nature portfolio

https://doi.org/10.1038/s41586-024-08246-7

Supplementary information

Transmission of a human isolate of clade 2.3.4.4b A(H5N1) virus in ferrets

In the format provided by the authors and unedited

Supplemental Table 1.

		Binding[a]				
Glycan Type	**Structure**	**A/Switzerland/9715293/2013 (H3)**	**A/Vietnam/1203/2004 (H5)**	**A/Astrakhan/3212/2020 (H5)**	**A/Sichuan/06681/2021 (H5)**	**A/Texas/37/2024 (H5)**
Sialic Acid						
1	Neu5Acα	nb	nb	nb	nb	nb
2	Neu5Acα	nb	nb	nb	nb	nb
3	Neu5Acβ	nb	nb	nb	nb	nb
α2-3 sialosides						
4	Neu5Acα2-3(6-O-Su)Galβ1-4(Fucα1-3)GlcNAcβ	nb	nb	+++	+++	+++
5	Neu5Acα2-3Galβ1-3(6OSO3)GalNAcα	nb	+++	+++	+++	+++
6	Neu5Acα2-3Galβ1-4(6OSO3)GlcNAcβ	nb	+++	+++	+++	+++
7	Neu5Acα2-3Galβ1-4(Fucα1-3)(6OSO3)GlcNAcβ	nb	+++	+++	+++	+++
8	Neu5Acα2-3Galβ1-3(6OSO3)GlcNAc	nb	+++	+++	+++	+++
9	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4)GlcNAcβ	nb	+++	+++	+++	+++
10	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcβ	nb	+++	+++	+++	+++
11	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ12Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	+++	+++	+++	+++
12	Neu5Acα2-3Galβ	nb	+++	+++	+++	+++
13	Neu5Acα2-3GalNAcα	nb	+++	+++	+++	+++
14	Neu5Acα2-3Galβ1-3GalNAcα	nb	+++	+++	+++	+++
15	Neu5Acα2-3Galβ1-3GlcNAcβ	nb	+++	+++	+++	+++
16	Neu5Acα2-3Galβ1-3GlcNAcβ	nb	+++	+++	+++	+++
17	Neu5Acα2-3Galβ1-4Glcβ	nb	+++	+++	+++	+++
18	Neu5Acα2-3Galβ1-4Glcβ	nb	+++	+++	+++	+++
19	Neu5Acα2-3Galβ1-4GlcNAcβ	nb	+++	+++	+++	+++
20	Neu5Acα2-3Galβ1-4GlcNAcβ	nb	+++	+++	+++	+++
21	Neu5Acα2-3GalNAcβ1-4GlcNAcβ	nb	+++	nb	nb	nb
22	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	nb	+++	+++	+++	+++

23	Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ	nb	+++	+++	+++	+++
24	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	nb	+++	+++	+++	+++
25	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ	nb	nb	nb	nb	nb
26	Neu5Acα2-3Galβ1-3GalNAcβ	nb	+++	+++	+++	++
27	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-3)GalNAcβ	nb	nb	nb	nb	nb
28	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ	nb	nb	+++	+++	+++
29	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ	nb	+	+++	+++	+++
30	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ	nb	nb	+++	+++	+++
31	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ	nb	nb	+++	+++	+++
32	Neu5Acα2-3-Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ	nb	nb	+++	+++	+++
33	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ	nb	+	+++	+++	+++
34	Neu5Aca2-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ	nb	nb	+++	+++	+++
35	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
36	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
37	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ	nb	nb	nb	nb	nb
38	Neu5Acα2-3(Galβ1-3GalNAcβ1-4)Galβ1-4Glcβ	nb	nb	nb	nb	nb
39	Neu5Acα2-3(Fucα1-2Galβ1-3GalNAcβ1-4)Galβ1-4Glcβ	nb	nb	nb	nb	nb
40	Neu5Acα2-3(Fucα1-2Galβ1-3GalNAcβ1-4)Galβ1-4Glcβ	nb	nb	nb	nb	nb
α2-6 sialosides						
41	Neu5Acα2-6Galβ1-4[6OSO3]GlcNAcβ	+++	nb	nb	nb	nb
42	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα16(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb

