

Group	Ferret #	Primary influenza strain	HA titer to primary strain	Second influenza strain	Length of time between infections	DPI after last strain inoculation	Viral Titer of last strain
Naïve	1	NA	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA	NA
	4417	NA	NA	NA	NA	NA	NA
	4420	NA	NA	NA	NA	NA	NA
H1N1	472	CA/09	NA	NA	NA	2	
	490	CA/09	NA	NA	NA	2	
	496	CA/09	NA	NA	NA	2	
	512	CA/09	NA	NA	NA	2	
	491	CA/09	NA	NA	NA	3	
	492	CA/09	NA	NA	NA	3	
	493	CA/09	NA	NA	NA	3	
	504	CA/09	NA	NA	NA	3	
	509	CA/09	NA	NA	NA	5	
	510	CA/09	NA	NA	NA	5	
	519	CA/09	NA	NA	NA	5	
	520	CA/09	NA	NA	NA	5	
	476	CA/09	NA	NA	NA	7	
	478	CA/09	NA	NA	NA	7	
	494	CA/09	NA	NA	NA	7	
	495	CA/09	NA	NA	NA	7	
	480	CA/09	NA	NA	NA	14	
	482	CA/09	NA	NA	NA	14	
	488	CA/09	NA	NA	NA	14	
	489	CA/09	NA	NA	NA	14	

Group	Ferret #	Primary influenza strain	HA titer to primary strain	Length of time between infections	Second influenza strain	DPI after last strain inoculation
H5N1	807	VN/04	NA	NA	NA	7
	793	VN/04	NA	NA	NA	6
	801	VN/04	NA	NA	NA	7
	541	VN/04	NA	NA	NA	6
	542	VN/04	NA	NA	NA	6
	539	VN/04	NA	NA	NA	7
	544	VN/04	NA	NA	NA	7
	285	VN/04	NA	NA	NA	2
	284	VN/04	NA	NA	NA	4
	806	VN/04	NA	NA	NA	4
	796	VN/04	NA	NA	NA	5
	797	VN/04	NA	NA	NA	5
	791	VN/04	NA	NA	NA	6
H1N1/ H5N1	754	Mex/09			VN/04	18
	74	Mex/09			VN/04	18
	376	Mex/09			VN/04	18
	764	Mex/09			VN/04	18
	557	Mex/09			VN/04	18
	646	Mex/09			VN/04	18
	56	Mex/09			VN/04	18
	779	Mex/09			VN/04	18
	560	Mex/09			VN/04	18
	718	Mex/09	1:160	>3 mo	VN/04	2
	785	Mex/09	1:640	>3 mo	VN/04	2
	605	Mex/09	1:1280	3 mo	VN/04	2

	615	Mex/09	1:1280	>3 mo	VN/04	4
	602	Mex/09	1:640	3 mo	VN/04	4
	601	Mex/09	1:640	3 mo	VN/04	4
	610	Mex/09	1:320	>3 mo	VN/04	6
	614	Mex/09	1:320	>3 mo	VN/04	6
	603	Mex/09	1:640	3 mo	VN/04	6
	612	Mex/09	1:320	>3 mo	VN/04	18
	611	Mex/09	1:640	>3 mo	VN/04	18
	290	Mex/09	1:2560	1.6 mo	VN/04	18
	287	Mex/09	1:2560	1.6 mo	VN/04	18
	616	Mex/09	1:2560	2 mo	VN/04	18
H3N2/ H5N1	299	Perth/09			VN/04	7
	3291	Vic/11	1:80	>3 mo	VN/04	2
	3290	Vic/11	1:80	>3 mo	VN/04	2
	3287	Vic/11	1:160	>3 mo	VN/04	2
	3289	Vic/11	1:160	>3 mo	VN/04	2
	1129	Vic/11	1:20	>3 mo	VN/04	4
	1098	Vic/11	1:80	>3 mo	VN/04	4
	1106	Vic/11	1:40	>3 mo	VN/04	4
	1103	Vic/11	1:160	>3 mo	VN/04	4
	1105	Vic/11		>3 mo	VN/04	4
	1123	Vic/11	1:40	>3 mo	VN/04	6
	1122	Vic/11	1:160	>3 mo	VN/04	6
	1131	Vic/11	1:80	>3 mo	VN/04	6
	277	Vic/11		1 mo	VN/04	23
	276	Vic/11*		1 mo	VN/04	23
	262	Vic/11*		1 mo	VN/04	23
	263	Vic/11*		1 mo	VN/04	23

