



OFFLU ANIMAL INFLUENZA REPORT

September 2019 to February 2020

Scope

In this document, we present a summary of H5, H7, and H9 avian influenza A virus events that were reported from September 2019 to February 2020, as well as a summary of H1 and H3 swine influenza A virus evolution from 2012 to present.

Swine influenza A viruses

Acknowledgements: Contributions were made from: USDA-ARS NADC: Tavis Anderson, Zebulun Arendsee, Jennifer Chang, Brian Kimble, Carine Kunzler-Souza, Amy Vincent; USDA-APHIS NVSL: Alicia Janas-Martindale; APHA: Ian Brown, Steve Essen, Susan Collins, Nicola Lewis; RVC: Divya Venkatesh, Nicola Lewis. Phylogenetic and sequence analyses were performed at NADC and Royal Veterinary College (RVC). We are very grateful to the OFFLU Swine Group for their expertise and for sharing data. We acknowledge NIH-CEIRS, APHA, Istituto Zooprofilattico Sperimentale, and the USDA IAV in Swine Surveillance Program for collection and sequencing of contemporary viruses. Antigenic data were generated by the Animal and Plant Health Agency (APHA), UK, and by the National Animal Disease Center (NADC), USDA-ARS, US. Ferret sera was kindly provided by the US-CDC.

Genetic and Antigenic Analyses - Brief Methods

Analyses were conducted by subtype and lineage or clade. Reference sequences and new data (deposited July 1, 2019 – December 31, 2019) downloaded from GISAID or GenBank were aligned with MAFFT (Kato and Standley, 2013) using automatic settings. Alignments for each segment were inspected manually on AliView (Larsson, 2014) and the ends trimmed to the starting ATG and end 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 US-CDC's amino acid substitution calculator. 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.

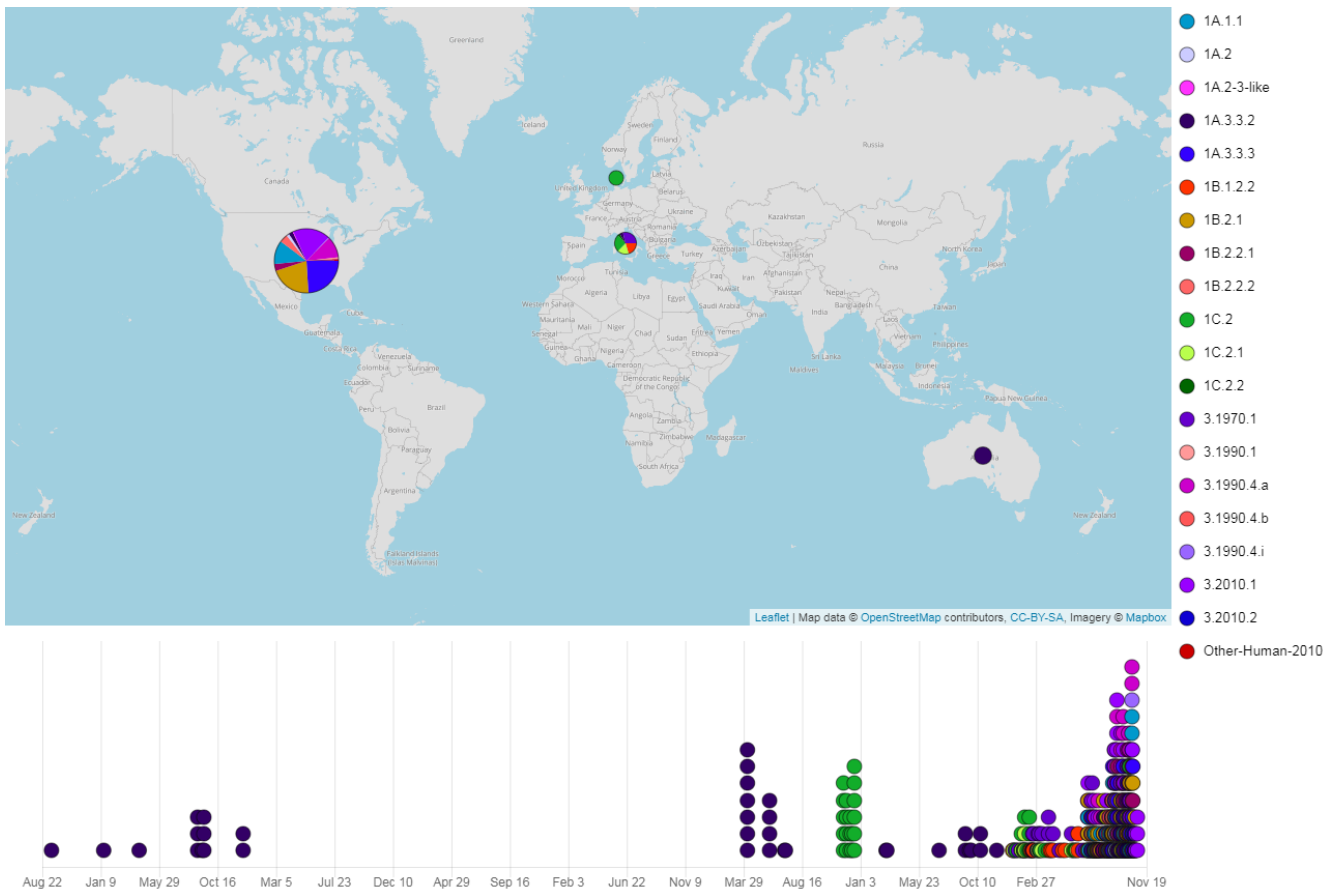


Figure 1. MicroReact map of swine IAV HA genes colored by phylogenetic clade for sequences deposited July 1, 2019 – December 31, 2019; however, collection date is represented along bottom time scale.

