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Table 1. Binding of A(H7N9) viruses to individual glycan structures.

#	Structure[a]	Anhui1	Shanghai1
1	Neu5Acα	nb	nb
2	Neu5Acα	++	nb
3	Neu5Acβ	nb	nb
4	Neu5Acα2-3(6-O-Su)Galβ1-4GlcNAcβ	nb	nb
5	Neu5Acα2-3Galβ1-3[6OSO3]GalNAcα	nb	nb
6	Neu5Acα2-3Galβ1-4[6OSO3]GlcNAcβ	++	++
7	Neu5Acα2-3Galβ1-4(Fucα1-3)[6OSO3]GlcNAcβ-propyl-NH2	+++	++
8	Neu5Acα2-3Galβ1-3[6OSO3]GlcNAc-propyl-NH2	++	+
9	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4)GlcNAcβ	+	++
10	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcα	+	++
11	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	++	+
12	Neu5Acα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[Neu5Acα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ	+	+
13	Neu5Acα2-3Galβ	nb	+++
14	Neu5Acα2-3Galβ1-3GalNAcα	+	+++
15	Neu5Acα2-3Galβ1-3GlcNAcβ	+	++
16	Neu5Acα2-3Galβ1-3GlcNAcβ	++	+++
17	Neu5Acα2-3Galβ1-4Glcβ	+	nb
18	Neu5Acα2-3Galβ1-4Glcβ	nb	+++
19	Neu5Acα2-3Galβ1-4GlcNAcβ	nb	nb
20	Neu5Acα2-3Galβ1-4GlcNAcβ	nb	+++
21	Neu5Acα2-3GalNAcβ1-4GlcNAcβ	nb	+
22	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	+	nb
23	Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ	nb	++
24	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	++	nb
25	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ	++	+++
26	Neu5Acα2-3Galβ1-3GalNAcα	nb	+++
27	Galβ1-3(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6)GalNAcα	++	+
28	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ	nb	+++
29	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ	nb	+++
30	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ	+	+++
31	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ	+	+++
32	Neu5Acα2-3Galβ1-3[Fucα1-4]GlcNAcβ1-3Galβ1-4[Fucα1-3]GlcNAcβ	+	+++
33	Neu5Acα2-3Galβ1-3[Fucα1-3]GlcNAcβ1-3Galβ1-4[Fucα1-3]GlcNAcβ	+	+++
34	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ	+	+++
35	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ	nb	nb
36	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ	+	+
37	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ	nb	nb
38	Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ	nb	nb
39	Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ	nb	nb
40	Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ	nb	nb
41	Neu5Acα2-6Galβ1-4[6OSO3]GlcNAcβ	+	nb

42	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb
43	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	++	nb
44	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]Manβ1-4GlcNAcβ1-4GlcNAcβ	+++	nb
45	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ	+++	++
46	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6]GalNAca	++	nb
47	Neu5Acα2-6Galβ1-4GlcNAcβ1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-6]GalNAca	++	nb
48	Neu5Acα2-6GalNAcα	+	+
49	Neu5Acα2-6Galβ	nb	nb
50	Neu5Acα2-6Galβ1-4Glcβ	nb	nb
51	Neu5Acα2-6Galβ1-4Glcβ	+	nb
52	Neu5Acα2-6Galβ1-4GlcNAcβ	nb	nb
53	Neu5Acα2-6Galβ1-4GlcNAcβ	nb	nb
54	Neu5Acα2-6GalNAcβ1-4GlcNAcβ	+	nb
55	Neu5Acα2-6Galβ1-4GlcNAcβ1-3GalNAcα	nb	nb
56	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	+	nb
57	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3GalNAcα	nb	nb
58	Neu5Aca2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	++	nb
59	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ	+	nb
60	Galβ1-3(Neu5Acα2-6)GlcNAcβ1-4Galβ1-4Glcβ-Sp10	+++	+
61	Neu5Acα2-6[Galβ1-3]GalNAca	nb	nb
62	Neu5Acα2-6Galβ1-4GlcNAcβ1-6[Galβ1-3]GalNAca	+	+
63	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6[Galβ1-3]GalNAca	+	nb
64	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	+	nb
65	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ	++	++
66	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα	++	+++
67	Neu5Acα2-3(Neu5Acα2-6)GalNAcα	+	nb
68	Neu5Gcα	+	nb
69	Neu5Gcα2-3Galβ1-3(Fucα1-4)GlcNAcβ	nb	nb
70	Neu5Gcα2-3Galβ1-3GlcNAcβ	nb	+
71	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ	nb	+++
72	Neu5Gcα2-3Galβ1-4GlcNAcβ	nb	++
73	Neu5Gcα2-6GalNAcα	nb	nb
74	Neu5Gcα2-6Galβ1-4GlcNAcβ	nb	nb
75	Neu5Acα2-8Neu5Acα	nb	nb
76	Neu5Acα2-8Neu5Acα2-8Neu5Acα	nb	nb
77	Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ	nb	nb
78	Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ	nb	nb
79	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ	+	nb
80	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ	+	nb
81	Neu5Acα2-8Neu5Acβ-Sp17	+	nb
82	Neu5Acα2-8Neu5Acα2-8Neu5Acβ	nb	nb
83	Neu5Acβ2-6GalNAcα	nb	nb

