	K	K	K	K	T	K	Sa
168	N	D						
175	V					I		
185	D			E				
195	N			S				Sb, RBS
196	H				N			
199	I				V	V	V	
202	G				E			
208	Q					K	K	
214	T	I	I	I	I	I	I	
215	V			I				
216	A	T						
220	I				V	V	V	RBS
222	E				K			RBS
227	I		M		M	M	M	
232	T				I			
235	D			N				
253	Y	H	H	H	X		H	
258	N				D		K	
262	S	N	N			N		
266	I				M	M	M	
267	M	T	T				R	
269	N	D	D	D	D	D	D	
271	R	H	H		H	N	Q	
273	H	Y	Y		Q			
278	M	K	K		K	K	K	
283	H	Y						
288	S						G	
298	I						V	
302	E			K				
311	H	Q	Q		Q	Q	Q	
321	I					T		
324	I					V		
aadiff		**26**	**20**	**15**	**32**	**34**	**29**	

***Within-lineage CVV in red, within-lineage variants in orange, swine strains in black.**

Table 19. Amino acid substitutions between recent swine 1C.2.4 strains compared to the CVV with the highest amino acid similarity, 1C.2.3 A/Hunan/42443/2015 CVV. Novel deletions observed in the swine 1C.2.4 strains are indicated by “-“ and visualized in Figure 7. Additional 1C lineage CVV strains 1C.2.1 A/Netherlands/3315/2016 and 1C.2.2 A/Hessen/47/2020, and 1C.2.1 variant A/Pavia/65/2016 are included for reference.

site	**A/Hunan/42443/2015**	**A/swine/Italy/69273/2020**	**A/Sw/France/29-200254/2020**	Consensus_1C.2.4	A/Pavia/65/2016	**A/Netherlands/3315/2016**	**A/Hessen/47/2020**	Annotations
5	V		I	I				
20	L		M	M		M		
31	N	D						
35	N	T	T	T			T	
36	S		N					
48	I	A	A	A	A	A	A	
53	G				E		K	
56	S	N	N	N	N	N	N	
57	V						L	
66	K	E	E	E	E	E	E	
69	L				F			Cb
71	L		V	V		I		
74	N				Y			
80	I						V	
83	S						P	
84	N						D	
89	A	I	T	T	T		T	
96	A	S			N			
97	D				N			
102	K		R	R	R	R	R	
104	Q	L	L	L				
106	S	G						
111	F				L			
116	I	V						
119	K	E						
120	A		-	-		E		
121	T		N	N				
124	P	K						Sa
125	N		S					Sa

127	D	E	E	E		E		
128	T	-	-	-				
129	T		-					
130	R	K	A	A	K	K		
132	T					S		RBS
134	V	G					I	RBS
135	A	S	S	S				RBS
138	H	R	K	K				
141	A	T				I		
142	N	K				K		
146	R	Q						
152	V	T	T	T				
153	K		S	S				Sb
155	G	-			E	E		Sa
156	N		K	K				Sb
157	S		P	P				Sa
159	P		S					Sa
161	L	I				I		
162	S						R	Sa
163	K					T	N	Sa
166	T		K	K				Ca1
172	E	K						
175	V					I		
185	D	Y	Y	Y				
190	T	A						Sb, RBS
194	N	S						RBS
196	H				N			
199	V						I	
202	G	V	V	V	E			
203	S	T						
208	K	Q	Q	Q	Q		Q	
214	I						T	
216	A	D	P	P				
219	K		E	E				RBS
220	V	I	I	I			I	RBS
222	E	G			K			RBS
224	A		R	R				RBS
227	M						I	
232	T				I			
235	D	G						
237	G		E					Ca1
239	T	N						

252	W	R	R	R				
253	H	Y	Y	Y	X	Y	Y	
258	K	D	D	D	D	N	N	
259	K	M						
260	G	S						
262	S		N	N		N		
266	M	L					I	
267	R	K	K	K	M	M	M	
269	D						N	
271	Q	H	H	H	H	N	R	
273	H				Q			
278	K						M	
288	G	S	S	S	S	S	S	
289	N	S	D	D				
290	L	R	R	R				
298	V	I	I	I	I	I	I	
311	Q						H	
321	I					T		
324	I					V		
aadiff		**48**	**44**	**39**	**25**	**25**	**29**	

***Within-lineage CVV in red, within-lineage variant in orange, swine strains in black.**

Table 20. Amino acid substitutions between recent swine 1C.2.5 strains compared to the CVV with the highest amino acid similarity, 1C.2.3 CVV A/Hunan/42443/2015. 1C lineage CVV strains 1C.2.1 A/Netherlands/3315/2016 and 1C.2.2 A/Hessen/47/2020, and 1C.2.1 variant A/Pavia/65/2016 are included for reference.

site	**A/Hunan/42443/2015**	**A/swine/Italy/127069/2020**	**A/swine/Italy/86554/2020**	Consensus_1C.2.5	A/Pavia/65/2016	**A/Netherlands/3315/2016**	**A/Hessen/47/2020**	Annotations
20	L					M		
30	V	I						
35	N						T	
44	L	M	M	M				
47	K	N	N	N				
48	I	A	A	A	A	A	A	
53	G				E		K	
56	S	D	D	D	N	N	N	
57	V						L	
66	K	E	E	E	E	E	E	
69	L	F	F	F	F			Cb
71	L					I		
74	N				Y			
80	I						V	
82	T	A	A	A				
83	S						P	
84	N						D	
85	S	P	P	P				
89	A				T		T	
96	A				N			
97	D		N		N			
102	K	R	R	R	R	R	R	
111	F	L	L	L	L			
120	A	R	R	R		E		
125	N	D	D	D				Sa
127	D	E	E	E		E		
130	R	K	K	K	K	K		
132	T					S		RBS
134	V	S	S	S			I	RBS
135	A	S	S	S				RBS
141	A					I		

142	N					K		
155	G				E	E		Sa
161	L					I		
162	S	N	N	N			R	Sa
163	K	N		N		T	N	Sa
166	T	I	I					Ca1
169	K	R	R	R				
170	G			E				Ca2
175	V					I		
190	T	A	A	A				Sb, RBS
196	H				N			
199	V						I	
202	G				E			
208	K	R	R	R	Q		Q	
214	I						T	
215	V	I	I	I				
220	V			I			I	RBS
222	E	N	N	N	K			RBS
227	M	I	I	I			I	
232	T				I			
233	L		I					
245	T	N	N	N				
253	H	Y	Y	Y	X	Y	Y	
258	K				D	N	N	
262	S					N		
266	M						I	
267	R	I	I	I	M	M	M	
269	D						N	
271	Q	H	H	H	H	N	R	
273	H				Q			
278	K						M	
283	H	N						
287	K	N	N	N				
288	G	N	N	N	S	S	S	
289	N	S						
298	V	I	I	I	I	I	I	
310	T	K	K	K				
311	Q						H	
321	I					T		
324	I					V		
aadiff		**36**	**34**	**34**	**25**	**25**	**29**	