Contemporary Global H1 swine IAV: genetic diversity

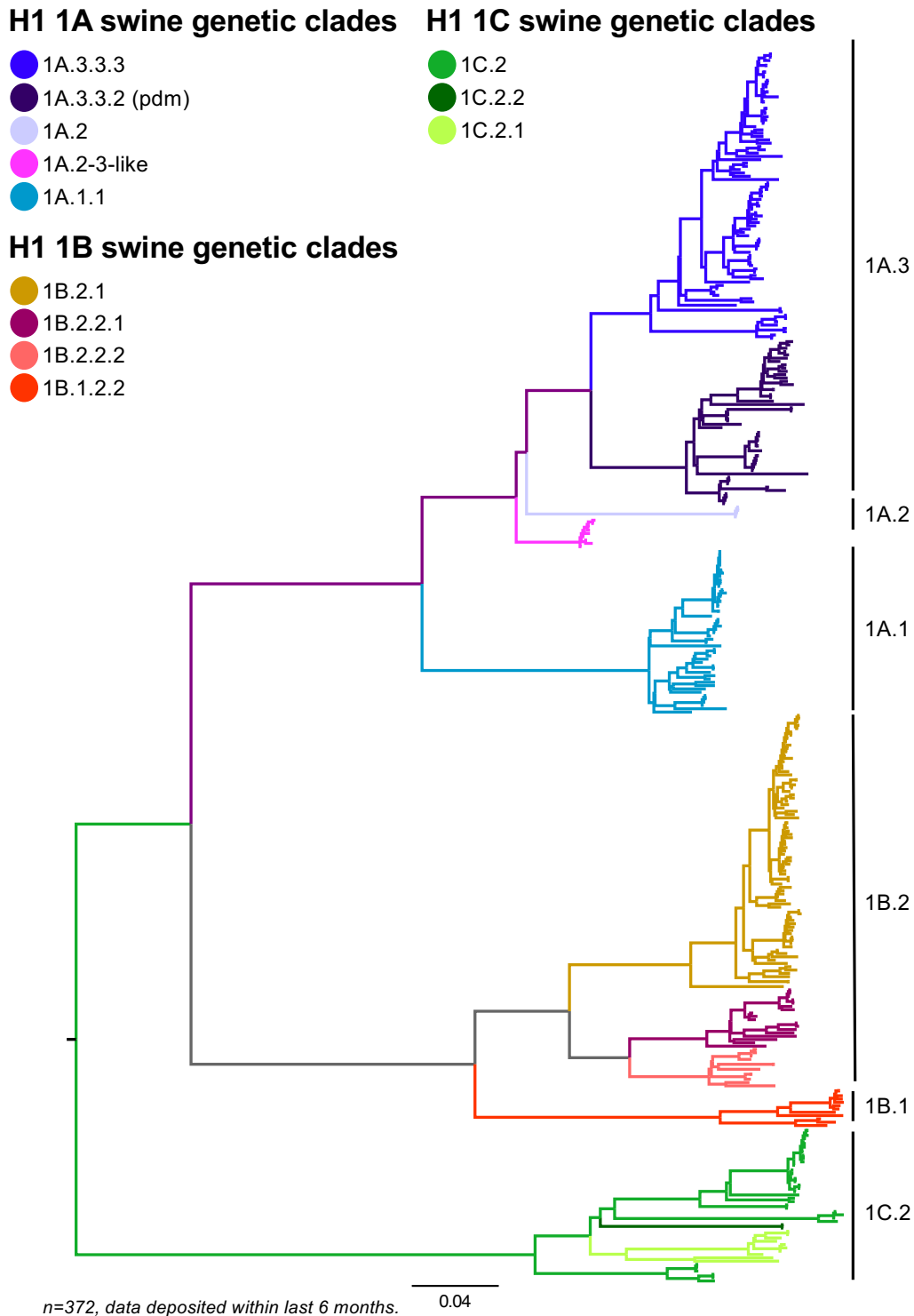


Figure 2. Global swine H1 phylogenetic tree colored by clade and annotated by global H1 lineage nomenclature. Analyses were conducted with reference sequences and new data (deposited July 1, 2019 – December 31, 2019) downloaded from GISAID or GenBank.

1A classical swine lineage

★ H1v candidate vaccine virus

■ A/Idaho/07/2018

H1N1pdm seasonal vaccines

Reported H1v cases

HI tested viruses

H1 1A swine genetic clades

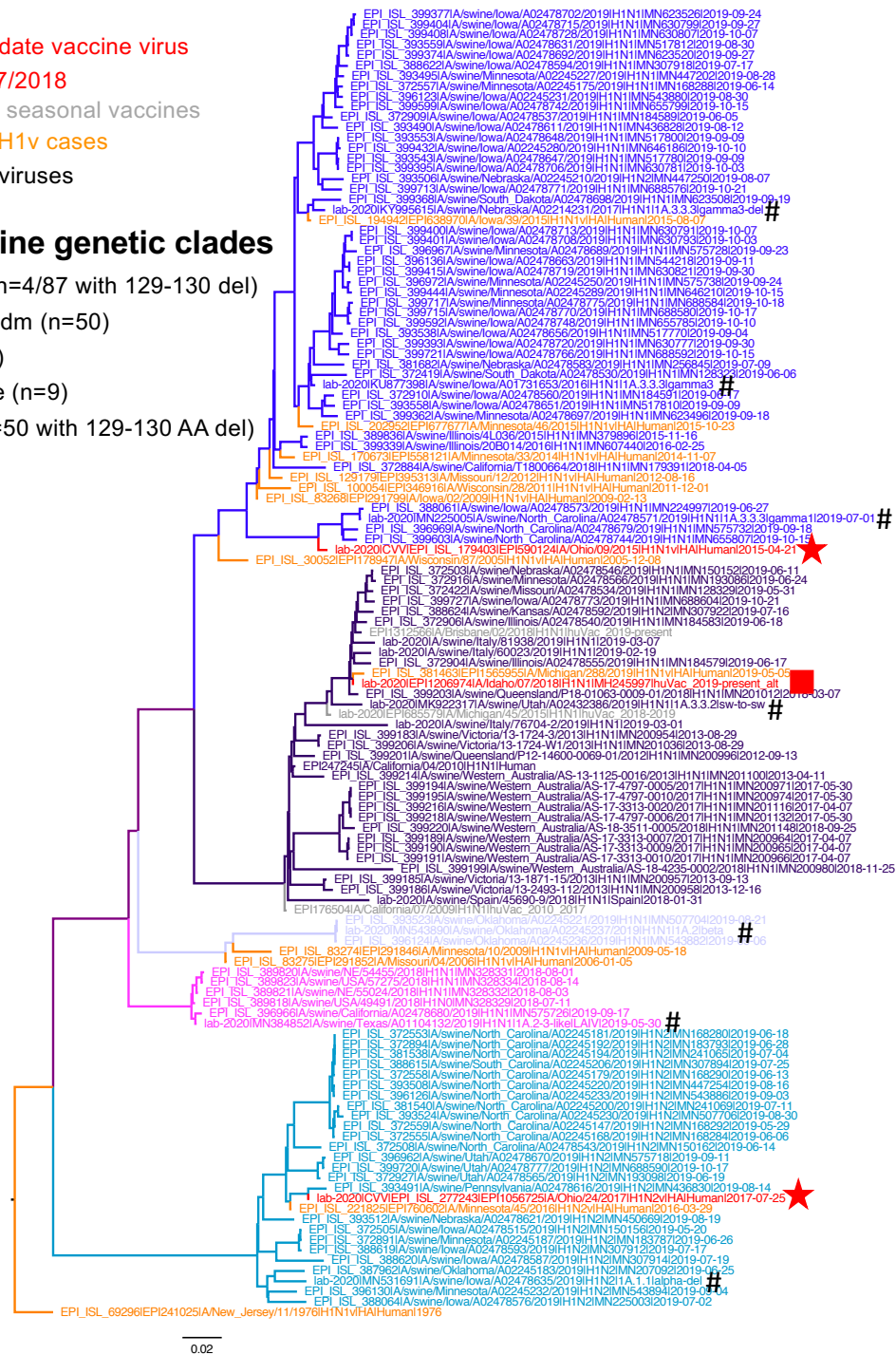
● 1A.3.3.3 (n=4/87 with 129-130 del)

● 1A.3.3.2/pdm (n=50)

● 1A.2 (n=4)

● 1A.2-3-like (n=9)

● 1A.1.1 (n=50 with 129-130 AA del)



n=100 randomly sampled from data deposited in last 6 months maintaining clade proportions, and n=25 reference genes.

Figure 3. Swine H1 HA genes of the 1A lineage. Tree was down-sampled to 100 from total of 200 swine 1A HA sequences deposited from July 1 – December 31, 2019. The most frequently deposited clades of the 1A lineage in descending order were: 1A.3.3.3 (gamma), 1A.3.3.2 (pdm), 1A.1.1 (alpha-del), 1A.2.-3-like (LAIV MN/99-like), and 1A.2 (beta).

Antigenic analysis: Swine 1A Lineage

Table 1. Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against clade consensus representative of contemporary swine 1A lineage strains.

	Global Clade	US Clade	A/Ohio/24/2017	IDCDC-RG59 A/Ohio/24/2017-like CVV	A/Ohio/9/2015 CVV RG48A	A/Idaho/7/2018
IDCDC-RG59 A/Ohio/24/2017-like CVV	1A.1.1	a-del H1N2v	5120	2560	<10	1280
A/swine/Iowa/A02478635/2019	1A.1.1	a-del H1N2	160	160	10	80
A/swine/Oklahoma/A02245237/2019	1A.2	b H1N1	40	20	320	2560
A/swine/Texas/A01104132/2019	1A.2-3-like	g2-b-like H1N1	20	20	80	1280
A/Ohio/9/2015 wt	1A.3.3.3-c1	g.1 H1N1v	<10	<10	1280	10
A/swine/North Carolina/A02478571/2019	1A.3.3.3-c1	g.1 H1N1	<10	10	1280	20
A/swine/Iowa/A01731653/2016	1A.3.3.3-c3	g.3 H1N1	10	<10	160	20
A/swine/Nebraska/A02214231/2017	1A.3.3.3-c3del	g.3-del H1N1	10	<10	320	10
A/Idaho/7/2018	1A.3.3.2	H1N1pdm09	80	160	160	10240
A/swine/Utah/A02432386/2019	1A.3.3.2	H1N1pdm09	80	40	160	5120

CVV in red and human seasonal vaccine strain in dark red.

- The contemporary swine 1A.1.1 (alpha-del) demonstrated a significant fold-decrease from CVV A/Ohio/24/2017.
- The contemporary swine 1A.2 (beta) does not have a CVV contained within clade and demonstrated limited antigenic similarity to any 1A CVV. There was cross-reactivity with the high-titer A/Idaho/7/2018 vaccine strain.
- The contemporary swine 1A.2.3-like (LAIV MN/99-like) does not have a CVV contained within clade and demonstrated limited antigenic similarity to any 1A CVV. There was cross-reactivity with the high-titer A/Idaho/7/2018, but at an 8-fold-decrease.
- The contemporary swine 1A.3.3.3 clade 1 (gamma-c1) retained cross-reactivity with CVV A/Ohio/9/2015; however, the clade 3 and clade 3-del strains had moderate to significant fold-decreases.
- The contemporary swine 1A.3.3.2 (pdm) with onward transmission in swine retained cross-reactivity to A/Idaho/7/2018 vaccine strain.