	279	Vic/11		1 mo	VN/04	7
	297	Vic/11		1 mo	VN/04	16

Supplementary Table 1. Individual ferret information. Six groups of ferrets were infected with the primary seasonal virus strain listed and HA titers to the corresponding primary influenza strain were measured. Ferrets recovered from the primary infection for the length of time listed and then challenged with H5N1 (VN/04). Ferrets were sacrificed at the time points listed in the last column.

Group	Ferret #	DPI	Trachea	Bronchi	Alveoli	Submucosal glands	Small bowel epithelia	Small bowel lamina propria	Small bowel ganglia	Liver	Spleen Red Pulp	Spleen White Pulp	Brain Olfactory Bulb	Brain Cerebral Cortex	Brain Deep Gray and White Matter	Brain Brainstem	Brain Cerebellum	Brain Meninges and Ependyma	Kidney
Naïve	1	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na
	2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na
	3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na
	4417	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na
	4420	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na
H1N1	472	2	0	2	1	3	0	0	0	na	na	na	0	0	0	0	0	0	na
	490	2	1	3	2	3	0	0	0	na	na	na	0	0	0	0	0	0	na
	496	2	1	0	0	0	0	0	0	na	na	na	0	0	0	0	0	0	na
	512	2	1	1	0	0	0	0	0	na	na	na	0	0	0	0	0	0	na
	491	3	0	1	0	0	0	1	0	na	na	na	0	0	0	0	0	0	na
	492	3	1	0	0	3	0	0	0	na	na	na	0	0	0	0	0	0	na
	493	3	0	2	1	3	0	0	0	na	na	na	0	0	0	0	0	0	na
	504	3	1	1	0	3	0	1	0	na	na	na	0	0	0	0	0	0	na
	509	5	2	2	0	3	0	0	0	na	na	na	0	0	0	0	0	0	na
	510	5	1	2	1	3	0	0	0	na	na	na	0	0	0	0	0	0	na
	519	5	2	1	0	2	0	0	0	na	na	na	0	0	0	0	0	0	na
	520	5	2	2	3	3	0	0	0	na	na	na	0	0	0	0	0	0	na
	476	7	0	0	0	0	0	1	0	na	na	na	0	0	0	0	0	0	na
	478	7	0	0	0	0	0	1	0	na	na	na	0	0	0	0	0	0	na
	494	7	0	0	0	0	0	0	0	na	na	na	0	0	0	0	0	0	na
	495	7	0	0	0	2	0	0	0	na	na	na	0	0	0	0	0	0	na
	480	14	0	0	0	0	0	0.5	0	na	na	na	0	0	0	0	0	0	na
	482	14	0	0	0	0	0	1	0	na	na	na	0	0	0	0	0	0	na
	488	14	0	0	0	0	0	1	0	na	na	na	0	0	0	0	0	0	na
	489	14	0	0	0	0	0	2	0	na	na	na	0	0	0	0	0	0	na
H5N1	807	6	2	2	1.5	0	0	0.5	0	1	0	0	3	3	2	3	2	0	na
	793	5	0.5	0	2	0	0	2	0	2	0	2	0	0	0	3	2	0	na
	801	8	0	0	1.5	0	0	3	0	3	0	0							na
	541	6	0	0	2.5	0	0	0	0	3	0	0	3	3	3	3	3	3	na
	542	5	0	0	3	0	0	0	0	2	0	2							na
	539	7	0	0	2	0	0	0	0	1	0	1							na
	544	5	1	0	2.5	0	0	0	0	3	0	3							na