***Within-lineage CVV in red, within-lineage variant in orange, swine strains in black.**

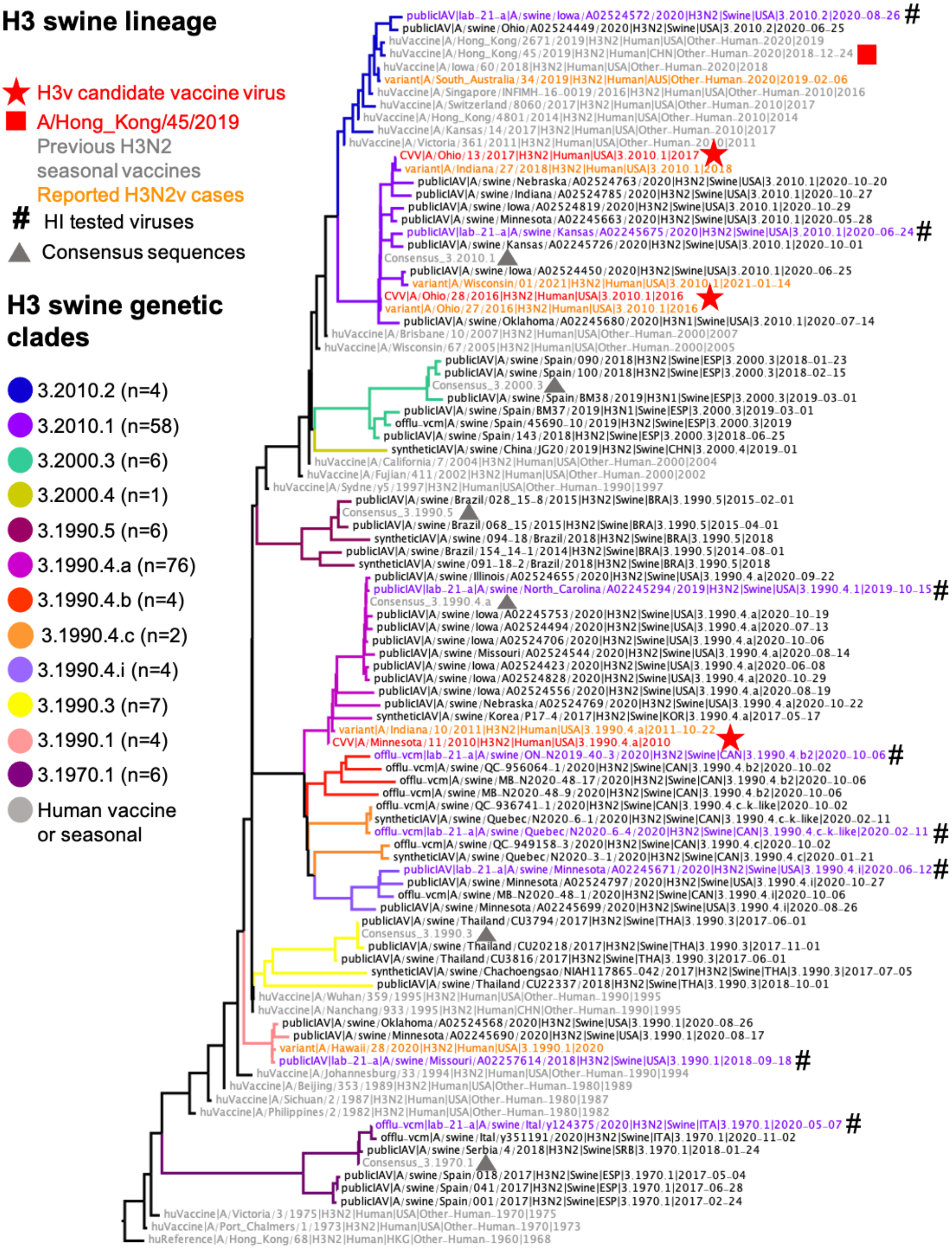


Figure 8. Swine H3 HA genes (tree was down sampled to 83 swine HA genes from 202). Detections of each clade deposited between July 1 to December 31, 2020 are presented adjacent to the clade name.

Antigenic analysis: Swine H3

Table 21. 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	1280	<10	20	160	20	40	<10
A/swine/Missouri/A02257614/2018	3. 1990.1	320	10	<10	20	<10	10	<10
A/Minnesota/11/2010 x 203	3.1990.4.a H3N2v	40	640	<10	20	<10	10	<10
A/swine/North Carolina/A02245294/2019	3.1990.4.a	20	40	20	40	20	10	<10
A/SW/ON/N-2019-40-3/2019	3.1990.4.b	40	10	20	20	20	20	10
A/swine/Quebec/N2020-6-4/2020	3.1990.4.c-k-like	40	20	20	40	10	20	<10
A/swine/Minnesota/A02245671/2020	3.1990.4.i	40	20	20	20	20	20	<10
IDCDC-RG55C A/Ohio/28/2016-like CVV	2010.1 H3N2v	20	<10	1280	40	20	20	<10
A/Indiana/27/2018*	2010.1 H3N2v	40	20	20	640	10	40	10
A/swine/Kansas/A02245675/2020	3.2010.1	20	10	40	80	20	20	<10
A/Kansas/14/2017 3C.3a	HuVacc H3N2	40	20	20	40	80	40	<10
A/Iowa/60/2018 3C.2a1b+T131K	HuVacc H3N2	20	<10	<10	10	20	160	<10
A/Hong Kong/45/2019 3C.2a1b/137F	HuVacc H3N2	20	20	20	40	40	80	80
A/swine/Iowa/A02524572/2020	3.2010.2	20	20	20	40	40	40	<10

CVV in red, seasonal vaccine strains in gray; Homologous titer is highlighted in gray. New swine strains in bold; variant in orange. *A/Indiana/27/2018 is a surrogate for CVV A/Ohio/13/2017 2010.1 H3N2v.

- The contemporary swine 1990.1 does not have a CVV contained within clade and demonstrated a significant >8-fold decrease from CVV A/Minnesota/11/2010 x 203 as well as a 4-fold decrease from the 1990.1 A/Hawaii/28/2020 H3N2v.
- 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.b, 1990.4.c-k-like, and 1990.4.i do not have within clade CVV and demonstrated significant >8-fold decreases from 1990.4a CVV A/Minnesota/11/2010 x 203.
- The contemporary swine 2010.1 representative strain had significant >8-fold decrease to CVV IDCDC-RG55C A/Ohio/28/2016-like. Reagents for the additional 2010.1 CVV A/Ohio/13/2017 were not available, so A/Indiana/27/2018 was used as a surrogate, and an 8-fold decrease was observed. Cross-reactivity was observed for HuVacc strains A/Kansas/14/2017 with a 4-fold decrease and A/Iowa/60/2019 with a significant 8-fold decrease.
- The contemporary swine 2010.2 representative strain does not have a CVV contained within clade but retained cross-reactivity to HuVacc A/Hong Kong/45/2019, and 4-fold decrease to HuVacc A/Iowa/60/2018.

Table 22. 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/Port Chalmers/1/1973	A/Ohio/28/2016_CVV
A/Port Chalmers/1/1973	1970.1	1280	<20
A/Ohio/28/2016_CVV	H3.2010.1	<20	640
A/swine/Italy/124375/2020|H3N2| 2020-05-07	3.1970.1	80	320
A/Victoria/3/1975	Human seasonal	320	<20

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

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

- The contemporary swine 3.1970.1 demonstrated poor cross-reactivity with HuVacc strain A/Port Chalmers/1/1973 and good reactivity with CVV strain A/Ohio/28/2016.

Table 23. Amino acid substitutions between swine 1990.1 strains compared to the most recent human seasonal vaccine A/Hong Kong/45/2019. A comparison to the prior human seasonal vaccine with highest amino acid similarity, A/Nanchang/933/1995, and within-clade variant case A/Hawaii/28/2020 are included This swine clade has fewer than five detections; a consensus sequence comparison was not conducted.

Relative to HI

site	A/Hong_Kong/45/2019	**A/swine/Missouri/A02257614/2018**	A/Hawaii/28/2020	A/Nanchang/933/1995	Annotations
3	I	L	L	L	
25	I	L	L	L	
33	R	Q	Q	Q	
42	L		M		
45	N	S	S	S	
48	I	T	T	T	
50	E	R	R	R	
57	Q	R	R	R	
62	G	K	K	K	
75	Q	H	H	H	
83	K	E	E	E	
88	V	I	I		
92	R	K	K	K	
106	A	S	S		
121	K	T	T	T	
124	S	D	D	G	
128	A	T	T	T	
131	T	A	A	A	
133	N	D	D	D	Site A
135	K	G	G	T	
137	F	Y	Y	Y	Site A
138	S			A	
140	I	K	K	K	
144	S	V	V	V	Site A
145	S	K	K	K	Site A
155	T	H	H	H	Site B

Relative to most similar vaccine

site	A/Nanchang/933/1995	**A/swine/Missouri/A02257614/2018**	A/Hawaii/28/2020	Annotations
42	L		M	
88	V	I	I	
106	A	S	S	
124	G	D	D	
135	T	G	G	
138	A	S	S	
190	V	E	E	
199	S	I	I	
208	R	S	S	Site D
221	P		L	
246	N	S	S	
262	S	N	N	
275	G	D	D	Site C
278	N	Y	Y	Site C
307	R	K	K	
312	N	K	K	
aadiff		**14**	**16**	