84	Neu5Acβ2-6Galβ1-4GlcNAcβ	+	++
85	Neu5Gcβ2-6Galβ1-4GlcNAc	nb	nb
86	Galβ1-3(Neu5Acβ2-6)GalNAcα	nb	nb
87	[9NAc]Neu5Acα	nb	nb
88	[9NAc]Neu5Acα2-6Galβ1-4GlcNAcβ	nb	nb
89	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ	nb	nb
90	Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ	+	nb
91	Galβ1-4GlcNAcβ1-2Manα1-3[Galβ1-4GlcNAcβ1-2Manα1-6]Manβ1-4GlcNAcβ1-4GlcNAcβ	nb	nb
92	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ	nb	nb
93	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ	nb	nb
94	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ	+	nb
95	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ	++	nb
96	Galβ1-3GalNAcα	nb	nb

[a]Glycan microarray differences between Anhui1 and Shanghai1. The color coding in the left hand column reflects the same coloring scheme used in Figure 1. Significant binding of samples to glycans were qualitatively estimated based on relative strength of the signal for the data shown in the figure; fluorescence intensity strong (+++), medium (++), weak (+), no binding (nb). Different categories of glycans on the array are color-coded in column 1 as follows: No color, sialic acid; blue, α2-3 sialosides; red, α2-6 sialosides, violet, mixed α2-3/α2-6 biantennaries; green, N-glycolylneuraminic acid-containing glycans; brown, α2-8 linked sialosides; pink, β2-6 linked and 9-O-acetylated sialic acids; grey, asialo glycans.

Table 2. Complete blood counts of H7N9 and H3N2 influenza virus-infected ferrets.

		Blood count ± SD (K/μl)[b]		
Cell type[a]	Day p.i.	Texas/50	Anhui/1	Shanghai/1
WBC	0	6.84 ± 1.6	6.82 ± 1.7	8.93 ± 4.0
	3	7.19 ± 1.3	7.52 ± 2.1	6.67 ± 2.1
	7	8.07 ± 2.8	8.93 ± 2.1	9.02 ± 2.9
LY	0	4.32 ± 1.0 (63.9%)	4.33 ± 1.3 (64.5%)	6.43 ± 3.2 (71.0%)
	3	4.13 ± 0.8 (51.0%)	3.12 ± 1.1 (41.4%)	3.67 ± 2.1 (53.4%)
	7	4.51 ± 2.5 (52.5%)	3.85 ± 1.3 (43.1%)	5.26 ± 2.3 (56.9%)
NE	0	1.97 ± 0.9 (27.9%)	1.75 ± 0.6 (26.0%)	1.68 ± 0.7 (19.8%)
	3	2.36 ± 1.4 (31.2%)	3.21 ± 1.2 (42.9%)	1.84 ± 0.8 (28.6%)
	7	2.64 ± 0.4 (35.5%)	3.40 ± 1.3 (38.4%)	2.13 ± 0.8 (25.3%)
MO	0	0.44 ± 0.2 (6.53%)	0.56 ± 0.3 (8.22%)	0.66 ± 0.2 (7.93%)
	3	0.56 ± 0.2 (8.28%)	0.87 ± 0.5 (12.9%)	1.11 ± 0.6 (16.8%)
	7	0.75 ± 0.1 (10.1%)	1.54 ± 0.7 (16.7%)	1.48 ± 0.6 (16.6%)
EO	0	0.11 ± 0.1 (1.49%)	0.11 ± 0.1 (1.69%)	0.12 ± 0.1 (1.03%)
	3	0.14 ± 0.1 (2.2%)	0.17 ± 0.2 (2.12%)	0.07 ± 0.1 (1.04%)
	7	0.16 ± 0.1 (1.94%)	0.13 ± 0.1 (1.55%)	0.02 ± 0.1 (1.0%)
BA	0	0.01 ± 0.1 (0.16%)	0.01 ± 0.1 (0.20%)	0.03 ± 0.1 (0.29%)
	3	0.01 ± 0.1 (0.08%)	0.06 ± 0.1 (0.56%)	0.01 ± 0.1 (0.22%)
	7	0.01 ± 0.1 (0.10%)	0.01 ± 0.1 (0.18%)	0.01 ± 0.1 (0.22%)
PLT	0	472 ± 146	435 ± 164	455 ± 102
	3	404 ± 115	299 ± 75	309 ± 62
	7	619 ± 103	708 ± 163	684 ± 83