Table 2. Amino acid substitutions between recent swine 1A.1.1 lineage strains compared to nearest CVV.

Mature H1 HA1	A/Ohio/24/2017 1A.1.1	A/swine/Iowa/A02478635/2019 1A.1.1	Annotation
48	A	S	
130	V	E	
136	D	Y	
139	A	K	
147	I	M	
153	G	D	Sb
154	N	D	
168	R	G	
207	E	K	
222	T	A	RBS
250	K	R	
308	T	R	
309	K	R	
aadiff		13	

*Reference CVV in red, swine strains in black.

Table 3. Amino acid substitutions between recent swine 1A.3.3.2, 1A.2, and 1A.2-3-like lineage strains compared to nearest seasonal vaccine strain.

Mature H1 HA1	A/Idaho/07/2018 1A.3.3.2	A/swine/Utah/A02432386/2019 1A.3.3.2	A/swine/Oklahoma/A02245237/2019 1A.2	A/swine/Texas/A01104132/2019 1A.2-3-like	Annotation
14	D		E		
24	V		I		
35	D		N	E	
40	K			R	
43	K		N		
47	V	I			
61	I		L		
71	S		F	F	
73	A			T	Cb
74	R	S	S	S	
83	S		P		
86	D		Y		
94	D		N		
96	I		N		
104	Q		H		
119	K			N	
120	T		A	A	
121	S		N		
126	H	X		Y	
127	D			E	
128	S		T	T	
129	N		D	S	
130	K			R	
138	H		Y	Y	
142	K		N	N	
146	K		R	R	

149	I			L	
162	N		S	S	Sa
163	Q		K	K	Sa
164	T	S	S	S	Sa
166	I		S		Ca1
168	D		N	N	
170	G		K	E	Ca2
173	V		I		
176	L		I		
183	P	S	S	S	
185	T		D	S	
186	A		T	T	
190	S		A		Sb, RBS
203	T		S	S	
205	R	K	K	K	
209	K		R		
216	T	S	A		
224	E		A	A	RBS
228	N		D		
234	V		I		
239	K		T	T	
240	I		V		
256	T		A	A	
258	E		K	K	
260	N		G	G	
261	A		S	S	
267	I		V		
270	T		A		
271	P			S	
278	T			N	
283	E		K	K	
295	V	I	I	I	
298	I		V	V	
302	K		E	E	
310	T			K	
314	L		M	M	
321	V		I	I	
aadiff		8	52	41	

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

Table 4. Amino acid substitutions of recent swine 1A.3.3.3 lineage strains compared to nearest CVV.

	Mature H1 HA1	A/Ohio/09/2015 1A.3.3.3-c1	A/swine/North_Carolina/A02478571/2019 1A.3.3.3-c1	A/swine/Iowa/A01731653/2016 1A.3.3.3-c3	A/swine/Nebraska/A02214231/2017 1A.3.3.3-c3-del	Annotation
2	K		T	T		
3	I		L	L		
22	K	R				
36	K		R	R		
61	L			I		
68	E	D				
71	A		S	S		
72	T	R			Cb	
84	S		N	N		
86	N		E	E		
113	K		R	R		
127	E		D	D		
129	N		D			
130	K		R	del		
142	N			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	I	Ca1	
169	R	K	K	K		
170	G		E	E	Ca2	

171	K			N	
183	P		S	S	
186	T		A	A	
193	Q		K	K	Sb, RBS
196	N		D	D	
197	S		A	A	
222	G	D	D	D	RBS
238	D			E	
250	V		A	A	
256	A			T	
269	E		D	D	
271	P	S	S	S	
283	N	S			
aadiff		8	28	31	

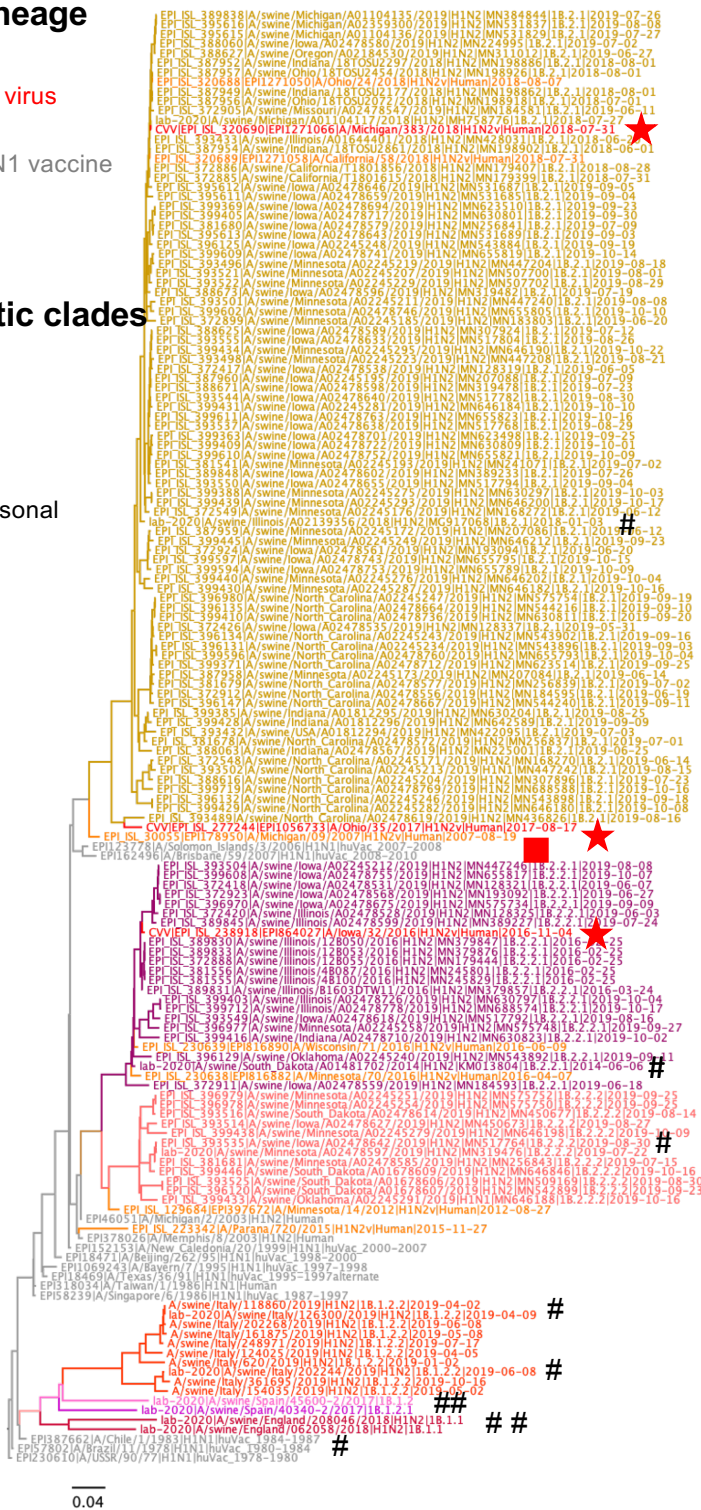
***Reference CVV in red, swine strains in black.**

1B human-like lineage

- ★ H1v candidate vaccine virus
- A/Brisbane/59/2007
- Previous seasonal H1N1 vaccine
- Reported H1v cases
- # HI tested viruses

H1 1B swine genetic clades

- 1B.2.1 (n=83)
- 1B.2.2.1 (n=18)
- 1B.2.2.2 (n=12)
- 1B.1.2.2 (n=10)
- Human vaccine or seasonal



n=125, data deposited within last 6 months, and n=19 reference genes.

Figure 4. Swine H1 HA genes of the 1B lineage. A total of 125 swine 1B HA sequences deposited from July 1 – December 31, 2019 were included. The most frequently deposited clades of the 1B lineage in descending order were: 1B.2.1 (delta-2); 1B.2.2.1 (delta-1a); 1B.2.2.2 (delta-1b); 1B.1.2.2 (European huH1).

Antigenic analysis: Swine 1B Lineage

Table 5. Hemagglutination inhibition of CVV vaccine ferret antisera against clade consensus representative of contemporary swine 1B lineage strains.

