Supporting Information

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Fig. S1. Respiratory droplet transmissibility of modern avian and human H1N1 viruses. Three ferrets were inoculated with 10⁶ ElD₅₀ of avian strains Dk/Alb/35/76 (*A*), Dk/NY/15024/96 (*B*), Mal/Alb/119/98 (*C*), Tk/SD/7034/86 (*D*), Tk/KS/4880/80 (*E*), or the human strain A/Solomon Islands/3/06 (*F*). All animals were housed individually in specialized cages that permit exchange of respiratory droplets, but prevent direct or indirect contact between inoculated-contact animal pairs. One day postinoculation (p.i.), a naive contact animal was placed in each adjacent cage. Viral replication was monitored by titration of nasal washes collected every other day in both inoculated (*Left*) and contact (*Right*) animals. The limit of detection was 10^{1.5} ElD₅₀/mL and is indicated by a solid line on each graph.



Fig. S2. Respiratory droplet transmissibility of wild-type, mutant, and reassortant avian H1N1 viruses. Three ferrets were inoculated with 10^6 ElD_{50} of Dk/NY/96 (*A*), Dk/NY/96-DD (*B*), 1918NA:Dk/NY/96-DD (*C*), or 1918HANA:Dk/NY/96 (*D*). All animals were housed individually in specialized cages that permit exchange of respiratory droplets, but prevent direct or indirect contact between inoculated-contact animal pairs. One day p.i., a naive contact animal was placed in each adjacent cage. Viral replication was monitored by titration of nasal washes collected every other day in both inoculated (*Left*) and contact (*Right*) animals. The limit of detection was $10^{1.5} \text{ ElD}_{50}$ /mL, and is indicated by a solid line on each graph.

Transmission Cages



Fig. 53. Transmission model: Respiratory droplet transmission and direct contact transmission. For the respiratory droplet transmission experiments (*Left*), ferrets were housed in adjacent transmission cages, each modified so that a side wall was replaced with a stainless-steel, perforated wall with holes 1–5 mm in diameter and spaced 3 mm apart to facilitate the transfer of respiratory droplets through the air while preventing direct contact between ferrets and indirect contact with the bedding and food of neighboring ferrets. The use of the term "respiratory droplet transmission" throughout this article refers to transmission in the absence of direct or indirect contact and does not imply an understanding of the droplet size involved in virus spread between ferrets. A total of 6 ferrets were used for each respiratory droplet transmission experiment. Three ferrets were inoculated with virus and each was placed in a separate cage. Twenty-four hours later (day 1 p.i. for the inoculated ferrets and day 0 p.c. for the contact ferrets), 3 naive ferrets were each placed in a cage adjacent to an inoculated ferret. To prevent inadvertent physical transmission of virus by the investigators, the contact ferrets were always handled first, and all items that came into contact with the ferrets or their bedding were decontaminated between each ferret. For direct contact transmission experiments, 3 ferrets were each placed in a unmodified cage with solid walls and inoculated with virus. Twenty-four hours later, a naive ferret was placed in the same cage with each inoculated ferret.

Table S1. Respiratory droplet transmission of wild-type avian and human H1N1 viruses

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Virus	Receptor preference	HA amino acid (190/225)	No. with clinical symptoms		No. with	No. with
			Weight loss (%)*	Sneezing (day of onset) [†]	virus detection (peak titer) [‡]	seroconversion (HI titer range) [§]
Inoculated animals						
A/Solomon Islands/3/06	α2,6	D/D	3/3 (7.8)	3/3 (3)	3/3 (7.3)	ND
A/Turkey/South Dakota/7034/86	α2,3	E/G	3/3 (10)	0/3	3/3 (5.9)	3/3 (640–1,280)
A/ Turkey/Kansas/4880/80	α2,3	E/G	1/3 (8)	0/3	3/3 (6.3)	3/3 (640–1,280)
A/Mallard/Alberta/119/98	α2,3	E/G	3/3 (7.9)	0/3	3/3 (6.5)	3/3 (1,280)
A/Duck/Alberta/35/76	α2,3	E/G	1/3 (8.2)	2/3 (5)	3/3 (5.9)	3/3 (1,280)
A/Duckk/NY/15024–21/96	α2,3	E/G	2/3 (6)	1/3 (3)	3/3 (6.3)	3/3 (1,280)
Contact animals						
A/Solomon Islands/3/06	α2,6	D/D	3/3 (7.9)	3/3 (7)	3/3 (7.8)	ND
A/Turkey/South Dakota/7034/86	α2,3	E/G	1/3 (7)	0/3	0/3	0/3
A/ Turkey/Kansas/4880/80	α2,3	E/G	0/3	0/3	0/3	0/3
A/Mallard/Alberta/119/98	α2,3	E/G	0/3	0/3	0/3	0/3
A/Duck/Alberta/35/76	α2,3	E/G	0/3	0/3	0/3	0/3
A/Duckk/NY/15024–21/96	α2,3	E/G	0/3	0/3	0/3	0/3

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*Number of animals in which weight loss was observed. Values in parentheses indicate percentage mean maximum weight loss observed during the first 9 days p.i.

[†]Number of animals in which sneezing was observed during the first 9 days of the experiment. Values in parentheses indicate day of onset.

[†]Number of animals in which virus was detected in nasal washes. Values in parentheses indicate nasal wash titers, which are expressed as mean log₁₀ peak virus titer observed within the first 9 days p.i.

⁵Number of animals in which seroconversion was observed. Values in parentheses indicate HI titer range. Shown are HI antibody titers in sera collected at least 18 days p.i. HI titers were determined by using the homologous virus as test antigen. ND, not determined.