43	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb
44	NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ	+++	nb	nb	nb	nb
45	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb
46	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ(1-3)(Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)GalNAcα	+++	nb	nb	nb	nb
47	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ12Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb
48	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ12Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	+	nb	nb	nb	nb
49	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα16)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb
50	Neu5Acα2-6GalNAcα	nb	nb	nb	nb	nb
51	Neu5Acα2-6Galβ	nb	nb	nb	nb	nb
52	Neu5Acα2-6Galβ1-4Glcβ	+++	nb	nb	nb	nb
53	Neu5Acα2-6Galβ1-4GlcNAcβ	+++	nb	nb	nb	nb
54	Neu5Acα2-6Galβ1-4GlcNAcβ	+++	nb	nb	nb	nb
55	Neu5Acα2-6GalNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb
56	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	+++	nb	nb	nb	nb
57	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3GalNAcα	++	nb	nb	nb	nb
58	Neu5Aca2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	+++	nb	nb	nb	nb

59	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ	+++	nb	nb	nb	nb
60	Neu5Acα2-6(Galβ1-3)GlcNAcβ1-4Galβ1-4Glcβ	nb	nb	nb	nb	nb
61	Neu5Acα2-6(Galβ1-3)GalNAcα	nb	nb	nb	nb	nb
62	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAcα	nb	nb	nb	nb	nb
63	NeuAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6(Galβ(1-3)GalNAcα	+++	nb	nb	nb	nb
Mixed α2-3 and α2-6 biantennaries						
64	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb
65	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	+++	+++	+++	+++
66	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα	nb	+++	+++	+++	+++
67	Neu5Acα2-3(Neu5Acα2-6)GalNAcα	nb	nb	nb	nb	nb
N-Glycolylneuraminic acid glycans						
68	Neu5Gcα	nb	nb	nb	nb	nb
69	Neu5Gcα2-3Galβ1-3(Fucα1-4)GlcNAcβ	nb	nb	nb	nb	nb
70	Neu5Gcα2-3-Galβ1-3GlcNAcβ	nb	nb	nb	nb	nb
71	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ	nb	nb	+++	+++	+++
72	Neu5Gcα2-3Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
73	Neu5Gcα2-6GalNAcα	nb	nb	nb	nb	nb
74	Neu5Gcα2-6Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
α2-8-linked sialosides						
75	Neu5Acα2-8Neu5Acα	nb	nb	nb	nb	nb
76	Neu5Acα2-8Neu5Acα2-8Neu5Acα	nb	nb	nb	nb	nb
77	Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ	nb	nb	nb	nb	nb
78	Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ	nb	nb	nb	nb	nb
79	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ	nb	nb	nb	nb	nb

80	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ	nb	nb	nb	nb	nb
81	Neu5Acα2-8Neu5Acβ	nb	nb	nb	nb	nb
82	Neu5Acα2-8Neu5Acα2-8Neu5Acβ	nb	nb	nb	nb	nb
β2-6-linked and 9-*O*-acetylated sialic acids						
83	Neu5Acβ2-6GalNAcα	nb	nb	nb	nb	nb
84	Neu5Acβ2-6Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
85	Neu5Gcβ2-6Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
86	Galβ1-3(Neu5Acβ2-6)GalNAcα	nb	nb	nb	nb	nb
87	9NAcNeu5Acα	nb	nb	nb	nb	nb
88	9NAcNeu5Acα2-6Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
Asialoglycans						
89	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
90	Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ	nb	nb	nb	nb	nb
91	Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb	nb	nb	nb
92	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ	nb	nb	nb	nb	nb
93	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ	nb	nb	nb	nb	nb
94	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ	nb	nb	nb	nb	nb
95	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ	nb	nb	nb	nb	nb
96	Galβ1-3GalNAcα	nb	nb	nb	nb	nb

[a] Binding of recombinant HA to glycans was qualitatively estimated based on relative strength of the signal for the data shown in Figure 1; Fluorescence Intensity >4000 (+++), 2000-3999 (++), 1000-1999 (+), <1000 (nb; no binding).
Key: Neu5Ac = Sialic acid; Neu5Gc = N-glycolylneuraminic acid; OSO3 = sulfate; Gal = galactose; Fuc = fucose; Glc = D-glucose; GlcNAc = N-Acetyl-D-glucosamine; GalNAc = N-acetyl-D-galactosamine; Man = D-mannose; 9NAc = 9-*O*-acetyl.

Supplemental Table 2.

