Equine Influenza A(H3N8) Virus Infection in Cats

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Interspecies transmission of equine influenza A(H3N8) virus has resulted in establishment of a canine influenza virus. To determine if something similar could happen with cats, we experimentally infected 14 cats with the equine influenza A(H3N8) virus. All showed clinical signs, shed virus, and transmitted the virus to a contact cohort.

Equine influenza A(H3N8) virus (EIV) remains a major cause of acute respiratory infections in horses (1). Epizootics are highly explosive and spread rapidly within and among equine premises. Virus transmission is by direct contact and inhalation. First isolated in 1963, EIV has evolved and diverged into American and Eurasian lineages (2,3). The American lineage has further diverged into multiple clades: Florida-1 clade predominates in North America and Florida-2 clade in Eurasia (4).

In Florida, USA, the etiologic agent of an outbreak of acute respiratory disease among greyhounds in 2004 was identified as EIV. Virologic and serologic analyses indicated that this virus had been circulating among greyhounds for several years before. Serologic evidence of infection was also found for pet dogs (5). In Great Britain, retrospective analysis showed that an outbreak of respiratory disease among English foxhounds in 2002 was caused by an EIV (6). Likewise, this virus was found to have circulated among greyhounds in the United States before 2004 (7). The virus has now been established as canine influenza virus and has spread to other breeds and pet dogs; the virus evolved independently from EIV as a monophyletic lineage (8).

During the 2003–2004 outbreak of highly pathogenic avian influenza virus (H5N1) infection in Asia, infections in feline species, including cats, were reported. (9). Previously, given the lack of circulating feline influenza virus, feral cats had been believed to be resistant to influenza virus, although an earlier report described susceptibility to A/Hong Kong/68 (H3N2) virus infection and prolonged virus shedding (10). Recent serologic and virus isolation studies have shown that cats are susceptible to multiple influenza viruses, e.g., avian-origin canine influenza (H3N2) (11), seasonal influenza A(H1N1), and influenza A/H1N1 pdm09 (12) viruses. To investigate cats’ susceptibility to EIV infection and virus transmissibility among cats, we conducted an infection and transmission experiment.

The Study

During 2013–2014, a total of 14 specific pathogen–free domestic shorthair cats, 9–12 weeks of age, were purchased and housed in an accredited Biosafety Level 2 facility at South China Agricultural University, Guangzhou. Results of virus isolation in eggs (nasal and rectal swab samples) and serologic testing determined that these cats were influenza virus free. Experiments were approved by the Institutional Animal Care and Use Committee and monitored by veterinarians.

The virus used was A/equine/Heilongjiang/SS1/2013, which had been isolated from a mule in northern China (S. Su et al., unpub. data). For virus inoculation, 6 cats were anesthetized with xylazine hydrochloride (30 mg/kg intraperitoneally), after which they were inoculated with virus (10 TCID₅₀ [median tissue culture infective dose]) in 1.0 mL of phosphate-buffered saline (0.5 mL in each nostril). One day after inoculation, 5 specific pathogen–free cats (contact cohort) were introduced into the same cages. Three noninfected cats (control cohort) were housed in a different room. Clinical monitoring began 1 day before virus inoculation and continued daily for the next 14 days. Nasal swab samples were collected daily for virus titration in MDCK cells. Serum was collected on postinoculation days 5, 7, 9, 12, and 14 and titrated by hemagglutination-inhibition assay with a 1% horse erythrocyte suspension. On postinoculation day 5, a total of 2 cats from the inoculated group were euthanized by intravenous pentobarbital, and on postinfection day 7, another 2 cats from the inoculated group plus 2 cats from the contact cohort were euthanized. Necropsies were performed, and trachea and lung sections were stained with hematoxylin and eosin and by an immunocytochemistry technique that involved a murine monoclonal antibody specific to EIV hemagglutinin.

The cats were susceptible to EIV infection; they showed overt clinical signs, virus shedding, and corresponding histopathologic changes in trachea and lung. Infected cats transmitted the virus to cats in the contact cohort. Overt clinical signs characteristic of acute influenza infection developed in inoculated cats during postinfection days 2–9 (peaking at day 4) and in contact cohort cats during days 4–9 (peaking at

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of lymphocyte infiltration on the same date (postinfection day 7), the lower intensity of lymphocytic infiltration was observed in samples from cats in the inoculated and contact cohorts; intensity was less for cats in the contact cohort (Figure 2). Likewise, EIV antigen was detected in cats in the inoculated and contact cohorts but not in the control cohort. These results indicated productive viral infection in cats in both cohorts. Because specimens were obtained from euthanized animals on the same date (postinfection day 7), the lower intensity of lymphocyte infiltration corresponded to the shift in virus titer (Figure 1) and clinical signs (Table).