	Global Clade	US Clade	A/Iowa/32/2016	A/Ohio/35/2017	A/Michigan/383/2018
A/Iowa/32/2016	1B.2.2.1	d1a H1N2v	640	20	20
A/swine/South Dakota/A01481702/2014	1B.2.2.1	d1a H1N2	80	20	80
A/swine/Minnesota/A02478597/2019	1B.2.2.2	d1b H1N2	40	20	20
A/Ohio/35/2017	1B.2.1	d2 H1N2v	80	640	160
A/Michigan/383/2018	1B.2.1	d2 H1N2v	40	160	1280
A/swine/Illinois/A02139356/2018	1B.2.1	d2 H1N2	20	320	1280

CVV in red.

- The contemporary swine 1B.2.2.1 (delta-1a) demonstrated a significant fold-decrease from CVV A/Iowa/32/2016.
- The contemporary swine 1B.2.2.2 (delta-1b) does not have a CVV contained within clade and demonstrated a significant fold-decrease from CVV A/Iowa/32/2016.
- The contemporary swine 1B.2.1 (delta-2) remained cross-reactive to CVV A/Ohio/35/2017 and A/Michigan/383/2018.

Table 6. Hemagglutination inhibition of CVV vaccine ferret antisera against clade consensus representative of contemporary swine 1B lineage strains from Europe.

	subtype	clade	A/Brazil/11/1978	A/Michigan/383/2018
A/Brazil/11/1978	H1N1	hu-vaccine	5120	40
A/Michigan/383/2018	H1N2	CVV	80	320
A/swine/Italy/126300/2019	H1N2	1B.1.2.2	160	160
A/swine/Italy/202244/2019	H1N2	1B.1.2.2	160	40
A/swine/England/20846/2018	H1N2	1B.1.1	1280	80
A/swine/England/062058/2018	H1N2	1B.1.1	640	80
A/swine/Spain/40340-2/2017	H1N2	1B.1.2.1	1280	40
A/swine/Spain/45600-2/2017	H1N2	1B.1.2	640	20

CVV in red, human vaccine strain in dark red

- The contemporary swine 1B.1.2.2 does not have a CVV contained within clade and demonstrated **some loss** of cross-reactivity to the CVV A/Michigan/383/2018 1B 2.1 and a **significant** fold-decrease from the ancestral human seasonal vaccine strain A/Brazil/11/1978.
- The contemporary swine 1B.1.1 does not have a CVV contained within clade and demonstrated a **significant** fold-decrease from **both** CVV A/Michigan/383/2018 1B 2.1 and the ancestral human seasonal vaccine strain A/Brazil/11/1978.
- The contemporary swine 1B.1.2.1 does not have a CVV contained within clade and demonstrated a **significant** fold-decrease from **both** the CVV A/Michigan/383/2018 1B 2.1 and the ancestral human seasonal vaccine strain A/Brazil/11/1978.
- The contemporary swine 1B.1.2. does not have a CVV contained within clade and demonstrated a **significant** fold-decrease from **both** the CVV A/Michigan/383/2018 1B 2.1 and the ancestral human seasonal vaccine strain A/Brazil/11/1978.

Table 7. Amino acid substitutions of recent swine 1B.2.2 (delta-1a and -1b) lineage strains compared to nearest CVV.

Mature H1 HA1	A/Iowa/32/2016 1B.2.2.1	A/swine/South_Dakota/A01481702/2014 1B.2.2.1	A/swine/Minnesota/A02478597/2019 1B.2.2.2	Annotation
19	L	V	V	
74	K	E		
82	A		T	
86	D		E	
94	Q		D	
96	T	A	A	
119	R		K	
127	T		N	
128	V		A	
132	K	V		RBS
141	E	K	K	
149	I		V	
152	T		K	
153	V		E	Sb
166	K	E		Ca1
168	E	D	N	
170	G		E	Ca2
183	S		P	
184	N		D	
186	G		E	
187	D		N	RBS
193	H		R	Sb, RBS
194	T		K	RBS
199	V		I	
202	M		A	
208	R		G	
222	D		N	RBS
244	T		A	

256	A		T	
261	L		H	
269	K		N	
277	A		T	
283	K		Q	
289	N		S	
310	T		A	
aadiff		7	32	

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

Table 8. Amino acid substitutions of recent swine 1B.2.1 (delta-2) lineage strains compared to nearest CVV.

Mature H1 HA1	A/Michigan/383/2018 1B.2.1	A/swine/Illinois/A02139356/2018 1B.2.1	Annotation
71	N	T	
169	E	K	
170	G	E	Ca2
173	V	I	
259	K	R	
260	S	G	
aadiff		6	

*Reference CVV in red, swine strains in black.

Table 9. Amino acid substitutions of contemporary swine 1B.1 strains from Europe compared to nearest seasonal vaccine strain.

Mature H1 HA1	A/Brazil/11/1978 H1N1 huVac_1980-1984	A/Michigan/383/2018 H1N2v CVV 1B.2.1	A/swine/Italy/126300/2019 H1N2 1B.1.2.2	A/swine/Italy/202244/2019 1B.1.2.2	A/swine/England/208046/2018 1B.1.1	A/swine/Spain/45600-2/2017 1B.1.2	A/swine/England/062058/2018 1B.1.1	A/swine/Spain/40340-2/2017 1B.1.2.1	Annotation
5	I	V							
19	V				I	I			
35	D		N		N	S			
36	S			N					
43	R	L	K	K	K				
44	L				M				
47	I		V	V		V			
48	A					D			
54	K	S		R					
57	I	V					V		
68	E						T		
69	S	L							Cb
70	L				S				Cb
71	F	N		L					
73	K				R		R	V	Cb
74	K	E							
80	A	V				T			
82	T		P	A					
83	P	S						T	
85	S	P	A	A	P	P	P	P	
88	G						D		
89	T		L	I	I	I	V	I	
94	Y			H			H		
96	A	E	S	S	S	S	S	S	
106	S						N		
109	S		A						

111	F			I	V				
112	E	K							
113	R	K							
116	I				M				
120	E	K	G						
121	R	S	S	S	S	G	S	S	
124	P							S	Sa
125	K	N				E	Q	N	Sa
127	N	T	S	S					
128	I	V	V		V	V	V	V	
129	T		del	del		N		N	
130	R	del	del	del		K	K	K	
132	V		S	A	K				RBS
133	T	S							RBS
134	A	S			V	K	V		RBS
137	S		P						
138	H		K	K					
139	K	N	Q	Q	N	R	N		
141	K	N		R		N		N	
146	R		T	T	K		Q		
149	L					I			
153	E	V					G		Sb
155	N				D		D		Sa
156	G	N							Sb
157	S	L			L	A		I	Sa
161	L				I				
162	S		T	T			N		Sa
163	K		M	M	N				Sa
164	S				H				Sa
166	V	T	M	M					Ca1
168	N	K	D	D			D		
169	K	E						R	
170	E	G		K			K	D	Ca2
171	K		R	R				E	
176	L				I				
179	V						I		
181	H				N				
183	S						A		
184	N						K		
185	I	M	M		M			M	
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
191	I	L							RBS
193	R	H					H		Sb, RBS
194	K	T							RBS
196	N		T	T	T	T	T	T	
199	V					I			
202	V		M	M					
205	N	H	Q	H	H	H	H	H	
207	N	S							
208	R			K				K	
211	T		A				I	I	
215	A				E	T	T		
216	K					R		R	
220	V		I		I	I		I	RBS
222	G	N	N	D	N	D	N	N	RBS
232	T						I		
235	E		D			N			
237	G		R	S		E		K	Ca4
238	D				E				
241	I		T				M		
245	N	S							
248	L				F	F		F	
249	I		V						
252	W	R					R	L	
256	A	E					S		
258	S		K	N					
259	R	K							
260	G	S					S		
261	F				L	S			
262	G		E	E	R	E	E	E	
267	T		V	V	I	V	V	V	
268	S				T				
270	A			V					
271	S	P	P						
272	M					T	L	V	
273	D	G						N	
276	D	N		N		N	N		
277	T		A	A		S			
278	K				E				
283	Q			R					
288	S		N	N	N				
290	L						R		

295	V			I					
298	V			I					
299	T				A				
307	V				I				
308	R		K	K	K				
310	T	A	A					K	
311	K				R				
313	R		K	K					
315	V		A	A					
321	I	T					V		
aadiff		49	47	47	45	36	43	34	