Glycan No.	Structure
3'-LN	Neu5Acα2-3Galβ1-4GlcNAcβ
3'-LNLN	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ
3'-LNLNLN	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ
Bi-3'-LN	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ
Bi-3'-LNLN	Neu5Acα2-3-Galβ1-4-GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ
Bi-3'-LNLNLN	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ
Bi-3'-LNLNLNLN	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4)GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ
6'-LN	Neu5Acα2-6Galβ1-4GlcNAcβ
6'-LNLN	Neu5Aca2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ
6'-LNLNLN	Neu5Aca2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ
Bi-6'-LN	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ
Bi-6'-LNLN	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]Manβ1-4GlcNAcβ1-4GlcNAcβ
Bi-6'-LNLNLN	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ
Bi-6'-LNLNLNLN	Neu5Acα2-6 Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ

Supplemental Table 3.

SRA Accession	Ferret #	Day p.i./p.c.	Specimen type	PB2	PB1	PB1-F2	PA	HA (H5 numbering)	NA	NS
			Inoculum				432I/V (95/5)	335K/I (76.5/23.5)	408V/A (85.2/14.8)	164P/S (90/10)
							659L/F (90/10)			
SRR29561775	DC_Inoculated_ferret1	1	Nasal wash					X335I (99.6)		
SRR29561774	DC_Inoculated_ferret1	3	Nasal wash	K702Q (26.4)				X335I (99.4)		
SRR29561772	DC_Inoculated_ferret2	1	Nasal wash					X335I (99.5)		
SRR29561819	DC_Inoculated_ferret2	3	Nasal wash	A707P (22.9)			L649Q (27.8)	X335I (98.8)		
							S652A (23.6)			
SRR29561817	DC_Inoculated_ferret3	1	Nasal wash					X335I (100)		
SRR29561814		3	Lung	K702Q (20.2)				A140S (24.7)		
								N165K (20.9)		
								X335I (98.7)		
SRR29561813			Nasal turbinates				L649Q (20.8)	X335I (95.9)		
SRR29561810			Trachea				L649Q (24.7)	X335I (99.6))		
SRR29561811			Soft palate					X335I (98.7)		
SRR29561816			Nasal wash					X335I (97.3)		
SRR29561815			Ethmoid turbinate					X335I (98.2)		
SRR29561773	DC_contact_ferret1	3	Nasal wash				D216Y (30.3)	X335I (99.6)		
							L683F			

							(99.2)			
SRR29561818	DC_contact_ferret2	3	Nasal wash					X335I (99.7)		
SRR29561807	RD_Inoculated_ferret1	1	Nasal wash					X335I (98.9)		
SRR29561806	RD_Inoculated_ferret2	1	Nasal wash					X335I (99.6)		
SRR29561805		3	Nasal wash	K702Q (34.1)				X335I (98.7)		
SRR29561803	RD_Inoculated_ferret3	1	Nasal wash					X335I (100)		
SRR29561786	RD_Inoculated_ferret4	1	Nasal wash		P72S (20.3)	C42S (61.5)				
SRR29561785		2	Nasal wash		P68L (35.9)	Q37S (35.9)		X335I (98.5)		
					I69T (36.4)	M39L (40.2)				
					D70P (37.5)					
SRR29561783	RD_Inoculated_ferret5	1	Nasal wash					X335I (98.8)		
SRR29561782		2	Nasal wash					X335I (99.7)		
SRR29561779	RD_Inoculated_ferret6	2	Nasal wash				L655F (21.6)	X335I (98.9)		
SRR29561804	RD_contact_ferret2	6	Nasal wash	N102K (37.4)	P72T (24.2)	H41Q (28.6)		X335I (96.8)		
				T105P/R (49/21)	L73M (28.6)					
				N659 (63.3)	F466P (32.5)					
					S482P (30.6)					
					F490L (33.3)					
					Y499H (34.8)					
					V502A (33.8)					
SRR29561784	RD_contact_ferret4	5	Nasal wash					R72K (48.8)		
								X335I (98.9)		