[a]WBC, total white blood cells; LY, total lymphocytes; NE, total neutrophils; MO, total monocytes; EO, total eosinophils; BA, total basophils; PLT, total platelets.
[b]Values are mean total count for each cell type ± standard deviation of 6-11 ferrets; the percentage of each cell subset among all circulating white blood cells is denoted in parentheses where appropriate.

Table 3. Replication of H7 influenza viruses following ocular inoculation in mice.

		Virus titer[a]		
Tissue	Day p.i.	Shv/Egypt/07	Anhui/1	Shanghai/1
Eye	3	2.3 (1/3)	<10	1.3 (1/3)
Nose	3	<10	3.3 ± 1.0 (2/3)	3.2 ± 0.2 (2/3)
Lung	3	<10	<10	<10
Eye	6	<10	<10	1.4 ± 0.1 (2/3)
Nose	6	2.5 ± 1.5 (2/3)	3.2 ± 0.5	2.0 ± 0.7 (2/3)
Lung	6	<10	<10	1.5 (1/3)

[a]Mean virus titers in mice inoculated with 10^6 PFU/5μl of virus following corneal scarification. Virus titers are expressed as the mean $\log_{10}$ PFU ± standard deviation among mice with positive virus detection (data are representative of all mice examined, unless denoted in parentheses, in which the number of mice with positive virus detection/total number of mice in the group are indicated). The limit of virus detection was 10 PFU.