Conclusions

That cats are susceptible to EIV by direct inoculation is not surprising because infection of cats with various influenza A viruses has been reported. Feline respiratory tract epithelial cells contain sialic acid α-2,3-galactose β-1,3-N-acetyl galactosamine (SA α2,3 gal) receptors for avian and equine influenza viruses and SA α2,6 gal receptors for mammalian influenza virus (13). However, our finding of horizontal transmission of EIV among cats is significant. If transmission occurs outside the laboratory, and if the basic reproduction rate is higher than 1.0, then EIV could potentially establish itself and circulate in this new host species. Why it has not yet happened naturally, as it did for canine influenza virus (H3N8), remains to be determined. Possibilities include lower transmission efficiency, lower probability of horse–cat contact, less virus shedding than in a laboratory, or feline behavior (less social contact than dogs).

Because cats in the inoculated and contact cohorts were housed in the same cages, our study could not delineate the route of transmission (direct contact or inhalation). Experiments to elucidate the transmission mechanism are being conducted.

Because we had used a contemporary strain of EIV, to rule out the possibility that the ability to cause clinical infection is unique to this strain, we repeated the experiment with the prototype EIV, A/equine/Miami/63 (H3N8). Although 6 of 6 infected cats showed no overt clinical signs, and virus shedding was not detectable (<1:10), susceptibility was evidenced by seroconversion for 2 of the 6 inoculated cats, although at a low hemagglutination-inhibition titer (1:40 on postinoculation day 14), thereby ruling out the possibility that A/equine/Heilongjiang/SS1/2013 is an aberrant virus. None of the 3 cats in the contact cohort showed clinical signs, shed virus, or had detectable hemagglutination-inhibition titers. This strain-dependent variation in virulence is not unusual for influenza virus. Interspecies transmission of EIV to dogs and establishment of a new lineage of equine influenza virus in dogs were probably a function of that particular EIV strain, as evidenced by finding that so far only 1 EIV-originated canine influenza virus lineage is circulating. However, transmission of EIV to dogs has occasionally occurred, including during the epizootic of EIV in Australia (14). Of note, the prototype canine influenza virus

| Cohort, cat | No. days after inoculation | –1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-------------|-----------------------------|----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inoculated  |                             |    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| A1          |                             | 0  | 0 | 0 | 2 | 2.5| 3.5| 0.5|†  |    |    |    |    |    |    |    |    |
| A2          |                             | 0  | 0 | 0.5|2.5| 2.5| 3  | 2  |1.5| 0.5| 0.5| 0  |    |    |    |    |    |
| A3          |                             | 0  | 0 | 0  |2.5| 2.5| 2  | 0.5|0  |†  |    |    |    |    |    |    |    |
| A4          |                             | 0  | 0 | 1  | 2 | 2  | 2.5| 1.5|0.5|*  |†  |    |    |    |    |    |    |
| A5          |                             | 0  | 0 | 0  | 2 | 2.5| 3  | 2  |†  |    |    |    |    |    |    |    |    |
| A6          |                             | 0  | 0 | 0  | 2.5|3  | 3  |1  |1.5| 1.5| 0.5| 0  |    |    |    |    |    |
| Contact     |                             |    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| B1          |                             | 0  | 0 | 0  | 0 | 0  | 2.5| 3  |2.5|†  |    |    |    |    |    |    |    |
| B2          |                             | 0  | 0 | 0  | 0 | 0  | 0  | 0  |0.5|0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| B3          |                             | 0  | 0 | 0  | 0 | 0  | 1.5|2.5|1.5|1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| B4          |                             | 0  | 0 | 0  | 0 | 0  | 0.5| 3  |2.5|1.5|†  |    |    |    |    |    |    |
| B5          |                             | 0  | 0 | 0  | 0 | 0  | 0  | 0  |0.5|0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Control     |                             |    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| C1          |                             | 0  | 0 | 0  | 0 | 0  | 0  | 0  |0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| C2          |                             | 0  | 0 | 0  | 0 | 0  | 0  | 0  |0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| C3          |                             | 0  | 0 | 0  | 0 | 0  | 0  | 0  |0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |

*Data indicate the sum of clinical scores, as follows: ocular and nasal discharge (0 = no discharge, 0.5 = serous, 1.0 = mild mucopurulent, 2.0 = severe mucopurulent); cough (0 = absent, 0.5 = mild, 1.0 = moderate, 1.5 = persistent, 2.0 = severe with choking or retching); sneezing, dyspnea, and depression (0 = absent, 2 = present); and body temperature (0 = <39.5°C, 2 = ≥39.5°C). Shading indicates days on which some animals had a clinical score ≥1, showing the shift between the 2 cohorts.