SRR29561781	RD_contact_ferret5	3	Nasal wash					X335I (100)		P164S (99.3)
SRR29561780		5	Nasal wash					X335I (98.5)		P164S (99.6)
SRR29561778	RD_contact_ferret6	3	Nasal wash						I443T (61.3)	
SRR29561777		4	Nasal wash						I443T (70.8)	
SRR29561787	Inoculated ferret1	3	Soft palate	K702Q (20.7)				X335I (99.8)		
SRR29561809			Nasal turbinates				L649Q (42.9)	X335I (96.5)		
SRR29561776			Trachea	K702Q (34)			L649Q (41.6)	E185K (23.6)		
								X335I (98.2)		
SRR29561821			Ethmoid turbinates	N137R (23)						
SRR29561798			Nasal wash					X335I (99.1)		
SRR29561799	Inoculated feret2	3	Nasal wash	K702Q (24.8)				X335I (98.1)		
SRR29561800			Nasal turbinates	K702Q (20.1)	A652G (32.6)		S648C (20.1)	X335I (97.0)		
							L649Q (53.9)			
SRR29561797			Soft palate	K702Q (31.3)			H41Q (24.6)	X335I (98.3)		
							L649Q (41.3)			
SRR29561796			Trachea	K702Q (28)				X335I (98.8)		
SRR29561801			Lung	K702Q (29.9)	T677I (21.2)		S632F (20)	X335I (95.9)		
SRR29561802			Ethmoid turbinates					X335I (91.0)		
SRR29561795	F_inoculated_ferret1	1	Nasal wash					X335I (99.5)		
SRR29561794		2	Nasal wash					X335I (99.8)		
SRR29561791	F_inoculated_ferret2	1	Nasal wash					X335I (97.7)		
SRR29561790		2	Nasal wash					X335I (99.8)		

SRR29561789	F_inoculated_ferret3	1	Nasal wash					X335I (99.2)		
SRR29561788		2	Nasal wash		T677I (23)			X335I (99.4)		
SRR29561793	F_contact_ferret1	1	Nasal wash					X335I (99.3)		
SRR29561792		7	Nasal wash					K234N (100)		
								X335I (100)		

Supplemental Table 4.

		Kruskal-Wallis chi-squared	df	p-value	Significant p < 0.05	Performed Dunn's Test
day 1 nasal washes	RNA copies	9.1358	3	**0.02754**	**Yes**	Yes (see below)
day 1 air samples	RNA copies	7.8283	3	**0.0497**	**Yes**	Yes (see below)
day 1 nasal washes	PFU	8.4984	3	**0.03676**	**Yes**	Yes (see below)
day 1 air samples	PFU	15.615	3	**0.00136**	**Yes**	Yes (see below)
day 2 nasal washes	RNA copies	4.0892	3	0.252	No	No
day 2 air samples	RNA copies	15.222	3	**0.001637**	**Yes**	Yes (see below)
day 2 nasal washes	PFU	3.4618	3	0.3257	No	No
day 2 air samples	PFU	10.145	3	**0.01737**	**Yes**	Yes (see below)

Dunn's post hoc pairwise comparison tests

		Virus Pairwise Comparison		Z	P.unadj	P.adj	Significant P.adj < 0.05
day 1 nasal washes	RNA copies	Chile/25945	MN/45	2.5311394	0.01136927	0.06821559	No
day 1 nasal washes	RNA copies	Chile/25945	NE/14	1.8786076	0.06029809	0.36178852	No
day 1 nasal washes	RNA copies	MN/45	NE/14	0.6166667	0.53745461	1	No
day 1 nasal washes	RNA copies	Chile/25945	TX/37	0.2738613	0.78419123	1	No
day 1 nasal washes	RNA copies	MN/45	TX/37	2.2249532	0.02608438	0.15650629	No
day 1 nasal washes	RNA copies	NE/14	TX/37	1.5858376	0.11277615	0.6766569	No

		Virus Pairwise Comparison		Z	P.unadj	P.adj	Significant P.adj < 0.05
day 1 air samples	RNA copies	Chile/25945	MN/45	2.390348	0.01683242	0.1009945	No
day 1 air samples	RNA copies	Chile/25945	NE/14	1.1130079	0.26570506	1	No
day 1 air samples	RNA copies	MN/45	NE/14	1.3350661	0.18185472	1	No
day 1 air samples	RNA copies	Chile/25945	TX/37	0.1311652	0.89564465	1	No
day 1 air samples	RNA copies	MN/45	TX/37	2.2437009	0.02485165	0.1491099	No
day 1 air samples	RNA copies	NE/14	TX/37	0.9727864	0.33065948	1	No

		Virus Pairwise Comparison		Z	P.unadj	P.adj	Significant P.adj < 0.05
day 1 nasal washes	PFU	Chile/25945	MN/45	2.2144489	0.02679792	0.1607875	No
day 1 nasal washes	PFU	Chile/25945	NE/14	2.4262083	0.01525751	0.09154507	No
day 1 nasal washes	PFU	MN/45	NE/14	0.351573	0.72515849	1	No
day 1 nasal washes	PFU	Chile/25945	TX/37	0.5501842	0.58219301	1	No
day 1 nasal washes	PFU	MN/45	TX/37	1.5993242	0.10974859	0.65849153	No
day 1 nasal washes	PFU	NE/14	TX/37	1.8380366	0.06605702	0.39634211	No