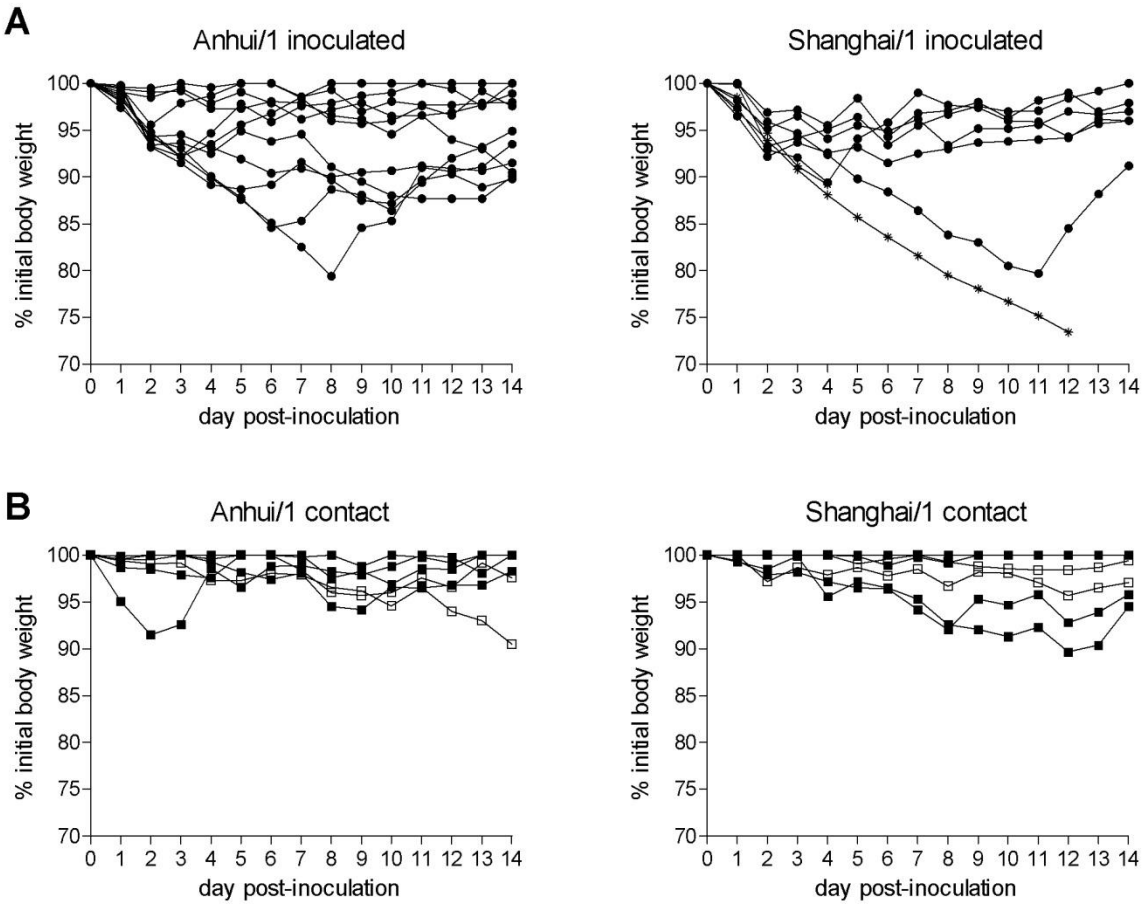

Figure 1. Morbidity following H7N9 virus infection in ferrets. Ferrets were intranasally inoculated with 10^6 PFU of Anhui/1 or Shanghai/1 (A) virus, and monitored daily for morbidity (as measured by weight loss) and mortality (filled circles). Any ferret which exhibited severe lethargy or lost >25% initial body weight was euthanized (denoted by *). A naïve ferret was placed in the same cage as each inoculated ferret 24 hours p.i. (DC transmission, filled squares) or in an adjacent cage with perforated side-walls (RD transmission, open squares) and were monitored daily for morbidity and mortality (B). Results from individual ferrets with positive virus isolation from nasal washes are presented.