156	H	K	K	K	
158	N	E	E	E	
160	T	K	K	K	
171	K	N	N	N	
172	E	D	D	D	
173	Q	K	K	K	
186	G	S	S	S	Site B
189	K	S	S	S	Site B
190	D	E	E	V	
192	I	T	T	T	
196	A	V	V	V	
198	S	A	A	A	
199	S	I	I		
202	I	V	V	V	
208	R	S	S		Site D
212	A	T	T	T	
221	P		L		
222	R	W	W	W	
223	I	V	V	V	
225	D	G	G	G	
227	P	S	S	S	
246	N	S	S		
262	S	N	N		
275	G	D	D		Site C
276	K	N	N	N	
278	K	Y	Y	N	Site C
307	R	K	K		
312	N	K	K		
aadiff		**51**	**53**	**43**	

***Reference human seasonal vaccines in gray, within-clade variant in orange, swine strains in black.**

Table 24. Amino acid substitutions between recent swine 1990.4 lineage strains compared to the CVV with highest amino acid similarity, 1990.4.a A/Minnesota/11/2010. The 1990.4.c-k-like swine clade, A/swine/Quebec/N2020-6-4/2020, has fewer than five detections; a consensus sequence comparison was not conducted.

site	**A/Minnesota/11/2010**	**A/swine/Quebec/N2020-6-4/2020**	**A/swine/North_Carolina/A02245294/2019**	Consensus_3.1990.4.a	**A/swine/ON-N2019-40-3/2020**	Consensus_3.1990.4.b2	**A/swine/Minnesota/A02245671/2020**	Consensus_3.1990.4.i	**A/Hong_Kong/45/2019**	Annotations
3	L								I	
6	S	G							N	
7	D				G					
8	N				S		K	T		
9	S				N		N			
10	M	T					T	T	T	
12	T						M			
25	L								I	
31	D								N	
33	Q				X				R	
45	S						X		N	
48	T								I	
49	G						D	D		
50	R						K		E	
53	N				S	S			D	Site C
57	Q					K	K	K		
58	I						V			
62	K				G		G	G	G	
75	H	N			L				Q	
78	D	E							G	
79	F				L					
80	Q						E	E		
82	K	E								
83	E				K	K			K	
92	T								R	
96	N		S	S			D			

101	Y								D	
107	T	S			S	S	S	S	S	
112	V	I								
117	N	T			T	T	T	T	T	
119	E	K	K	K						
121	T					N	N	N	K	
122	Q	P			H		L	L	N	Site A
124	S		I	I			N	N		
128	T								A	
131	A		T	T			D		T	
133	D								N	Site A
135	S								K	
137	Y				N	N	F	F	F	Site A
138	A		S	S			S	S	S	
140	R						K	K	I	
142	G	K			E		N	N		
144	V	I			N	N			S	Site A
145	N	K			K	K			S	Site A
155	Y				H		H	H	T	Site B
156	N	H	H	H	H	H	Q	H	H	
159	Y				N	N	H	H		
160	K								T	
163	E	A			A	A	A	A	A	
164	Q	L			L	L	L	L	L	
171	N								K	
172	D								E	
173	K	E				N			Q	
189	K						M	M		Site B
192	T								I	
193	N				Y	Y	S	S	S	Site B
196	V	I				A			A	
198	A						E	E	S	
199	S				P	P				
202	V								I	
203	I	A			T	T	T	T	T	
204	V				I	I				
207	K		R	R						Site D
210	Q							L		
212	T								A	
213	V	I								
217	I				V	V				Site D
222	W								R	

223	V								I	
225	G								D	
226	V								I	
227	S								P	
229	I								R	
233	Y				H					
260	I						M	M		
261	Q						R	R	R	
262	S						I	I		
269	R						K	K		
273	H	P			P	P	L	L	P	
275	D	G					G	G	G	Site C
276	E	I			N	N	N	N	K	
278	N								K	Site C
280	E				G					
289	P		S	S						
299	K	R			R	R	R	R	R	
304	A	T								
323	V		I	I						
328	T				N					
aadif		**25**	**9**	**9**	**32**	**23**	**39**	**33**	**56**	

***Reference human seasonal vaccines in gray, within-clade CVV in red, swine strains in black.**

Table 25. Amino acid substitutions between recent swine 2010.1 clade consensus A/swine/Kansas/A02245675/2020 and A/Wisconsin/01/2021 H3N2v compared to the within-clade CVV A/Ohio/28/2016, CVV A/Ohio/13/2017, and within-clade variant case A/Indiana/27/2018.

site	**A/Ohio/13/2017**	**A/Ohio/28/2016**	**A/swine/Kansas/A02245675/2020**	A/Indiana/27/2018	Consensus	**A/Wisconsin/01/2021|2021-01-14|HA**	Annotations
56	Y	H	H		H	H	
88	V					I	
92	K					R	
138	S	A					
142	R			G			
144	S					I	Site A
145	N	K					Site A
156	H			R		Q	
158	N			D			
167	T					A	
188	D		E				Site B
189	K			T			Site B
198	A		T				
209	N	S	S		S	G	
280	A		E				
312	N					R	
aadiff		4	5	4	2	8	

***Reference human seasonal vaccines in gray, within-clade CVV in red, within-clade variant in orange, swine strains in black.**

Table 26. Amino acid substitutions between recent swine 2010.2 strains compared to the most recent human seasonal vaccine A/Hong Kong/45/2019, additional human vaccines A/Iowa/60/2018 or A/Kansas/14/2017, and a comparison to the prior human seasonal vaccine with the highest amino acid similarity, A/Kansas/14/2017. This swine clade has fewer than five detections; a consensus sequence comparison was not conducted.

Relative to HI

site	A/Hong_Kong/45/2019	A/swine/Iowa/A02524572/2020	A/Kansas/14/2017	A/Iowa/60/2018	Annotations
9	S	G			
31	N	D			
58	I	V			
62	G	E	E		
91	S	N	N		
92	R		K		
121	K		N		
128	A	T		T	
131	T			K	
135	K	E	T	T	
137	F	S	S	S	Site A
138	S			A	
144	S		K		Site A
145	S	R			Site A
159	Y		S		
160	T	K	K		
167	T	I			
171	K	N	N		
189	K	N			Site B
193	S			F	Site B
197	Q	R			
203	T	I			
227	P	S			
312	N	S	S	S	
326	K		R		
aadiff		**17**	**12**	**7**	

Relative to most similar vaccine

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
197	Q	R	
203	T	I	
227	P	S	
326	R	K	
aadiff		**16**	

***Reference human seasonal vaccines in gray, swine strains in black.**

Table 27. Amino acid substitutions between recent swine 1970.1 strains compared to the human seasonal vaccine A/Hong Kong/45/2019, and the prior human seasonal vaccine with the highest amino acid similarity, A/Victoria/3/1975.

Relative to HI

site	A/Hong_Kong/45/2019	**A/swine/Italy/124375/2020**	Consensus_3.1970.1	A/Victoria/3/1975	Annotations
2	K	D	D	D	
3	I	F	F	L	
5	G	R			
6	N	K	K		
7	D	G	G		
7+1	-			N	
25	I	L	L	L	
31	N	D	D		
33	R	Q	Q	Q	
45	N			S	
46	S	F	F		
48	I	M	M	T	
50	E	K	K	K	
53	D	N	K	N	Site C
54	S	N	N	N	Site C
57	Q	R	R	R	
62	G	A	A	I	
69	A	S	S		
75	Q	H	H	H	
82	K	E	E	E	
83	K	R	R		
88	V	I	I		
92	R	K	K	K	
94	Y	F	F	F	
104	D	E	E		
106	A	T	T		
112	V	I	I		
121	K	T	T	I	
124	S	N	N	G	
128	A	T	T	T	

Relative to most similar vaccine

site	A/Victoria/3/1975	**A/swine/Italy/124375/2020**	Consensus_3.1970.1	Annotations
3	L	F	F	
5	G	R		
6	N	K	K	
7	D	G	G	
7+1	N	-	-	
31	N	D	D	
45	S	N	N	
46	S	F	F	
48	T	M	M	
53	N		K	Site C
62	I	A	A	
69	A	S	S	
83	K	R	R	
88	V	I	I	
104	D	E	E	
106	A	T	T	
112	V	I	I	
121	I	T	T	
124	G	N	N	
132	Q	P		
133	N	D	D	Site A
138	A	S		
143	P	I		Site A
144	D	N	N	Site A
145	S	K	K	Site A
146	G	S	S	Site A
159	S	N	N	
163	V	M	M	
171	N	S	S	
173	N	D	D	

