

## Supplementary Information

### Title:

US feral swine were exposed to both avian and swine influenza A viruses

### Running Title:

US feral swine exposed to influenza A viruses

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**Table S1.** Hemagglutination inhibition cross-reactivity among reference influenza A viruses and homologous antisera.

**Table S2.** Total number of influenza A virus-positive (N=406) and total number of feral swine serum samples collected during fiscal years 2010-2013<sup>a</sup> for month, age group, and sex.

Month	No. positive samples/total no. samples <sup>b</sup> (%)				
	2010	2011	2012	2013	Total
January	13/220 (5.91)	16/373 (4.29)	4/80 (5.00)	10/188 (5.32)	43/861 (4.99)
February	16/235 (6.81)	19/337 (5.64)	25/208 (12.02)	9/274 (3.28)	69/1054 (6.55)
March	14/250 (5.60)	18/378 (4.76)	8/245 (3.27)	3/364 (0.82)	43/1237 (3.48)
April	20/162 (12.35)	7/253 (2.77)	1/159 (0.63)	16/218 (7.34)	44/792 (5.56)
May	5/162 (3.09)	2/234 (0.85)	1/131 (0.76)	22/185 (11.89)	30/712 (4.21)
June	4/81 (4.94)	4/134 (2.99)	1/153 (0.65)	2/99 (2.02)	11/467 (2.36)
July	9/133 (6.77)	5/184 (2.72)	0/86 (0.00)	0/101 (0.00)	14/504 (2.78)
August	9/246 (3.66)	4/170 (2.35)	47/309 (15.21)	2/201 (1.00)	62/926 (6.70)
September	1/57 (1.75)	0/112 (0.00)	18/280 (6.43)	0/67 (0.00)	19/516 (3.68)
October	6/107 (5.61)	6/110 (5.45)	1/91 (1.10)	18/187 (9.63)	31/495 (6.26)
November	6/80 (7.50)	9/56 (16.07)	1/26 (3.85)	4/83 (4.82)	20/245 (8.16)
December	9/85 (10.59)	5/126 (3.97)	4/78 (5.13)	2/141 (1.42)	20/430 (4.65)
Age group	2010	2011	2012	2013	Total
Juvenile	1/7 (14.29)	0/63 (0.00)	1/97 (1.03)	1/23 (4.35)	3/190 (1.58)
Subadult	15/526 (2.85)	17/658 (2.58)	17/451 (3.77)	3/406 (0.74)	52/2041 (2.55)
Adult	98/1281 (7.49)	78/1737 (4.49)	93/1288 (7.22)	83/1678 (4.95)	350/5984 (5.85)
Unknown	0/0 (0.00)	0/9 (0.00)	0/10 (0.00)	1/1 (100.00)	1/24 (4.17)
Sex		47/1287		59/1101	233/4333
Female	63/968 (6.51)	(3.65)	64/977 (6.55)	(5.36)	(5.38)
Male	46/843 (5.81)	48/1170 (4.10)	47/858 (5.48)	29/1000 (2.90)	173/3871 (4.47)
Unknown	0/7 (0.00)	0/10 (0.00)	0/11 (0.00)	0/7 (0.00)	0/35 (0.00)

Total	112/1818 (6.16)	95/2467 (3.85)	111/1846 (6.01)	88/2108 (4.17)	406/8239 (4.93)
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<sup>a</sup> Fiscal years run from October 1st of one year through September 30th of the next year; the FY is named according to the second year.

<sup>b</sup> A total of 8,239 samples were tested using the IDEXX AI MultiS-Screen Ab Test (IDEXX, Westbrook, Maine, USA).

**Table S3.** Total number and number of influenza A virus–positive feral swine serum samples collected from each of 35 US states during fiscal years 2010–2013.<sup>a</sup>

State	Total no. samples/no. (%) positive <sup>b</sup>
Alabama	268/11 (4.10)
Arkansas	437/7 (1.60)
Arizona	100/1 (1.00)
California	499/39 (7.82)
Colorado	3/0 (0.00)
Florida	916/17 (1.86)
Georgia	553/12 (2.17)
Hawaii	559/36 (6.44)
Iowa	1/1 (100.00)
Idaho	1/0 (0.00)
Illinois	41/4 (9.76)
Indiana	22/0 (0.00)
Kansas	274/10 (3.65)
Kentucky	41/0 (0.00)
Louisiana	390/5 (1.28)
Michigan	47/0 (0.00)
Missouri	188/4 (2.13)
Mississippi	440/7 (1.59)
North Carolina	211/34 (16.11)
North Dakota	5/0 (0.00)
New Hampshire	20/0 (0.00)
New Jersey	8/0 (0.00)
New Mexico	224/2 (0.89)
Nevada	16/0 (0.00)
New York	63/2 (3.17)

Ohio	45/0 (0.00)
Oklahoma	735/27 (3.67)
Oregon	94/1 (1.06)
Pennsylvania	5/0 (0.00)
South Carolina	274/14 (5.11)
Tennessee	127/4 (3.15)
Texas	1,561/164 (10.51)
Virginia	52/3 (5.77)
Wisconsin	4/0 (0.00)
West Virginia	15/1 (6.67)
<b>Total</b>	<b>8,239/406 (4.93)</b>

<sup>a</sup> Fiscal years run from October 1st of one year through September 30th of the next year; the FY is named according to the second year.