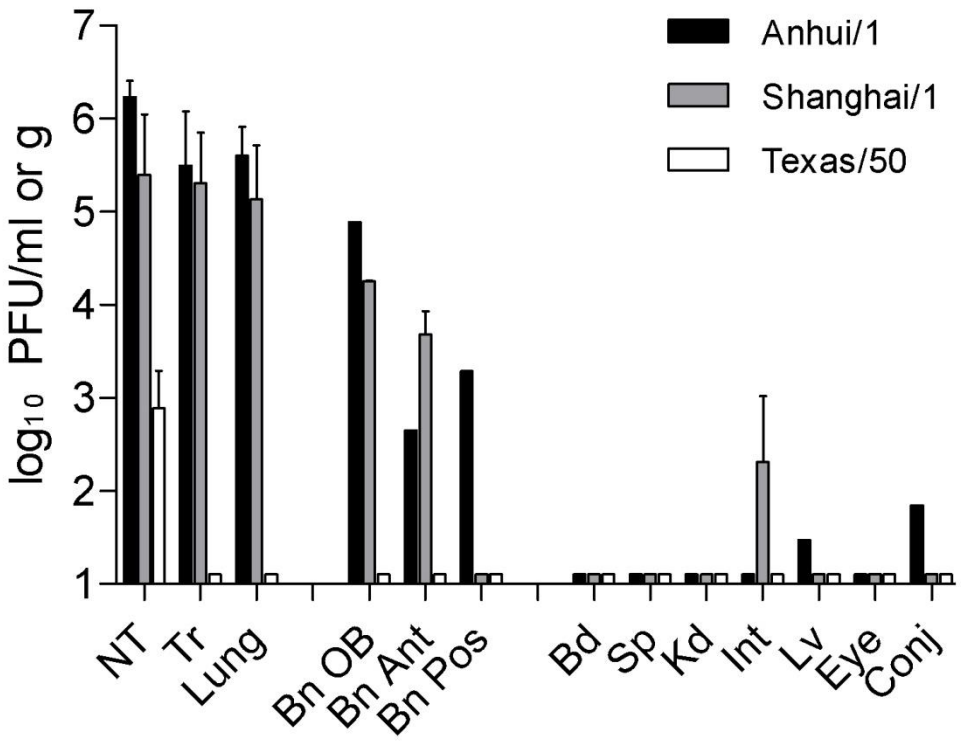

Figure 2. Detection of H7N9 and H3N2 viruses in ferret tissues. Three ferrets were intranasally inoculated with 10^6 PFU of Anhui/1 (H7N9), Shanghai/1 (H7N9), or Texas/50 (H3N2) virus. Tissues were collected 3 days p.i. for determination of virus titer. NT, nasal turbinates; Tr, trachea; Bn OB, olfactory bulb; Bn Ant, anterior brain; Bn Pos, posterior brain; Bd, blood; Sp, spleen; Kd, kidney; Int, intestine (pooled ducdenum, jejuno-ileal loop, and descending colon); Lv, liver; Eye, pooled right and left eyes; Conj, pooled right and left conjunctiva. Mean virus titers of positive samples only are presented as $\log_{10}$ PFU/g plus standard deviation for all tissues except NT, Bd, Eye, Conj which are expressed as PFU/ml. Anhui/1 brain, liver, and conjunctivitis titers are reflective of 1/3 positive ferrets; Shanghai/1 brain titers are reflective of 2/3 positive ferrets; Texas/50 nasal turbinate titers are reflective of 2/3 positive ferrets. The limit of detection was 10 PFU.