132	Q	P			
133	N	D	D		Site A
135	K	G	G	G	
137	F	S	S	S	Site A
138	S		A	A	
140	I	K	K	K	
143	S	I	P	P	Site A
144	S	N	N	D	Site A
145	S	K	K		Site A
146	S			G	Site A
155	T	Y	Y	Y	Site B
156	H	K	K	K	
157	L	S	S	S	
158	N	G	G	G	
159	Y	N	N	S	
163	A	M	M	V	
164	L	Q	Q	Q	
171	K	S	S	N	
172	E	D	D	D	
173	Q	D	D	N	
174	F	Y	Y	S	
186	G	S	S	S	Site B
190	D	E	E	E	
192	I	T	T	T	
193	S	D	D	N	Site B
196	A	I	I	V	
198	S	A	A	A	
201	R	K	K	K	Site D
202	I			V	
203	T	I	I		
212	A	I	I	T	
213	V	I		I	
217	I			V	Site D
219	S	P			
222	R	W	W	W	
223	I	V	V	V	
225	D	G	G	G	
226	I	L	L	L	
227	P	S	S	S	
242	I	T	T		
244	L	I	I	V	
248	T	N	N	N	

174	S	Y	Y	
193	N	D	D	Site B
196	V	I	I	
202	V	I	I	
203	T	I	I	
212	T	I	I	
213	I		V	
217	V	I	I	Site D
219	S	P		
242	I	T	T	
244	V	I	I	
260	M	I	I	
261	R	Q	Q	
267	I	V	V	
278	S	N	N	Site C
299	K	R	R	
307	K	H	H	
309	V	I	I	
323	V	I	I	
aadiff		**47**	**44**	

260	I			M	
261	R	Q	Q		
262	S	T	T	T	
267	I	V	V		
276	K	T	T	T	
278	K	N	N	S	Site C
299	R			K	
307	R	H	H	K	
309	V	I	I		
323	V	I	I		
aadiff		**74**	**71**	**61**	

***Reference human seasonal vaccines in gray, swine strains in black.**

Table 28. Amino acid substitutions between recent swine 2000.3 strains compared to the human seasonal vaccine with highest amino acid similarity, A/California/7/2004.

site	A/California/7/2004	**A/swine/Spain/BM38/2019**	**A/swine/Spain/090/2018**	**A/swine/Spain/45690-10/2019**	Consensus_3.2000.3	Annotations
5	G	R	R	E	R	
6	N			H		
8	N	S	S	S	S	
25	I		L			
34	I			V		
45	S	N	N		N	
49	G	D	D	D	D	
50	G	R	R	R	R	
56	H	Y	Y	Y	Y	
57	Q		R			
69	A	S	S		S	
92	K			R		
102	V		I			
112	V	I	I	I	I	
124	S	N	D	N	N	
133	N			D		Site A
135	T	E	E	K	E	
142	R	K		K		
144	N	K		K		Site A
145	N		S	S	S	Site A
155	T	Y	Y		Y	Site B
158	K	G	G	G	G	
159	F	I	I		I	
163	A	M	M		M	
167	T			V	V	
168	M	I				
171	N	S		S		
173	K	A	A	G	A	
186	G	S	S	S	S	Site B

188	N	D	D	D	D	Site B
192	I			V		
196	T	A	A	A	A	
197	Q	K	K	K	K	
201	R	K	K	K	K	Site D
203	T	I	I	I	I	
214	I	T	T	T	T	
216	N	S	S	S	S	
225	D	G	G		G	
241	D		E			
242	I			V		
244	L	I	I		I	
248	T	N	N			
260	I		V			
261	R	H	H		H	
262	S		T			
268	M			I		
269	R	K	K		K	
275	G	E	E		E	Site C
276	K	T	T		T	
280	E	K	K	K	K	
304	A			D		
326	K	R	R		R	
aadiff		**36**	**39**	**32**	**33**	

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

Summary and Risk Assessment

Europe:

- The contemporary swine 1A.3.3.2 (pdm) demonstrated no cross-reactivity to three previous human seasonal vaccine strains including A/California/04/2009 (see table 2)
- The contemporary swine 1B.1.2.2. strain demonstrated reduced cross-reactivity to A/Michigan/383/2018 and poor cross-reactivity to A/Brazil/11/1978 (see table 8)
- The contemporary swine 1B.1.2.3 strain demonstrated good cross-reactivity to both A/Michigan/383/2018 and to A/Brazil/11/1978.
- The contemporary swine clades 1C demonstrate variable cross-reactivity to CVV strains A/Netherlands/3115/2016 and A/Hunan/42443/2015 CNIC-1601 (see table 16).
- The contemporary swine 3.1970.1 demonstrated poor cross-reactivity with HuVacc strain A/Port Chalmers/1/1973 and good cross reactivity with CVV strain A/Ohio/28/2016 (See table 22).

North America:

- 1A 1.1 lineage viruses (alpha or alpha-del) 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. and Canada pigs (Figure 1, 4 and A1-2).
- 1A.3.3.2 (pdm) circulating in swine demonstrated a significant loss of cross-reactivity against the current human seasonal vaccine A/Wisconsin/488/2019 (Table 1) and represent a consistent proportion of 1A lineage viruses detected in U.S. and Canada pigs (Figure 1, 4 and A1-2).
- 1A.3.3.3 (gamma) HA genes diversified into additional statistically supported clades that may require new clade designations based on the demonstrated loss in cross-reactivity of the clade-3 virus against the 1A.3.3.3-clade 1 CVV A/Ohio/24/2015 (Table 1). 1A.3.3.3 clade 3 represent a substantial proportion of 1A lineage viruses detected in U.S. pigs (Figures 1, 4, and A1-2).
- 1B.2.2.1 (delta-1a) demonstrated cross-reactivity to CVV A/Iowa/32/2016 (Table 7) and represent a consistent proportion of 1B lineage viruses detected in U.S. pigs (Figures 1, 5, and A1-2)
- 1B.2.2.2 (delta-1b) demonstrated a significant loss in cross-reactivity against the CVV A/Iowa/32/2016 (Table 7) and represent a consistent proportion of 1B lineage viruses detected in U.S. pigs (Figures 1, 5, and A1-2).
- 1B.2.1 (delta-2) retained cross-reactivity to CVV A/Michigan/383/2018 RG58A and represent a substantial proportion of 1B lineage viruses detected in U.S. pigs (Figures 2, 5, and A1-2).
- H3.1990.1 (Cluster I) demonstrated a significant loss of cross-reactivity against the CVV A/Minnesota/ 11/2010 (Table 21) and represent a small proportion of H3 viruses detected in U.S. pigs (Figures 1, 8, and A1-2).
- H3.1990.4.a (Cluster IV-A) demonstrated a significant loss of cross-reactivity against the CVV A/Minnesota/ 11/2010 (Table 21) and represent a substantial proportion of H3 viruses detected in U.S. pigs (Figures 1, 8, and A1-2).
- H3.1990.4.b, H3.1990.4.c-k-like, and H3.1990.4.i demonstrated significant losses in cross-reactivity against the CVV A/Minnesota/ 11/2010 (Table 21) and represent a small proportion of H3 viruses detected in U.S. pigs (Figures 1, 8, and A1-2).
- H3.2010.1 demonstrated significant loss in cross-reactivity with the 2010.1 IDCDC-RG55C A/Ohio/28/2016-like CVV and 4-fold loss to A/Indiana/27/2018 (Table 21). H3.2010.1 represent a substantial proportion of H3 viruses detected in U.S. pigs (Figures 1, 8, and A1-2).
- H3.2010.2 demonstrated cross-reactivity to human vaccine strain A/Kansas/14/2017 (Table 21) and are a small but consistent detection in U.S. pigs (Figures 1, 8, and A1-2).