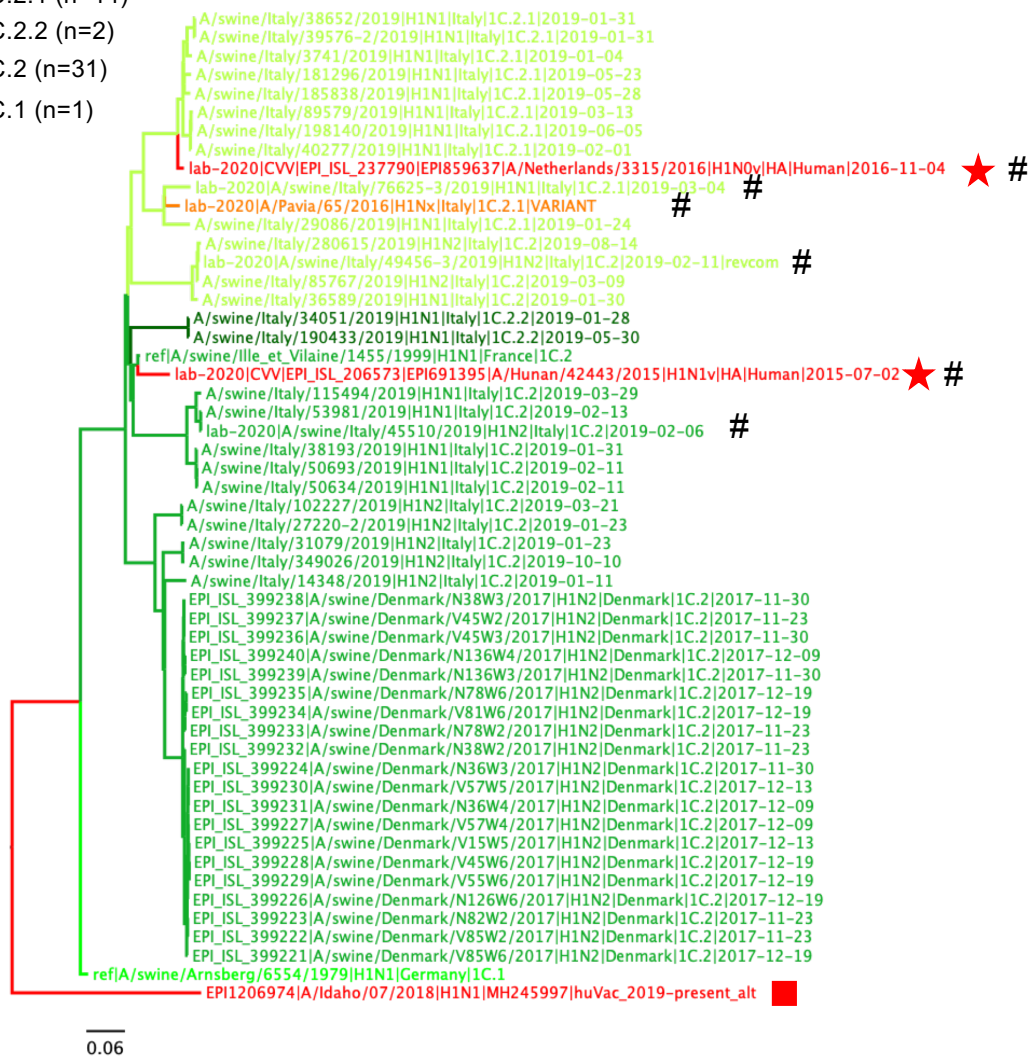
*Nearest CVV in red, swine strains in black.

1C Eurasian avian lineage

- ★ H1v candidate vaccine virus
- A/Idaho/07/2018 (H1N1pdm vaccine)
- Reported H1v cases
- # HI tested viruses

H1 1C swine genetic clades

- 1C.2.1 (n=14)
- 1C.2.2 (n=2)
- 1C.2 (n=31)
- 1C.1 (n=1)



n=47, data deposited within last 6 months, and n=6 reference genes.

Figure 5. Swine H1 HA genes of the 1C lineage. A total of 47 swine 1C HA sequences deposited from July 1 – December 31, 2019 were included. The most frequently deposited clades of the 1C lineage in descending order were: 1C.2, 1C.2.1, 1C.2.2, and 1C.1 (Eurasian avian H1 subclades).

Table 10. Hemagglutination inhibition of CVV vaccine ferret antisera against clade consensus representative of contemporary swine 1C lineage strains from Europe.

	subtype	clade	A/Hunan/42443/2015	A/Netherlands/3315/2016	A/Pavia/65/2016
A/Hunan/42443/2015	H1N1v	CVV	640	80	20
A/Netherlands/3315/2016	H1N1v	CVV	40	640	40
A/Pavia/65/2016	H1N1v	1C.2.1	20	20	640
A/swine/Italy/45510/2019	H1N2	1C.2	160	40	80
A/swine/Italy/76625-3/2019	H1N1	1C.2.1	160	80	40
A/swine/Italy/49456-3/2019	H1N2	outgroup	160	80	40

CVV in red, human vaccine strains in dark red.

- The contemporary swine 1C 2 does not have a CVV contained within clade and demonstrated a **significant** fold-decrease to the 1C CVV's and A/Pavia/65/2016.
- The contemporary swine 1C 2.1 does have a CVV contained within clade and demonstrated a **significant** fold-decrease to the 1C CVV's and A/Pavia/65/2016.
- The contemporary swine 1C outlier group does not have a CVV contained within clade and demonstrated a **significant** fold-decrease to the 1C CVV's and A/Pavia/65/2016.

Table 11. Amino acid substitutions of recent swine 1C lineage strains compared to nearest CVV.

Mature H1 HA1	A/Hunan/42443/2015 1C.2	A/Netherlands/3315/2016 1C.2	A/swine/Italy/45510/2019 1C.2	A/swine/Italy/49456-3/2019 1C.2	1C.2_consensus	1C.2_consensus_Denmark	1C.2_consensus_Italy	Annotation
2	T		I					
5	V			I				
20	L	M		M				
35	N			T	T	T	T	
44	L		M					
47	K		N					
48	I	A	A	A	A	A	A	
56	S	N	D	N	N	N	N	
61	I				V	V		
66	K	E	E	E	E	E	E	
69	L		F					Cb
71	L	I		I				
82	T		A		A	A		
83	S				P	P		
84	N				S	S		
85	S		P					
86	K			R				
89	A			T	T	T	T	
96	A			T	T	T		
102	K	R	R	R	R	R	R	
104	Q			L				
106	S			N				
111	F		L					
113	R			K				
119	K			E				
120	A	E	R	-			R	
124	P			L				Sa
125	N		D		H	H	D	Sa
126	H			Y				
127	D	E		K	E	E	E	
129	T		A					
130	R	K	K	-	N	N	K	
132	T	S						RBS
134	V		S				S	RBS
135	A		S				S	RBS

137	S			P				
138	H			N				
141	A	I						
142	N	K		S	K	K		
152	V			T	R	R		
153	K				G	G		Sb
155	G	E			K	K		Sa
156	N				G	G		Sb
157	S			T				Sa
159	P		S					Sa
161	L	I		I				
162	S		N					Sa
163	K	T	N					Sa
166	T			K				Ca1
169	K		R					
170	G			R				Ca2
175	V	I			I	I		
185	D			Y	Y	Y	Y	
190	T		A	A			A	Sb, RBS
196	H		R					
202	G			V	E	E		
208	K		R		R	R	R	
215	V		I					
216	A			S	T	T		
218	P					Q		RBS
220	V			I	I	I	I	RBS
221	R			N				RBS
222	E		N					RBS
224	A			E		T		RBS
227	M		I	I			I	
232	T			I				
236	Q			R				
245	T		N					
252	W			R				
253	H	Y	Y	Y	Y	Y	Y	
258	K	N		N	D	D	N	
259	K		R					
261	S				L	L		
262	S	N						
266	M		L	I	V	V	V	
267	R	M	I	K	M	M	I	
269	D				E	E		
270	A		V					
271	Q	N	H	L	H	H	H	
273	H			Q				
287	K		N	E				
288	G	S	N	S			N	
289	N		S	S	S	S	S	