	285	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	na
	284	4	0	1	1	0	0	2	0	1	0	0	0	0	0	0	0	0	na
	806	4	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	na
	796	5	0	0	0	0	0	0.5	0	2	0	0	0	2	0	0	0	0	na
	797	5	0	0	0	0	0	0	0	2	0	0	NA	2	0	0	0	1	na
	791	6	0	0	0	0	0	0	0	3	0	0	3	3	2	2	1	0	na
H1N1/ H5N1	754	18	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	na
	74	18	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0	na
	376	18	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	na
	764	18	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	na
	557	18	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	na
	646	18	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	na
	56	18	0	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0	na
	779	18	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0	na
	560	18	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	na
	718	2	0	0	0	0	0	1	0	0			0	0	0	0	0	0	na
	785	2	0	0	0	0	0	0	0	0			0	0	0	0	0	0	na
	605	2	0	0	0	0	0	0	0	1			0	0	0	0	0	0	na
	615	4	0	0	0.5	0	0	0	0	0.5			0	0	0	0	0	0	na
	602	4	0	0	0	0	0	0	0	0			0	0	0	0	0	0	na
	601	4	0	0	0	0	0	0	0	2			0	0	0	0	0	0	na
	610	6	0	0	0	0	0	0	0	0			0	0	0	0	0	0	na
	614	6	0	0	0	0	0	1	0	0			0	0	0	0	0	0	na
	603	6	0	0	0	0	0	0	0	0			0	0	0	0	0	0	na
	612	18	0	0	0	0	0	0.5	0	0.5	1	2	0	0	0	0	0	0	na
	611	18	0	0	0	0	0	2	0	2	1	0	0	0	0	0	0	0	na
	290	18	0	0	0	0	0	1	0	0.5	0.5	0	0	0	0	0	0	0	na
	287	18	0	0	0	0	0	2	0	0.5	0	0.5	0	0	0	0	0	0	na
	616	18	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	na
H3N2/ H5N1	299	7	0	0	1	0	0	2	0	0	2	1							na
	3291	2	0.5	0	1	0	0	0	0	3	0.75	1	0	0	0	0	0	0	0
	3290	2	0	0.5	1	0	0	1	0	0.5	0.5	1	0	0	0	0	0	0	0
	3287	2	0	0	0.5	0	0	1	0.5	0.5	0	1	0	0	0	0	0	0	0
	3289	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1129	4	0	0.5	3	0	0	1	0	3	3	1.5	0	.5	0	1	1	0	2

	1098	4	0	0	0	0	0	0	0	1	0	1	2.5	0	0	0	0	0	0
	1106	4	1	0	0.75	0	0	0	0	1	0	2	3	0	0	1	0	1	0
	1103	4	0	0	1	0	0	0	0	3	0	.5	0	1.5	0	0	0	0	0
	1105	4	0	0	0	0	0	0	0	1	0	2	3	2	2	1	2	0	0
	1123	6	0	0	0	0	0	0	0	2	0	0.5	0	0	1	2	0	2	0
	1122	6	0	0	0	0	0	0	0	0.5	0	0	0.5	2	2	1	0	0.5	0
	1131	6	0	0	0	0	0	0	0	1	0.5	0	3	0	0	0	0	0	0
	277	23	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	276	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	262	23	0	0	1	0	0	0	0	2	0	2	0	0	0	0	0	0	0
	263	23	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
	279	7	2	3	2	3	0	1	0	2	0	2		1	0	0	0	3	0
	297	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Suppelementary Table 2. Distribution of viral infection in various organs as assessed by in situ hybridization (ISH) in individual ferrets. Column 1 (**Group**) describes viral infection groups. Column 2 (**Ferret #**) describes the individual ferret number, and column 3 (**DPI**) the number of days post infection that the animals were sacrificed. Remaining columns indicate the ISH score of the organ or sub-region that showed influenza RNA. Scoring was performed as described in the materials and methods. Abbreviations: NA, not applicable; na, not available.