<sup>b</sup> A total of 8,239 samples were tested using the IDEXX AI MultiS-Screen Ab Test (IDEXX, Westbrook, Maine, USA).

**Table S4.** Virus neutralization assay results for avian influenza–positive feral swine serum samples collected from swine in 35 US states, 2010–2013.

**Table S5.** Titration of virus loads in nasal washes from the feral swine infected with A/Mallard/WI/A00661712/20092009 (H3N2). Treatment feral swine were intranasally inoculated with  $10^6$  TCID<sub>50</sub> A/Mallard/WI/A00661712/20092009 (H3N2), meanwhile, control feral swine were intranasally inoculated with 1 mL PBS. Nasal wash were collected from 1 ~10 DPI. The virus shedding titers were detected in SPF eggs. Virus titer was expressed as log<sub>10</sub>EID<sub>50</sub>/mL.

DPI	Treatment swine								Control swine			
	37	38	39	40	41	45	47	48	35	36	42	46
1	ND	ND	ND	ND	2	1	ND	2	ND	ND	ND	ND
2	ND	ND	ND	ND	2	ND	ND	2.5	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	0.83	ND	ND	0.625	ND	ND	ND	ND

5	ND	ND	ND	0.833	ND	ND	0.625	2.222	ND	ND	ND	ND
6	ND	- <sup>a</sup>	ND	ND	ND	-	ND	2.25	ND	-	ND	ND
7	ND	-	ND	ND	ND	-	ND	ND	ND	-	ND	ND
8	ND	-	-	ND	-	-	ND	ND	ND	-	-	ND
9	ND	-	-	ND	-	-	ND	ND	ND	-	-	ND
10	ND	-	-	ND	-	-	ND	ND	ND	-	-	ND

<sup>a</sup>:- samples were not available

**Table S6.** Serological responses in the feral swine inoculated with A/mallard/WI/A00661712/2009 (H3N2). Treatment feral swine were intranasally inoculated with  $10^6$  TCID<sub>50</sub> A/Mallard/WI/A00661712/20092009 (aH3N2), meanwhile, control feral swine were intranasally inoculated with 1 mL PBS. Swine 38, swine 45 and swine 36 were necropsied at 5 DPI, swine 39, swine 41 and swine 42 were necropsied at 7 DPI. Sera were collected at 0, 10, 14, and 21 DPI, respectively. HI assay was conducted with 0.5% turkey red blood cell.

DPI	Treatment swine								Control swine			
	37	38	39	40	41	45	47	48	35	36	42	46
0	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
21	<10	- <sup>a</sup>	-	<10	-	-	<10	80	<10	-	-	<10

<sup>a</sup>:- samples were not available

**Table S7.** Viral titers in different tissues of feral swine. Treatment feral swine were intranasally inoculated with  $10^6$  TCID<sub>50</sub> A/Mallard/WI/A00661712/2009 (H3N2), meanwhile, control feral swine were intranasally inoculated with 1 mL PBS. Swine 38, swine 45, and swine 36 were necropsied at 5 DPI, swine 39, swine 41, and swine 42 were necropsied at 7 DPI. Turbinate, trachea and lung of these swine were collected and virus titer in these tissues were detected in SPF eggs. Virus titers are expressed as log<sub>10</sub>EID<sub>50</sub>/gram/mL.

Tissues	Treatment swine				Control swine	
	5 DPI		7 DPI		5 DPI	7 DPI
	38	45	39	41	36	42
Turbinate	2.5	ND	ND	ND	ND	ND
Trachea	2.5	1.333	ND	0.667*	ND	ND
Lung	2.5	3.5	ND	ND	ND	ND

\*Below the detection limit.

**Table S8.** Serological responses in feral swine inoculated with A/mallard/Ohio/648/2002 (H6N2). Sera were collected at 0, 5, 7, and 21 DPI, respectively. HI assay was conducted with 0.5% turkey red blood cell.

DPI	Treatment swine			
	116	117	121	122
0	<10	<10	<10	<10
21	20	80	20	40