†Euthanized on the day indicated.
is phylogenetically related to the Florida-1 clade of EIV. Whether viruses in this clade have characteristics considered to be “promiscuous” and “plastic” (15) remains to be determined. On the basis of our results, we conclude that cats are susceptible to EIV and that the infection can be transmitted by close contact.

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References

1. Cullinane A, Newton JR. Equine influenza—a global perspective. Vet Microbiol. 2013;167:205–14. http://dx.doi.org/10.1016/j.vetmic.2013.03.029
2. Daly JM, Lai AC, Binns MM, Chambers TM, Barrandeguy M, Mumford JA. Antigenic and genetic evolution of equine H3N8 influenza A viruses. J Gen Virol. 1996;77:661–71. http://dx.doi.org/10.1099/0022-1317-77-4-661
3. Lai AC, Chambers TM, Holland RE Jr, Morley PS, Haines DM, Townsend HG, et al. Diverged evolution of recent equine-2 influenza (H3N8) viruses in the Western Hemisphere. Arch Virol. 2001;146:1063–74. http://dx.doi.org/10.1007/s007050170106
4. Bryant NA, Rash AS, Russell CA, Ross J, Cooke A, Bowman S, et al. Antigenic and genetic variations in European and North American equine influenza virus strains (H3N8) isolated from 2006 to 2007. Vet Microbiol. 2009;138:41–52. http://dx.doi.org/10.1016/j.vetmic.2009.03.004
5. Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP, Chen L, et al. Transmission of equine influenza virus to dogs. Science. 2005;310:482–5. http://dx.doi.org/10.1126/science.1117950
6. Daly JM, Blunden AS, Macrae S, Miller J, Bowman SJ, Kolodziejek J, et al. Transmission of equine influenza virus to English foxhounds. Emerg Infect Dis. 2008;14:461–4. http://dx.doi.org/10.3201/eid1403.070643

7. Anderson TC, Bromfield CR, Crawford PC, Dodds WJ, Gibbs EP, Hernandez JA. Serological evidence of H3N8 canine influenza-like virus circulation in USA dogs prior to 2004. Vet J. 2012;191:312–6. http://dx.doi.org/10.1016/j.tvjl.2011.11.010.

8. Rivaille P, Perry IA, Jang Y, Davis CT, Chen LM, Dubovi EJ, et al. Evolution of canine and equine influenza (H3N8) viruses co-circulating between 2005 and 2008. Virology. 2010;408:71–9. http://dx.doi.org/10.1016/j.virol.2010.08.022.

9. Kuiken T, Rimmelzwaan G, van Riel D, van Amerongen G, Baars M, Fouchier R, et al. Avian H5N1 influenza in cats. Science. 2004;306:241. http://dx.doi.org/10.1126/science.1102287

10. Paniker CK, Nair CM. Infection with A2 Hong Kong influenza virus in domestic cats. Bull World Health Organ. 1970;43:859–62.

11. Song DS, An DJ, Moon HJ, Yeom MJ, Jeong HY, Jeong WS, et al. Interspecies transmission of the canine influenza H3N2 virus to domestic cats in South Korea, 2010. J Gen Virol. 2011;92:2350–5. http://dx.doi.org/10.1099/vir.0.033522-0.

12. McCullers JA, Van De Velde LA, Schultz RD, Mitchell CG, Halford CR, Boyd KL, et al. Seroprevalence of seasonal and pandemic influenza A viruses in domestic cats. Arch Virol. 2011;156:117–20. http://dx.doi.org/10.1007/s00705-010-0809-7.

13. Wang H, Wu X, Cheng Y, An Y, Ning Z. Tissue distribution of human and avian type siaic acid influenza virus receptors in domestic cat. Acta Vet Hung. 2013;61:537–46. http://dx.doi.org/10.1556/Avet.2013.030.

14. Kirkland PD, Finlaison DS, Crespe E, Hurt AC. Influenza virus transmission from horses to dogs, Australia. Emerg infect Dis.2010;16:699–702. DOI: 10.3201/eid1604.091489

15. Gibbs EP, Anderson TC. Equine and canine influenza: a review of current events. Anim Health Res Rev. 2010;11:43–51. http://dx.doi.org/10.1017/S1466252310000046.

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