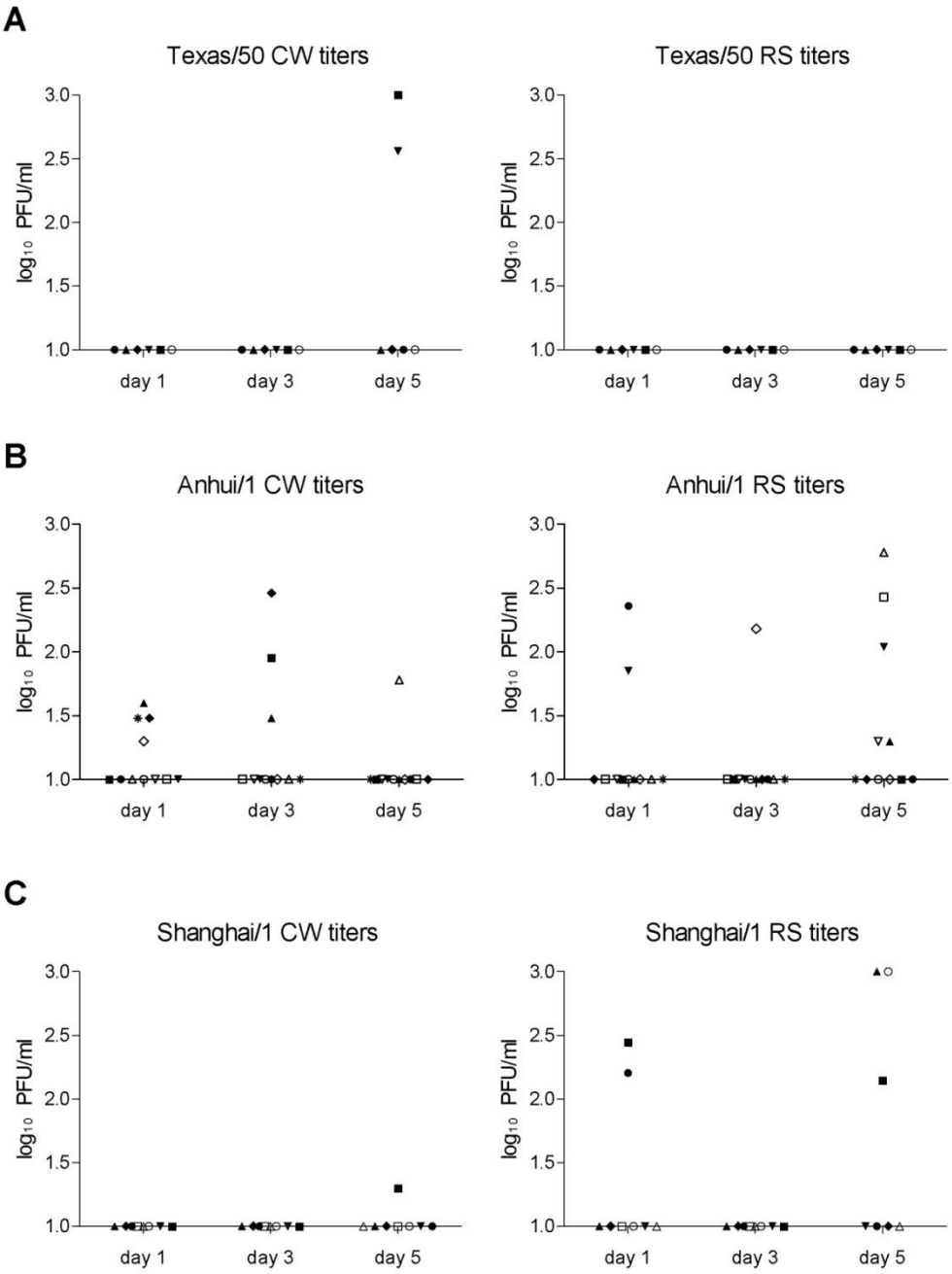

Figure 3. Comparison of H7N9 and H3N2 virus recovery in conjunctival wash and rectal swab samples in ferrets. Ferrets were inoculated with 10^6 PFU of Texas/50 (H3N2, n=6) (A), Anhui/1 (H7N9, n=11) (B) or Shanghai/1 (H7N9, n=8) (C) virus. Viral titers were measured in conjunctival washes (CW) or rectal swabs (RS) collected on indicated days p.i. Results from individual ferrets are expressed as $\log_{10}$ PFU/ml. The limit of virus detection was 10 PFU.