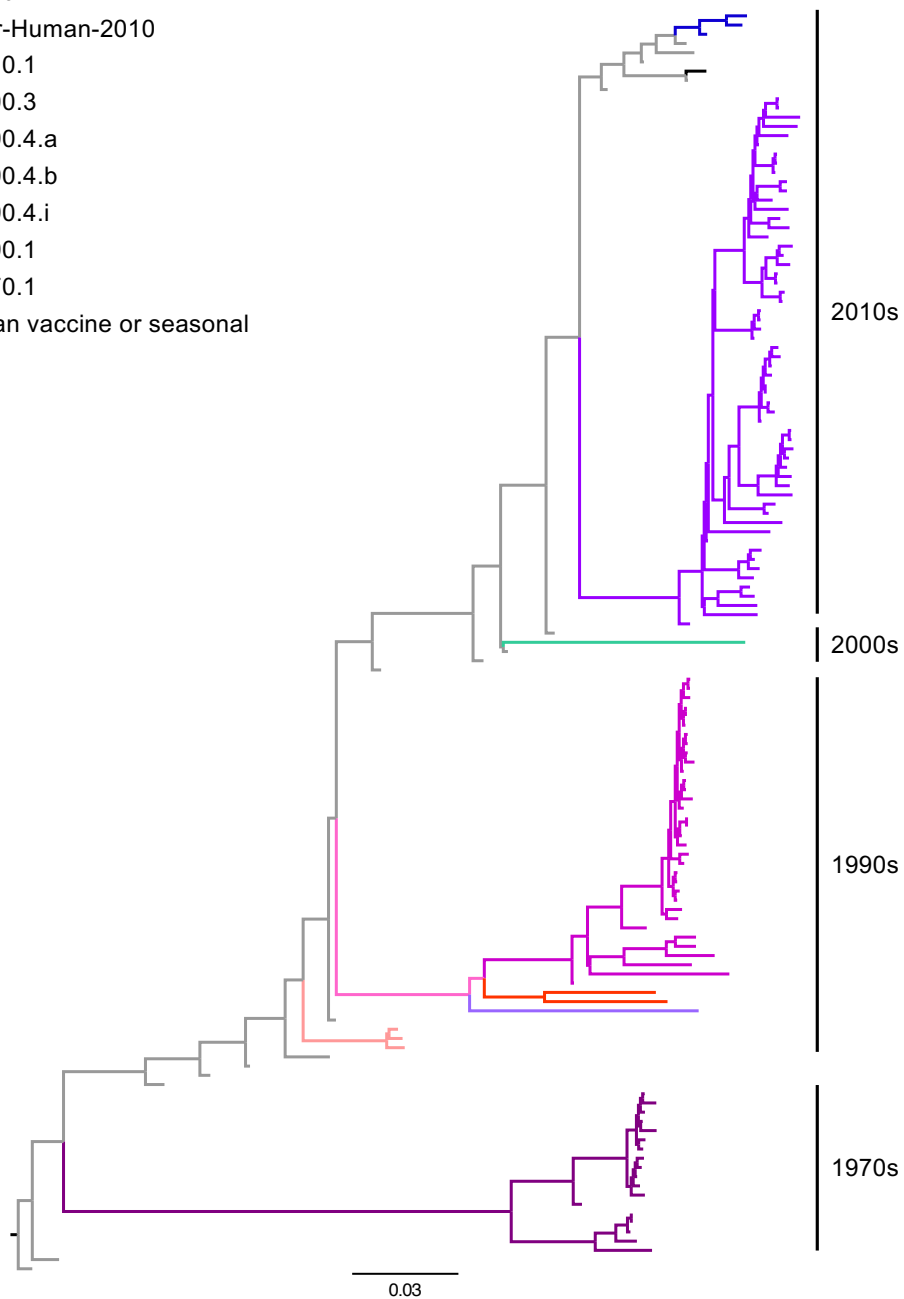
290	L			R				
298	V	I	I	I	I	I	I	
310	T		K					
313	R			K				
321	I	T						
324	I	V						
aadiff		25	39	51	34	36	25	

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

Contemporary Global H3 swine IAV: genetic diversity

H3 swine genetic clades

- 3.2010.2
- Other-Human-2010
- 3.2010.1
- 3.2000.3
- 3.1990.4.a
- 3.1990.4.b
- 3.1990.4.i
- 3.1990.1
- 3.1970.1
- Human vaccine or seasonal



n=108, data deposited within last 6 months, and n=44 reference genes.

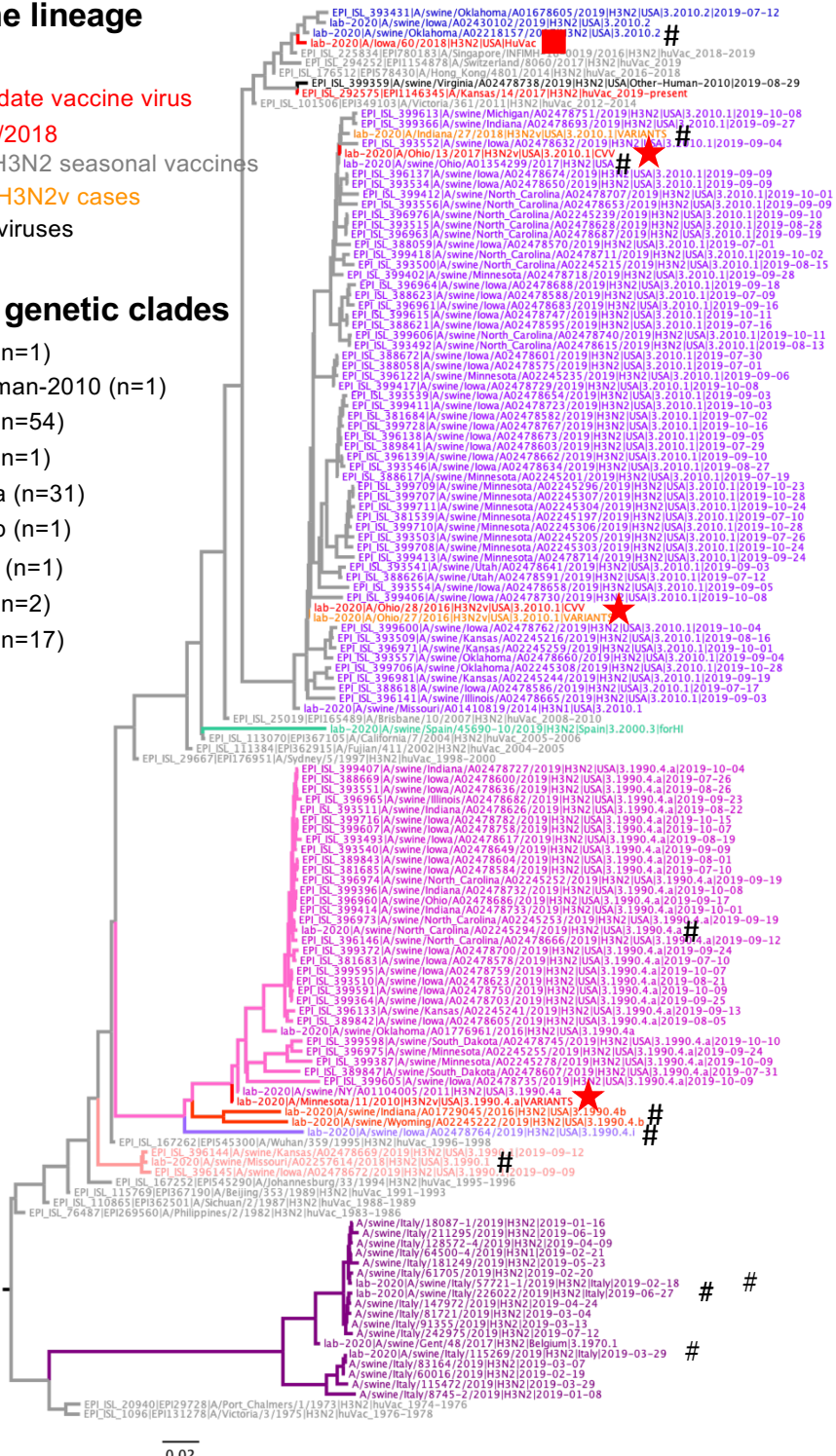
Figure 6. Global swine H3 phylogenetic tree colored by clade and annotated by decade of introduction of human seasonal ancestor. Analyses were conducted with reference sequences and new data (deposited July 1, 2019 – December 31, 2019) downloaded from GISAID or GenBank.

H3 swine lineage

- ★ H3v candidate vaccine virus
- A/Iowa/60/2018
- Previous H3N2 seasonal vaccines
- Reported H3N2v cases
- # HI tested viruses

H3 swine genetic clades

- 3.2010.2 (n=1)
- Other-Human-2010 (n=1)
- 3.2010.1 (n=54)
- 3.2000.3 (n=1)
- 3.1990.4.a (n=31)
- 3.1990.4.b (n=1)
- 3.1990.4.i (n=1)
- 3.1990.1 (n=2)
- 3.1970.1 (n=17)



n=108, data deposited within last 6 months, and n=32 reference genes.

Figure 7. Swine H3 HA genes. A total of 108 swine H3 sequences deposited from July 1 – December 31, 2019 were included. The most frequently deposited clades of the H3 subtype in descending order were: 3.2010.1; 3.1990.4.a (IV-A); 3.1970.1; 3.1990.1 (I, LAIV TX/98-like); 3.1990.4.i (IV-I); 3.1990.4.b (IV-B); 3.2000.3; 3.2010.2.