Annex 1 – Variant Strains

Phylogenetic Tree – 1A.3.3.2

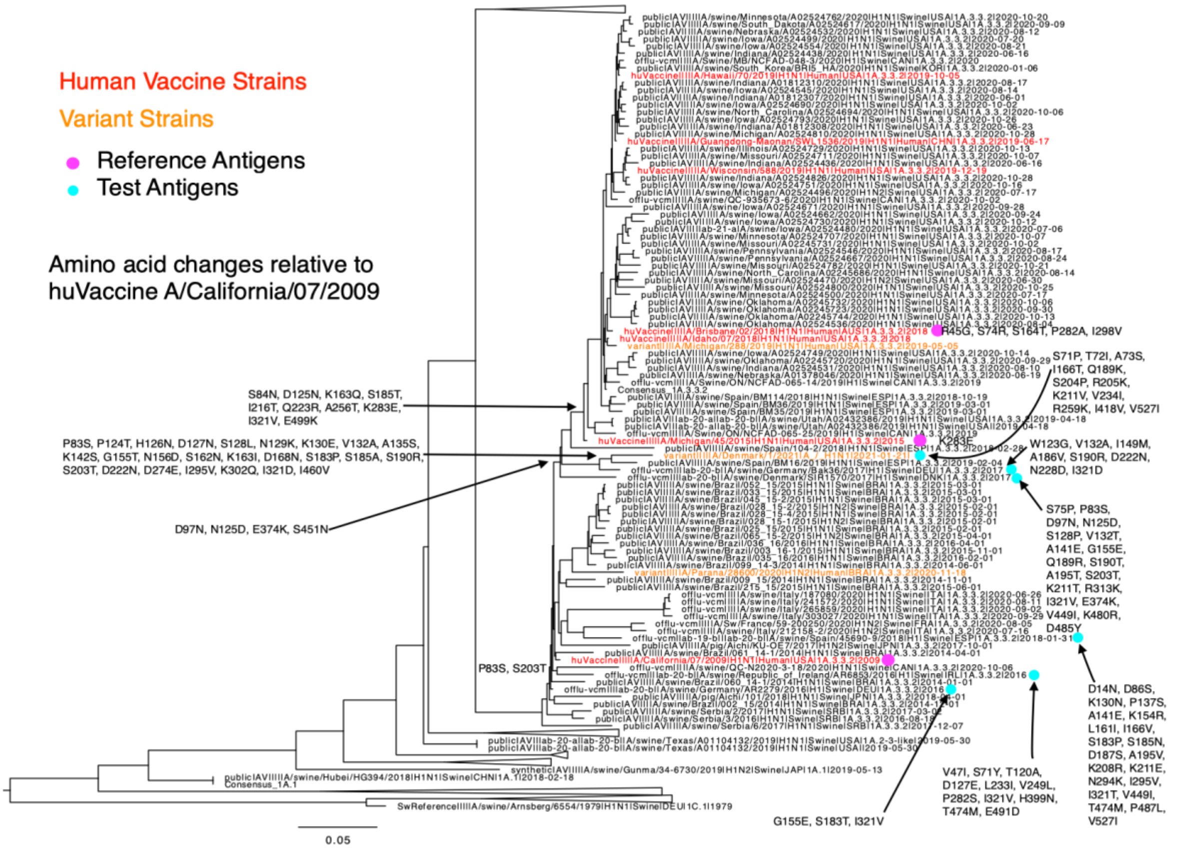


Figure A1: Maximum likelihood phylogenetic tree of the 1A 3.3.2 lineage. Vaccine strains are coloured red, variant strains coloured orange and amino acid differences in the HA1 and HA2 are annotated next to strains used in HI testing comparative to the human vaccine strain A/California/07/2009.

Amino Acid difference table 1A3.3.2 Lineage

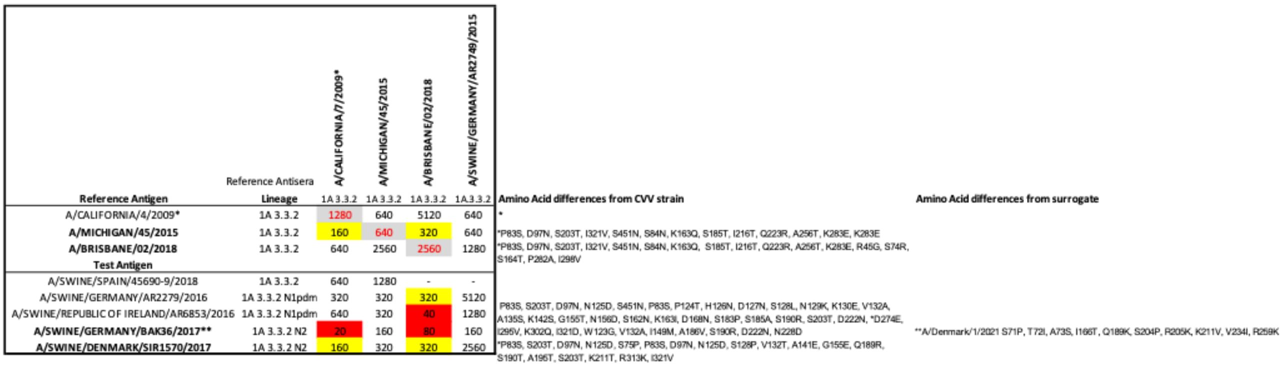
Table A1: Amino acid substitutions between recent swine and variant 1A3.3.2 strains compared to the human seasonal vaccine with highest amino acid similarity, A/California/7/2009 and comparisons between the variant strain and strains most similar used in Hemagglutination inhibition assays.

Site	hu-vaccine|map|AGSR|A/CALIFORNIA/4/2009|npdm|2009_1A.3.3.2	hu-vaccine|map|AGSR|A/MICHIGAN/45/2015|npdm|2015_1A.3.3.2	huVaccine|A/Brisbane/02/2018|H1N1|human|human|Australia|2018	forHI|EPI_ISL_304240_A/swine/Denmark/SIR1570/2017_|_HA_1A.3.3.2	forHI|EPI_ISL_264252_A/swine/Germany/Bak36/2017_|_HA_1A.3.3.2	variant||||A/Denmark/1/2021|A_/_H1N1|2021-01-21|
45	R		G			
71	S					P
72	T					I
73	A					S
74	S		R			
75	S			P		
83	P	S	S	S	S	S
84	S	N	N			
97	D	N	N	N	N	N
123	W				G	
124	P				T	T
125	N			D	D	D
126	H				N	N
127	D				N	N
128	S			P	L	L
129	N				K	K
130	K				E	E
132	V			T	A	I
135	A				S	S
141	A			E		
142	K				S	S
149	I				M	
155	G			E	T	T
156	N				D	D
162	S	N	N		N	N
163	K	Q	Q		I	I
164	S		T			
166	I					T
168	D				N	N
183	S		P		P	P
185	S	T	T		A	A
186	A				V	
189	Q			R		K
190	S			T	R	W
191	L					
195	A			T		
197	A					
203	S	T	T	T	T	T
204	S					P
205	R					K
211	K			T		V
216	I	T	T			
222	D				N	N
223	Q	R	R			
228	N				D	
234	V					I
256	A	T	T			
259	R					K
274	D				E	E
282	P		A			
283	K	E	E			
295	I		V		V	V
298	I		V			
302	K				Q	Q
313	R			K		
321	I	V	V	V	D	E
Total	X	12	19	15	30	36

Site	variant||||A/Denmark/1/2021|A_/_H1N1|2021-01-21|	forHI|EPI_ISL_304240_A/swine/Denmark/SIR1570/2017_|_HA_1A.3.3.2	forHI|EPI_ISL_264252_A/swine/Germany/Bak36/2017_|_HA_1A.3.3.2
71	P	S	S
72	I	T	T
73	S	A	A
75	S	P	
123	W		G
124	T	P	
126	N	H	
127	N	D	
128	L	P	
129	K	N	
130	E	K	
132	I	T	A
135	S	A	
141	A	E	
142	S	K	
149	I		M
155	T	E	
156	D	N	
162	N	S	
163	I	K	
166	T	I	I
168	N	D	
183	P	S	
185	A	S	
186	A		V
189	K	R	Q
190	W	T	R
195	A	T	
204	P	S	S
205	K	R	R
211	V	T	K
222	N	D	
228	N		D
234	I	V	V
259	K	R	R
274	E	D	
295	V	I	
302	Q	K	
313	R	K	
321	E	V	D
Total		36	17