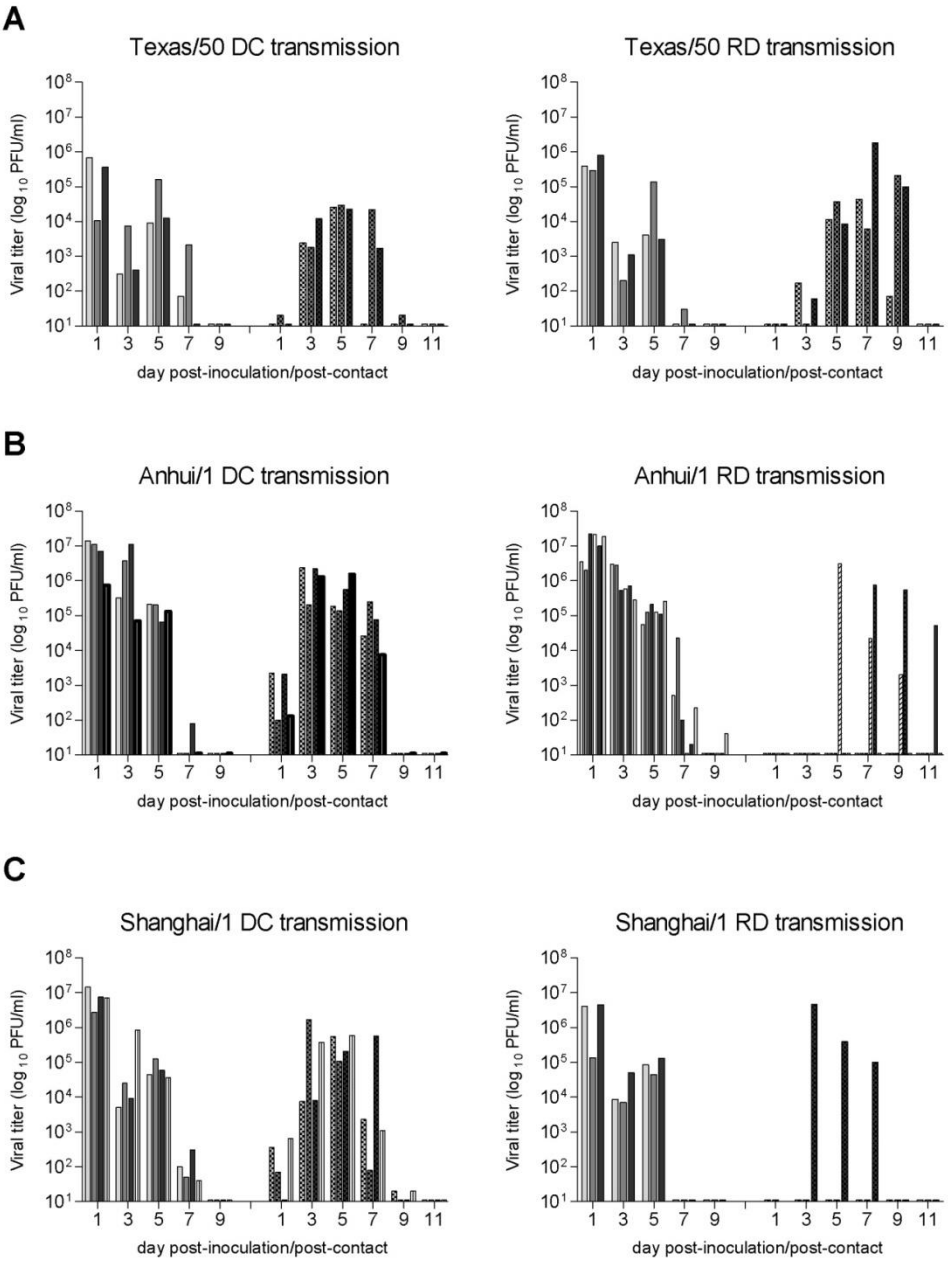

Figure 4. Transmissibility of H7N9 and H3N2 viruses in the ferret model. Groups of ferrets (6 to 10) were intranasally inoculated with 10^6 PFU of Texas/50 (A), Anhui/1 (B) virus, or Shanghai/1 (C) virus, respectively. Nasal washes were collected from each ferret on indicated days p.i. (Left). A naïve ferret was placed in the same cage as each inoculated ferret 24 hours p.i. (DC transmission) or in an adjacent cage with perforated side-walls (RD transmission) and nasal washes were collected from each contact ferret on indicated days p.c. (Right). Results from individual ferrets are presented. The limit of virus detection was 10 PFU.