Antigenic analysis: Swine H3

Table 12. Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine H3 strains selected to represent clade consensus.

	Lineage	A/Minnesota/11/2010 x 203	IDCDC-RG55C A/Ohio/28/2016-like CVV	A/Ohio/27/2016	A/Indiana/27/2018*	A/Iowa/60/2018(H3N2)
A/Minnesota/11/2010 x 203	1990.4.A H3N2v	640	<10	<10	10	<10
A/swine/Missouri/A02257614/2018	1990.1 H3N2	40	<10	20	20	<10
A/swine/North_Carolina/A02245294/2019	1990.4.A H3N2	80	<10	10	20	<10
A/swine/Wyoming/A02245222/2019	1990.4.B H3N2	40	<10	20	20	<10
A/swine/Iowa/A02478764/2019	1990.4.I H3N2	10	<10	20	10	<10
IDCDC-RG55C A/Ohio/28/2016-like CVV	2010.1 H3N2v	<10	1280	1280	40	10
A/Ohio/27/2016	2010.1 H3N2v	<10	640	1280	80	10
A/swine/Ohio/A01354299/2017	2010.1 H3N2	<10	80	160	80	20
A/Indiana/27/2018*	2010.1 H3N2v	<10	10	40	640	10
A/Iowa/60/2018 cell	HuVacc H3N2	<10	<10	10	10	320
A/swine/Iowa/A02430102/2019	2010.2 H3N2	<10	<10	20	20	40

CVV in red, human vaccine strain in dark red. *A/Indiana/27/2018 H3N2v (equivalent to A/Ohio/13/2107) is also representative of contemporary swine strains.

- The contemporary swine 1990.1 (C-I, LAIV TX/98-like) does not have a CVV contained within clade and demonstrated a significant fold-decrease from CVV A/Minnesota/11/2010.
- The contemporary swine 1990.4.A (IV-A) demonstrated a significant fold-decrease from CVV A/Minnesota/11/2010.
- The contemporary swine 1990.4.B does not have a CVV contained within clade and demonstrated a significant fold-decrease from CVV A/Minnesota/11/2010.
- The contemporary swine 1990.4.I does not have a CVV contained within clade and demonstrated a significant fold-decrease from CVV A/Minnesota/11/2010.
- The contemporary swine 2010.1 A/swine/Ohio/A01354299/2017 demonstrated a significant fold-decrease from CVV A/Ohio/28/2016.
- The contemporary swine 2010.2 does not have a CVV contained within clade and demonstrated a significant fold-decrease from CVV A/Ohio/28/2016 and seasonal vaccine strain A/Iowa/60/2018.

Table 13. Hemagglutination inhibition of CVV or human seasonal vaccine ferret antisera against contemporary swine H3 strains from Europe selected to represent clade consensus.

	subtype	clade	A/Port Chalmers/1/1973
A/Port Chalmers/1/1973	H3N2	hu-vaccine	1280
A/swine/Italy/57721-1/2019	H3N2	3.1970.1	>10
A/swine/Italy/226022/2019	H3N2	3.1970.1	160
A/swine/Italy/115269/2019	H3N2	3.1970.1	>10

Human vaccine strain in dark red

- The contemporary swine 3.1970.1 do not have a CVV contained within clade and demonstrated a **significant and heterogenous** fold-decrease to the clade ancestral vaccine strain A/Port Chalmers/1/1973

Table 14. Amino acid substitutions of recent swine 1990 lineage H3 strains compared to nearest CVV.

Mature H3 HA1	A/Minnesota/11/2010	A/swine/North_Carolina/A02245294/2019 3.1990.4.a	A/swine/Wyoming/A02245222/2019 3.1990.4.b	A/swine/Iowa/A02478764/2019 3.1990.4.i	A/swine/Missouri/A02257614/2018 3.1990.1	Annotation
6	S				N	
8	N			K		
10	M			T	T	
31	D				N	
33	Q		K			
49	G			D		
53	N		S		D	Site C
57	Q		K	K	R	
62	K			G		
78	D				G	
80	Q			E		
83	E		K			
88	V		I		I	
92	T		K		K	
96	N	S				
101	Y				D	
106	A				S	
107	T		S	S	S	
112	V			I		
117	N		T	T	T	
119	E	K				
121	T			N		
122	Q			P	N	Site A
124	S	I	V	N	D	

131	A	T				
135	S				G	
137	Y			F		Site A
138	A	S		S	S	
140	R			K	K	
142	G		K	N		
144	V		A			Site A
145	N				K	Site A
155	Y			H	H	Site B
156	N	H	H	H	K	
158	N				E	
159	Y			H		
160	K		N			
163	E		A	A	A	
164	Q		L	L	L	
173	K		N			
186	G				S	Site B
189	K			M	S	Site B
190	D				E	
193	N		S	S	S	Site B
198	A			G		
199	S				I	
203	I		T	T	T	
207	K	R	R			Site D
208	R				S	Site D
210	Q			L		
214	I		V			
217	I		V			Site D
223	V		I			
226	V				I	
229	I				R	
239	P		S			
246	N				S	
261	Q			R	R	
262	S			I	N	
269	R		K	K		
273	H		P	L	P	
275	D		S	G		Site C
276	E		S	N	N	
278	N				Y	Site C
289	P	S				
299	K		R	R	R	

307	R				K	
312	N				K	
323	V	I				
aadiff		9	28	33	41	

*Reference CVV in red, swine strains in black.

Table 15. Amino acid substitutions of recent swine 2010.1 H3 strains compared to nearest CVV.

Mature H3 HA1	A/Ohio/28/2016	A/Ohio/27/2016*	A/Indiana/27/2018	A/swine/Ohio/A01354299/2017	3.2010.1_consensus_54seqs	Annotation
56	H		Y	Y		
138	A		S	S	S	
142	R		G			
145	K		N	N	N	Site A
156	H		R			
158	N		D			
167	T				A	
189	K		T			Site B
209	S		N	N		
aadiff		0	8	4	3	

*Reference CVV in red, swine strains in black, A/Ohio/27/2016 represents contemporary swine strains in the USA in addition to A/swine/Ohio/A01354299/2017.

Table 16. Amino acid substitutions of recent swine 2010.2 H3 strains compared to human seasonal vaccine strain.

Mature H3 HA1	A/Iowa/60/2018	A/swine/Iowa/A02430102/2019	Annotation
9	S	G	
31	N	D	
58	I	V	
62	G	E	
131	K	T	
135	T	K	
138	A	S	
167	T	I	
189	K	N	Site B
197	Q	R	
203	T	I	
aadiff		11	

*Reference seasonal vaccine strain in red, swine strains in black.

Table 17. Amino acid substitutions of recent swine 1970.1 H3 strains compared to nearest ancestral human seasonal vaccine strain.

Mature H3 HA1	A/Port_Chalmers/1/1973	A/swine/Italy/57721-1/2019 1970.1	A/swine/Italy/226022/2019 1970.1	A/swine/Italy/115269/2019 1970.1	3.1970.1_consensus_17seqs	Annotation
5	G	R	R		R	
6	N	K	K	K	K	
7	D	G	G	G	G	
9	S			N		
31	N	D	D	D	D	
45	S	N	N	N	N	
46	S	V	F	F	F	
48	T	I	M	M	M	
53	N	K	K	K	K	Site C
62	I	A	A	A	A	
67	I			V		
69	A	S	S	S	S	
83	T	R	R	K	R	
88	V	I	I	I	I	
92	K	R	R	R	R	
104	D	E	E	E	E	
106	A	T	T	T	T	
112	V	I	I	I	I	
117	T			N		
121	I	T	T	T	T	
124	G	N	N	N	N	
126	T	N	N	N	N	
133	N		D			Site A
137	N	S	S	S	S	Site A
143	P	I	I		I	Site A
144	D		N	N	N	Site A
145	S	K	K	N	K	Site A
146	G	S	S	S	S	Site A
159	S	N	N	N	N	

160	A	T	T	T	T	
163	V	M	M	M	M	
164	L	Q	Q		Q	
171	N	S	S	S	S	
173	N	D	D	D	D	
174	F	Y	Y		Y	
189	Q	K	K	R	K	Site B
193	N	D	D		D	Site B
196	V	I	I	I	I	
201	R	K	K	K	K	Site D
202	V	I	I	I	I	
203	T	I	I	M	I	
208	R			K		Site D
212	T	I	I		I	
213	I	V	A		V	
242	I	T	T	V	T	
244	V	I	I	I	I	
260	M	I	I	I	I	
261	R	Q	Q	Q	Q	
267	I	V	V	V	V	
269	R			K		
278	I	N	N	N	N	Site C
299	K	R	R	R	R	
307	K	H	H	H	H	
309	V	I	I	I	I	
323	V	I	I	I	I	
aadiff		48	50	47	49	

*Reference seasonal vaccine strain in red, swine strains in black.