Antigenic analysis: 1A 3.3.2 lineage

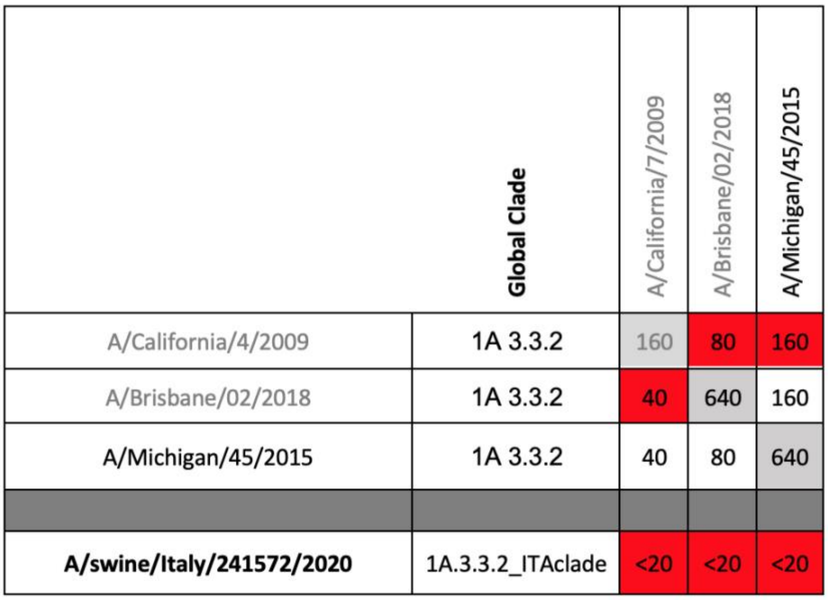
Table A2: EU Data: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine 1A3.2.2 strains selected to represent variant strains. Amino acid differences from the CVV A/California/4/2009 in the HA1 are annotated. Additional differences from A/Swine/Germany/BAK36/2017 acting as a surrogate for A/Denmark/1/2021 are also included.



	Reference Antisera	A/CALIFORNIA/7/2009*	A/MICHIGAN/45/2015	A/BRISBANE/02/2018	A/SWINE/GERMANY/AR2749/2015		
Reference Antigen	**Lineage**	1A 3.3.2	1A 3.3.2	1A 3.3.2	1A.3.3.2	**Amino Acid differences from CVV strain**	**Amino Acid differences from surrogate**
A/CALIFORNIA/4/2009*	1A 3.3.2	1280	640	5120	640	*	
A/MICHIGAN/45/2015	1A 3.3.2	🟨 160	640	🟨 320	640	*P83S, D97N, S203T, I321V, S451N, S84N, K163Q, S185T, I216T, Q223R, A256T, K283E, K283E	
A/BRISBANE/02/2018	1A 3.3.2	640	2560	2560	1280	*P83S, D97N, S203T, I321V, S451N, S84N, K163Q, S185T, I216T, Q223R, A256T, K283E, R45G, S74R, S164T, P282A, I298V	
Test Antigen							
A/SWINE/SPAIN/45690-9/2018	1A 3.3.2	640	1280	-	-		
A/SWINE/GERMANY/AR2279/2016	1A 3.3.2 N1pdm	320	320	🟨 320	5120		
A/SWINE/REPUBLIC OF IRELAND/AR6853/2016	1A 3.3.2 N1pdm	640	320	🟥 40	1280	P83S, S203T, D97N, N125D, S451N, P83S, P124T, H126N, D127N, S128L, N129K, K130E, V132A, A135S, K142S, G155T, N156D, S162N, K163I, D168N, S183P, S185A, S190R, S203T, D222N, *D274E, I295V, K302Q, I321D, W123G, V132A, I149M, A186V, S190R, D222N, N228D	
A/SWINE/GERMANY/BAK36/2017**	1A 3.3.2 N2	🟥 20	160	🟥 80	160		**A/Denmark/1/2021 S71P, T72I, A73S, I166T, Q189K, S204P, R205K, K211V, V234I, R259K
A/SWINE/DENMARK/SIR1570/2017	1A 3.3.2 N2	🟨 160	320	🟨 320	2560	*P83S, S203T, D97N, N125D, S75P, P83S, D97N, N125D, S128P, V132T, A141E, G155E, Q189R, S190T, A195T, S203T, K211T, R313K, I321V	

Antigenic analysis: Swine 1A Lineage

Table A3. EU Data: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine 1A lineage strains.



	Global Clade	A/California/7/2009	A/Brisbane/02/2018	A/Michigan/45/2015
A/California/4/2009	1A 3.3.2	160	🟥 80	🟥 160
A/Brisbane/02/2018	1A 3.3.2	🟥 40	640	160
A/Michigan/45/2015	1A 3.3.2	40	80	640
A/swine/Italy/241572/2020	1A.3.3.2_ITAclade	🟥 <20	🟥 <20	🟥 <20

- 🟥 >8 fold reduction
- 🟧 8 fold reduction
- 🟨 4 fold reduction

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

Phylogenetic tree 1C lineage

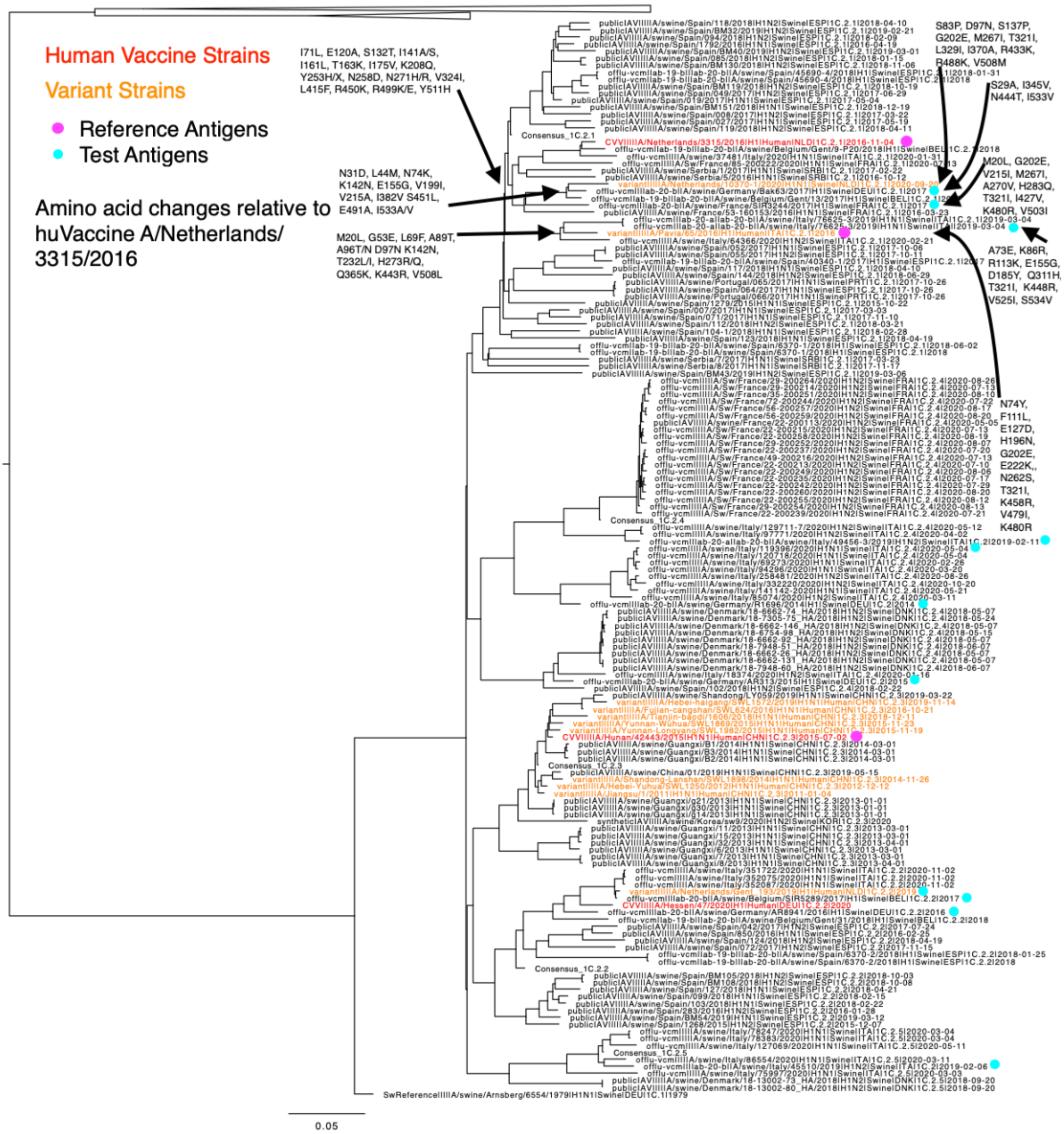


Figure A2: Maximum likelihood phylogenetic tree of the 1C lineage. Vaccine strains are coloured red, variant strains coloured orange and amino acid differences in the HA1 and HA2 are annotated next to strains used in HI testing comparative to the CVV A/Netherlands/3315/2016.