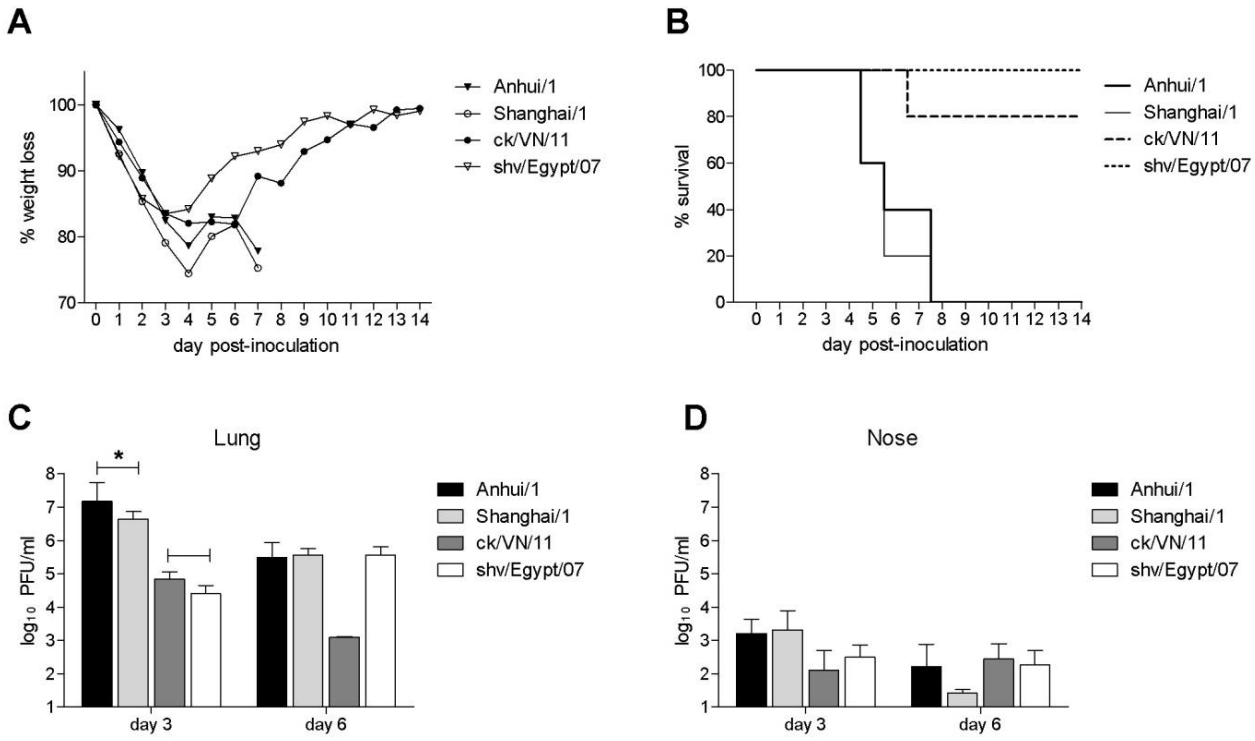

Figure 5. Disease outcome and replication following H7N9 and H9N2 virus infection in mice. Groups of five mice were intranasally inoculated with 10^6 PFU of Anhui/1 (H7N9), Shanghai/1 (H7N9), shv/Egypt/07 (H7N9), or ck/VN/11 (H9N2) virus and monitored daily for morbidity (as measured by weight loss), (A) and mortality (B). Any mouse which lost >25% initial body weight was euthanized. Lungs (C) and noses (D) were collected days 3 and 6 p.i. from three mice per group for determination of viral titer. Titers are expressed as mean $\log_{10}$ PFU/ml plus standard deviation. The limit of detection was 10 PFU. *, $p<0.05$ by one-way ANOVA with a Bonferroni post-test.

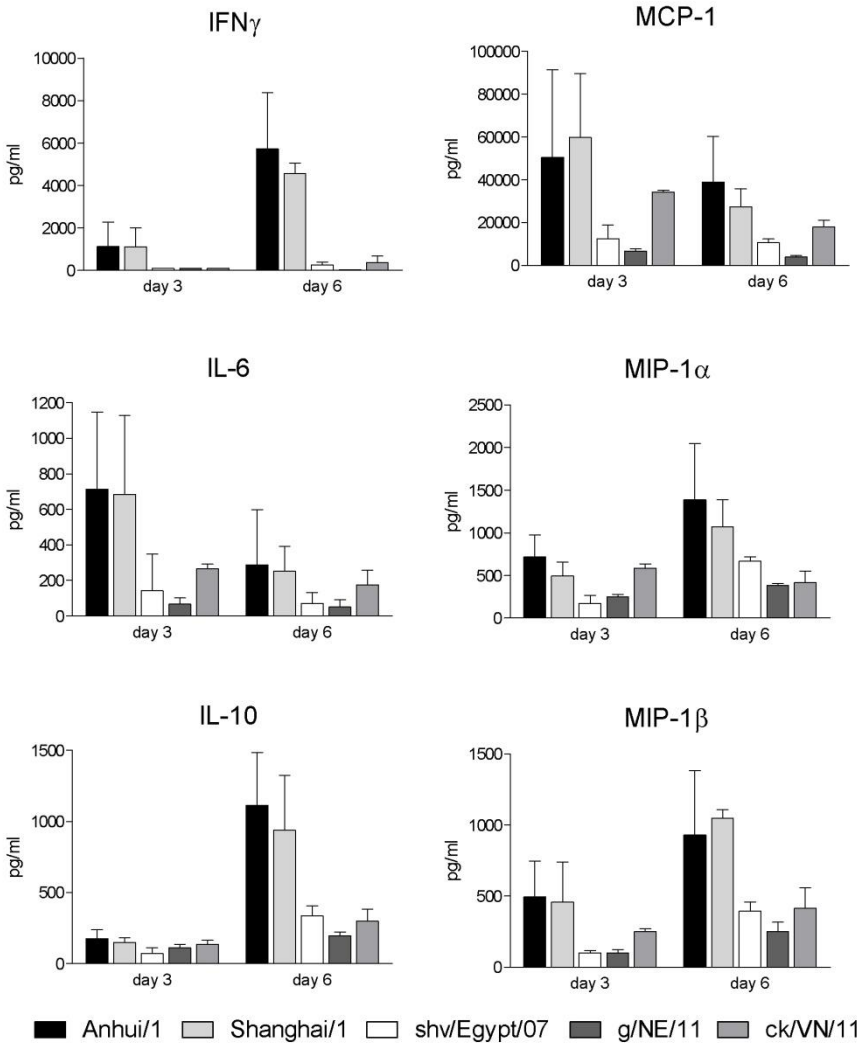

Figure 6. Cytokine and chemokine production in influenza virus infected mouse lungs. Groups of three mice were intranasally inoculated with influenza virus, and lungs were removed at the indicated days p.i. and frozen at -70°C until processed. Clarified cell lysates from lungs homogenized in 1ml cold PBS were analyzed with the BioPlex Mouse Cytokine 23-Plex panel (BioRad). The constitutive cytokine levels present in the lung were determined by harvesting lungs of mice inoculated with PBS as a mock control. The mean cytokine levels plus standard deviation are shown. Baseline levels in mock: IL-6 and IFNγ, undetectable; IL-10, 65.7pg; MCP-1, 987.8pg; MIP-1α, 98.9pg; MIP-1β, 472pg.