Annex 1

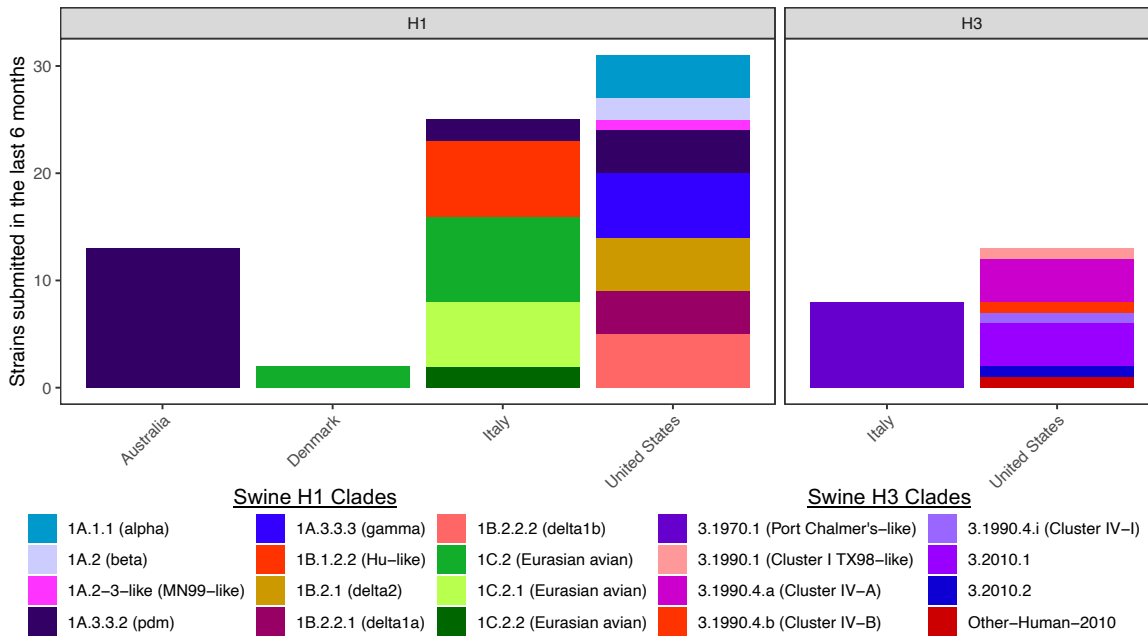


Figure A1. Geographic distribution of swine HA phylogenetic clades for HA sequences deposited July 1, 2019 – December 31, 2019. Y-axis represents cumulative count for each country, separated by HA subtype across the top headers.

Table A1. Summary of swine H1 clades by country and month of collection.

Subtype	Country	Clade	Year-Month	Count
H1N2	United States	1A.1.1	2019-07	8
H1N2	United States	1A.1.1	2019-08	8
H1N2	United States	1A.1.1	2019-09	9
H1N2	United States	1A.1.1	2019-10	9
H1N1	United States	1A.2	2019-08	1
H1N1	United States	1A.2	2019-09	3
H1N1	United States	1A.2-3-like	2019-09	1
H1N1	Australia	1A.3.3.2	2012-09	1
H1N1	Australia	1A.3.3.2	2013-04	1
H1N1	Australia	1A.3.3.2	2013-08	3
H1N1	Australia	1A.3.3.2	2013-09	4
H1N1	Australia	1A.3.3.2	2013-12	3
H1N1	Australia	1A.3.3.2	2017-04	7
H1N1	Australia	1A.3.3.2	2017-05	4
H1N1	Australia	1A.3.3.2	2017-07	2
H1N1	Australia	1A.3.3.2	2018-03	2
H1N1	Australia	1A.3.3.2	2018-07	1
H1N1	Australia	1A.3.3.2	2018-09	4
H1N1	Australia	1A.3.3.2	2018-10	2
H1N1	Australia	1A.3.3.2	2018-11	1
H1N1	Italy	1A.3.3.2	2019-02	1
H1N1	Italy	1A.3.3.2	2019-03	2
H1N2	United States	1A.3.3.2	2019-07	1
H1N1	United States	1A.3.3.2	2019-08	3
H1N1	United States	1A.3.3.2	2019-09	1
H1N1	United States	1A.3.3.2	2019-10	1
H1N1	United States	1A.3.3.3	2019-07	4
H1N1	United States	1A.3.3.3	2019-08	9
H1N2	United States	1A.3.3.3	2019-08	1
H1N1	United States	1A.3.3.3	2019-09	28
H1N1	United States	1A.3.3.3	2019-10	26
H1N2	United States	1A.3.3.3	2019-10	1
H1N2	Italy	1B.1.2.2	2019-01	1
H1N2	Italy	1B.1.2.2	2019-02	2
H1N2	Italy	1B.1.2.2	2019-04	3
H1N2	Italy	1B.1.2.2	2019-05	2
H1N2	Italy	1B.1.2.2	2019-06	2
H1N2	Italy	1B.1.2.2	2019-07	1
H1N2	Italy	1B.1.2.2	2019-10	1
H1N2	United States	1B.2.1	2019-07	14
H1N1	United States	1B.2.1	2019-08	1
H1N2	United States	1B.2.1	2019-08	11
H1N2	United States	1B.2.1	2019-09	18
H1N2	United States	1B.2.1	2019-10	16
H1N2	United States	1B.2.2.1	2019-07	1

H1N2	United States	1B.2.2.1	2019-08	2
H1N2	United States	1B.2.2.1	2019-09	3
H1N2	United States	1B.2.2.1	2019-10	4
H1N2	United States	1B.2.2.2	2019-07	2
H1N2	United States	1B.2.2.2	2019-08	4
H1N2	United States	1B.2.2.2	2019-09	3
H1N1	United States	1B.2.2.2	2019-10	1
H1N2	United States	1B.2.2.2	2019-10	2
H1N2	Denmark	1C.2	2017-11	9
H1N2	Denmark	1C.2	2017-12	11
H1N1	Italy	1C.2	2019-01	2
H1N2	Italy	1C.2	2019-01	3
H1N1	Italy	1C.2	2019-02	3
H1N2	Italy	1C.2	2019-02	2
H1N1	Italy	1C.2	2019-03	1
H1N2	Italy	1C.2	2019-03	2
H1N2	Italy	1C.2	2019-08	1
H1N2	Italy	1C.2	2019-10	1
H1N1	Italy	1C.2.1	2019-01	4
H1N1	Italy	1C.2.1	2019-02	1
H1N1	Italy	1C.2.1	2019-03	1
H1N2	Italy	1C.2.1	2019-03	1
H1N1	Italy	1C.2.1	2019-05	2
H1N1	Italy	1C.2.1	2019-06	1
H1N1	Italy	1C.2.2	2019-01	1
H1N2	Italy	1C.2.2	2019-05	1

Table A2. Summary of swine H3 clades by country and month of collection.

Subtype	Country	Clade	Year-Month	Count
H3N2	Italy	3.1970.1	2019-01	2
H3N1	Italy	3.1970.1	2019-02	1
H3N2	Italy	3.1970.1	2019-02	3
H3N2	Italy	3.1970.1	2019-03	5
H3N2	Italy	3.1970.1	2019-04	2
H3N2	Italy	3.1970.1	2019-05	1
H3N2	Italy	3.1970.1	2019-06	2
H3N2	Italy	3.1970.1	2019-07	1
H3N2	United States	3.1990.1	2019-09	2
H3N2	United States	3.1990.4.a	2019-07	4
H3N2	United States	3.1990.4.a	2019-08	6
H3N2	United States	3.1990.4.a	2019-09	10
H3N2	United States	3.1990.4.a	2019-10	11
H3N2	United States	3.1990.4.b	2019-08	1
H3N2	United States	3.1990.4.i	2019-10	1
H3N2	United States	3.2010.1	2019-07	12
H3N2	United States	3.2010.1	2019-08	5
H3N2	United States	3.2010.1	2019-09	20
H3N2	United States	3.2010.1	2019-10	17
H3N2	United States	3.2010.2	2019-07	1
H3N2	United States	Human	2019-08	1