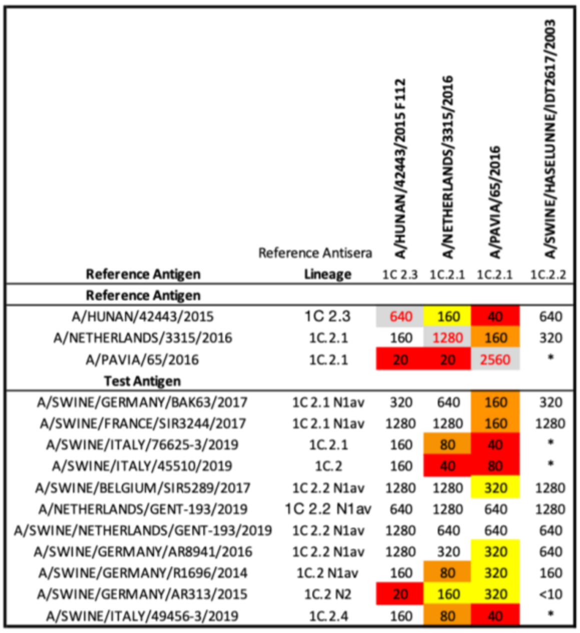
Amino Acid Difference tables 1C.2.1 Lineage

Table A3: Amino acid substitutions between recent swine 1C.2.1 strains compared to the within-clade 1C.2.1 CVV A/Netherlands/3315/2016.

site	A/Netherlands/3315/2016	A/swine/Italy/64366/2020	A/swine/Spain/007/2017	A/Sw/France/85-200222/2020	A/Pavia/65/2016
20	M	L	L	L	L
31	N				
35	N		S	T	
44	L				
48	A				
51	Q			H	
53	G				E
56	N				
57	V				
66	E				
68	D			E	
69	L	F			F
71	I	L	L	L	L
74	N				Y
80	I	V			
82	T	N		N	
83	S			P	
84	N			K	
89	A	T		T	T
95	F			L	
96	A	T		T	N
97	D			N	N
102	R				
111	F				L
120	E		A	A	A
127	E				D
130	K				
132	S	T	T		T
134	V		I		
137	S			T	
141	I	V	A	V	A
142	K	N		N	N
155	E	G	G	A	
161	I	L	L		L
162	S			N	
163	T	K	N	K	K
168	N			D	
175	I	V	V	V	V
190	T	A			
196	H				N
199	V	I			
202	G			E	E
208	K	Q	R		Q
214	I				
215	V				
220	V	I			
222	E				K
227	M				
230	Y			H	
232	T	V			I
253	Y	H	H		X
258	N	E			D
259	K			E	
261	S			P	
262	N	S		S	S
266	M			L	
267	M		I	V	
269	D				
271	N	Q	H		H
272	V	L			
273	H	Q			Q
274	N	D			
278	K				
288	S				
298	I				
311	Q				
321	T	I	I	I	I
324	V	I	I	I	I
Tot		**28**	**17**	**30**	**29**

Antigenic analysis: 1C.2.1 lineage

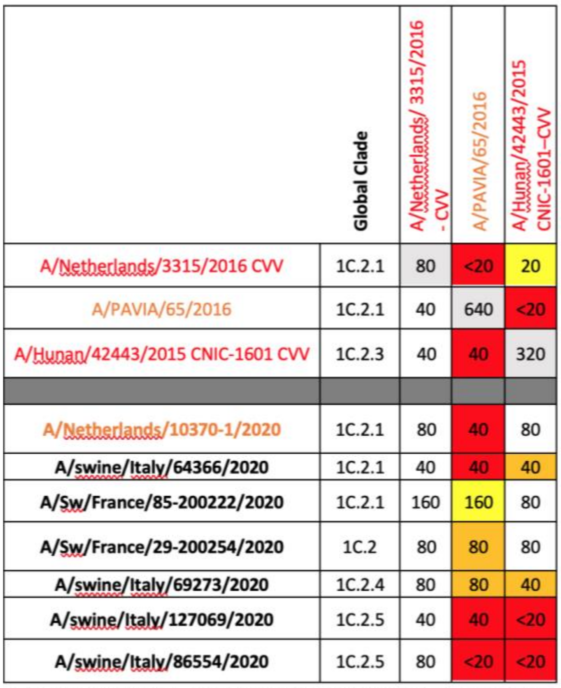
Table A4: Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine 1C strains



Reference Antigen	Reference Antisera Lineage	A/HUNAN/42443/2015 F112 1C 2.3	A/NETHERLANDS/3315/2016 1C.2.1	A/PAVIA/65/2016 1C.2.1	A/SWINE/HASELUNNE/IDT2617/2003 1C.2.2
Reference Antigen					
A/HUNAN/42443/2015	1C 2.3	640	160	40	640
A/NETHERLANDS/3315/2016	1C.2.1	160	1280	160	320
A/PAVIA/65/2016	1C.2.1	20	20	2560	*
Test Antigen					
A/SWINE/GERMANY/BAK63/2017	1C 2.1 N1av	320	640	160	320
A/SWINE/FRANCE/SIR3244/2017	1C 2.1 N1av	1280	1280	160	1280
A/SWINE/ITALY/76625-3/2019	1C.2.1	160	80	40	*
A/SWINE/ITALY/45510/2019	1C.2	160	40	80	*
A/SWINE/BELGIUM/SIR5289/2017	1C 2.2 N1av	1280	1280	320	1280
A/NETHERLANDS/GENT-193/2019	1C 2.2 N1av	640	1280	640	1280
A/SWINE/NETHERLANDS/GENT-193/2019	1C 2.2 N1av	1280	640	640	640
A/SWINE/GERMANY/AR8941/2016	1C 2.2 N1av	1280	320	320	640
A/SWINE/GERMANY/R1696/2014	1C.2 N1av	160	80	320	160
A/SWINE/GERMANY/AR313/2015	1C.2 N2	20	160	320	<10
A/SWINE/ITALY/49456-3/2019	1C.2.4	160	80	40	*

Antigenic analysis: Swine 1C Lineage

Table A5. UK & EU Data: Hemagglutination inhibition of CVV vaccine ferret antisera against contemporary swine 1C lineage strains selected to represent clade consensus.



	Global Clade	A/Netherlands/ 3315/2016 - CVV	A/PAVIA/65/2016	A/Hunan/42443/2015 CNIC-1601–CVV
A/Netherlands/3315/2016 CVV	1C.2.1	80	<20	20
A/PAVIA/65/2016	1C.2.1	40	640	<20
A/Hunan/42443/2015 CNIC-1601 CVV	1C.2.3	40	40	320
A/Netherlands/10370-1/2020	1C.2.1	80	40	80
A/swine/Italy/64366/2020	1C.2.1	40	40	40
A/Sw/France/85-200222/2020	1C.2.1	160	160	80
A/Sw/France/29-200254/2020	1C.2	80	80	80
A/swine/Italy/69273/2020	1C.2.4	80	80	40
A/swine/Italy/127069/2020	1C.2.5	40	40	<20
A/swine/Italy/86554/2020	1C.2.5	80	<20	<20

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

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

Annex 2

Geographic Distribution of swine HA phylogenetic clades by country

Figure A3. Summary of swine HA genes colored by phylogenetic clade for sequences collected July 1, 2020 – December 31, 2020 (n=416).