		Virus Pairwise Comparison		Z	P.unadj	P.adj	Significant P.adj < 0.05
day 1 air samples	PFU	Chile/25945	MN/45	2.4009681	0.016351763	0.09811058	No
day 1 air samples	PFU	**Chile/25945**	**NE/14**	3.4849566	0.000492217	**0.002953302**	**Yes**
day 1 air samples	PFU	MN/45	NE/14	1.3539546	0.175750849	1	No

day 1 air samples	PFU	Chile/25945	TX/37	0.5426279	0.587386008	1	No
day 1 air samples	PFU	MN/45	TX/37	1.7942916	0.072766638	0.436599825	No
day 1 air samples	PFU	**NE/14**	**TX/37**	2.9048629	0.00367414	**0.022044839**	**Yes**
		Virus Pairwise Comparison		**Z**	**P.unadj**	**P.adj**	**Significant P.adj < 0.05**
day 2 air samples	RNA copies	**Chile/25945**	**MN/45**	3.4168778	0.000633437	**0.003800623**	**Yes**
day 2 air samples	RNA copies	**Chile/25945**	**NE/14**	2.6729716	0.007518261	**0.045109567**	**Yes**
day 2 air samples	RNA copies	MN/45	NE/14	0.6765133	0.498714802	1	No
day 2 air samples	RNA copies	Chile/25945	TX/37	0.7541997	0.450729311	1	No
day 2 air samples	RNA copies	MN/45	TX/37	2.5736569	0.010063002	0.060378011	No
day 2 air samples	RNA copies	NE/14	TX/37	1.8666982	0.061943744	0.371662462	No
		Virus Pairwise Comparison		**Z**	**P.unadj**	**P.adj**	**Significant P.adj < 0.05**
day 2 air samples	PFU	**Chile/25945**	**MN/45**	2.7691937	0.005619522	**0.03371713**	**Yes**
day 2 air samples	PFU	**Chile/25945**	**NE/14**	2.8274167	0.004692523	**0.02815514**	**Yes**
day 2 air samples	PFU	MN/45	NE/14	0.2044393	0.838010255	1	No
day 2 air samples	PFU	Chile/25945	TX/37	2.3732225	0.017633636	0.10580182	No
day 2 air samples	PFU	MN/45	TX/37	0.1158502	0.907771258	1	No
day 2 air samples	PFU	NE/14	TX/37	0.290335	0.771559932	1	No

Summary statistics of the Kruskal Wallis and Dunn's post hoc tests examining the differences in viral RNA copy and PFU titers in nasal wash and air samples collected on day 1 and day 2 post inoculation of ferrets. Kruskal Wallis shows the comparisons, the test chi-squared statistic, degrees of freedom (df) and the p-value. Dunn's test shows pairwise comparisons for statistically significant Kruskal Wallis tests. The comparisons are shown along with the Z test statistic, unadjusted p-value (P.unadj) and Bonferroni multiple comparisons adjusted p-value (P.adj). Statistically significant values ($p < 0.05$) are highlighted in green.

Supplemental Table 5.

		PB2						PB1				PA					HA†		NS		
	GISAID Isolate ID	**340**	**389**	**591**	**627**	**631**	**701**	**384**	**392**	**581**	**741**	**13**	**142**	**219**	**497**	**613**	**195**	**320**	**40**	**77**	**229**
A/Texas/37/2024*	EPI_ISL_19027114	R	R	Q	K	M	D	S	V	E	A	I	E	L	K	E	I	S	R	L	E
A/dairy_cow/New_Mexico/A240920343-93/2024*	EPI_ISL_19091702	R	R	Q	E	L	D	P	I	E	A	V	K	I	K	K	I	N	Q	R	K
A/Michigan/90/2024*	EPI_ISL_19162802	R	R	Q	E	L	D	S	I	K	V	I	K	I	R	E	I	S	Q	L	E
A/Chile/25945/2023#	EPI_ISL_17468386	K	K	K	E	M	N	S	I	E	A	I	K	L	K	E	T	S	Q	L	E

* Isolates associated with outbreaks on dairy farms; all amino acid differences across all proteins are shown.
# Selected amino acids are shown. Total number of amino acid differences between Chile/25945 and TX/37: 16 in PB2, 12 in PB1, 8 in PA, 6 in NS, 5 in NP, 2 in NA, 3 in M1 and M2, 1 in HA
† H5 numbering