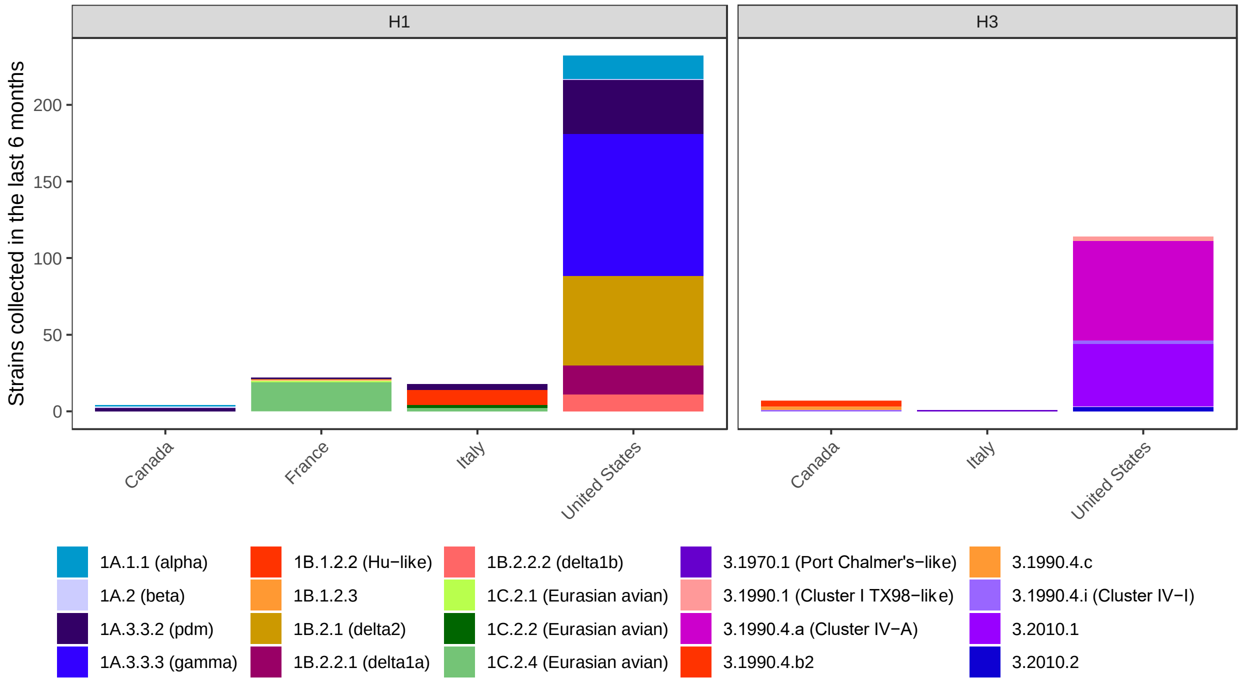


Figure A4. Summary of swine HA clades by country, colored by phylogenetic clade for sequences deposited July 1, 2020 – December 31, 2020 (n=662).

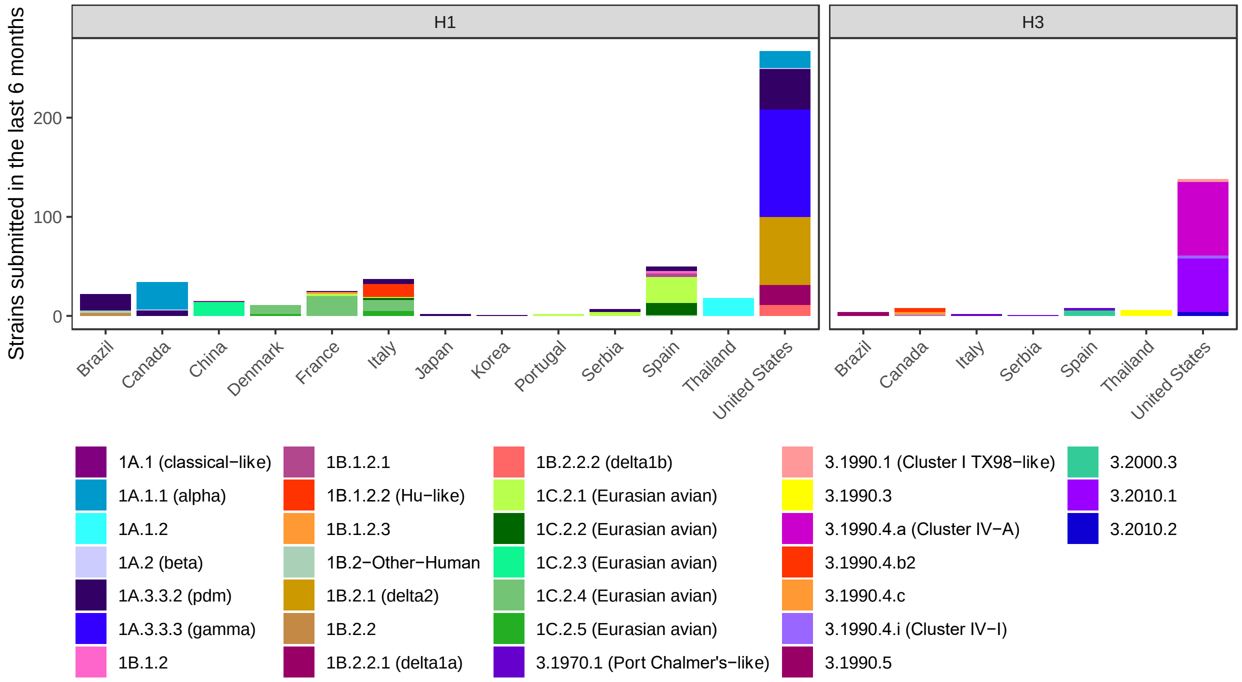


Table A5. Summary of swine H1 clades by country, data collected July 1 - December 31, 2020.

Clade	Country	Count
1A.1	CHN	1
1A.1	JAP	1
1A.1.1	CAN	30
1A.1.1	USA	19
1A.1.2	THA	18
1A.2	CAN	3
1A.2	USA	4
1A.3.3.2	AUS	1
1A.3.3.2	BRA	17
1A.3.3.2	CAN	5
1A.3.3.2	CHN	1
1A.3.3.2	ESP	5
1A.3.3.2	FRA	1
1A.3.3.2	ITA	5
1A.3.3.2	JPN	2
1A.3.3.2	KOR	1
1A.3.3.2	SRB	3
1A.3.3.2	USA	46
1A.3.3.3	USA	114
1B.1.2	ESP	2
1B.1.2.1	ESP	4
1B.1.2.2	ITA	13
1B.1.2.3	FRA	2
1B.2.1	USA	74
1B.2.2	BRA	5
1B.2.2	USA	1
1B.2.2.1	USA	22
1B.2.2.2	USA	11
1C.2.1	ESP	26
1C.2.1	FRA	2
1C.2.1	ITA	2
1C.2.1	NLD	1
1C.2.1	PRT	2
1C.2.1	SRB	4
1C.2.2	DEU	1
1C.2.2	ESP	12
1C.2.2	ITA	2
1C.2.2	NLD	1
1C.2.3	CHN	23
1C.2.3	KOR	1
1C.2.4	DNK	9
1C.2.4	ESP	1
1C.2.4	FRA	20
1C.2.4	ITA	11
1C.2.5	DNK	2
1C.2.5	ITA	5
Other-Human-1B.2	BRA	4
Other-Human-1B.2	CHL	1

Table A6. Summary of swine H3 clades by country, data collected July 1 - December 31, 2020.

Clade	**Country**	**Count**
3.1970.1	ESP	3
3.1970.1	ITA	2
3.1970.1	SRB	1
3.1990.1	USA	4
3.1990.3	THA	7
3.1990.4.a	KOR	1
3.1990.4.a	USA	76
3.1990.4.b2	CAN	4
3.1990.4.c	CAN	2
3.1990.4.c-k-like	CAN	3
3.1990.4.i	CAN	1
3.1990.4.i	USA	3
3.1990.5	BRA	6
3.2000.3	ESP	5
3.2000.4	CHN	1
3.2010.1	USA	58
3.2010.2	USA	4