Seroepidemiological Evidence of Subtype H3N8 Influenza Virus Infection among Pet Dogs in China

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Abstract

The H3N8 virus and the H3N2 virus are the main subtypes of canine influenza virus (CIV). H3N8 CIV mainly circulates in America, and H3N2 CIV mainly circulates in Asia. However, there was an outbreak of the Asian H3N2 virus in the United States (US) in 2015. Thus, it is important to evaluate the presence of subtype H3N8 virus in dogs in China. From May 2015 to November 2015, 600 sera from pet dogs were collected from Guangzhou, Shanghai, Beijing and Shenzhen for hemagglutination inhibition (HI) assays and microneutralization (MN) assays. Fifty-two (8.66%) of the 600 sera were positive for the subtype H3N2 virus, which matched the previous reports. Five (0.83%) of 600 sera were positive for the subtype H3N8 virus (H3N8 EIV or H3N8 AIV or H3N8 CIV), which is the first report of subtype H3N8 virus infection among dogs in China and remind us to pay more attention to this subtype virus. Therefore, further serological and virological surveillance of influenza virus infection among dogs in China is imperative.

Introduction

Under most circumstances, there are species barriers that hamper interspecies transmission of influenza viruses. However, evolution can help viruses surmount species barriers to sustain transmission in a new host species [1]. Recently, influenza A virus has been shown to infect various hosts, from birds to mammals, and to have varying degrees of adaptation in different hosts [2].

The research history of CIV is relatively short because dogs were long regarded as unsusceptible to influenza viruses. This perception did not change until H3N8 CIV was first identified in the US from what was known as an equine-origin H3N8 influenza virus in January 2004 [3]. The persistence of this subtype H3N8 virus in dogs suggests that the virus has become enzootic in the US [3, 4]. In 2008, the avian-origin H3N2 CIV was first isolated in South Korea [5], and...
this subtype H3N2 virus was later reported in China [6]. Since then, in China, epidemiological studies of dogs have focused on the subtype H3N2 virus [7–11] and subtype H1N1, H5N1, H7N9, H10N8 [12–16] viruses, which have public health significance. H3N8 CIV had mainly circulated in America, and H3N2 CIV had mainly circulated in Asia. However, this changed during the outbreak of H3N2 CIV in Chicago, and the virus then rapidly spread to numerous states in the US in 2015 [17]. Therefore, it remains possible that H3N8 CIV infection has spread among dogs to reach China or that H3N8 EIV or H3N8 AIV has surmounted species barriers to sustain transmission among dogs. To examine this possibility, we conducted serological surveillance from May 2015 to November 2015 in Guangzhou, Shanghai, Beijing, and Shenzhen, which are the four biggest international cities in China, to evaluate whether the subtype H3N8 virus has infected dogs in China.

Materials and Methods

Sample collection, viral antigens, and sera

From May 2015 to November 2015, sera from 600 pet dogs (150 specimens per city) were collected for serology from animal hospitals in Guangzhou, Shanghai, Beijing and Shenzhen and were preserved at -80°C for future testing. The dogs’ characteristics were recorded by the research staff. The samples were tested for EIV-H3N8: A/equine/Heilongjiang/SS1/2013 (H3N8); AIV-H3N8: A/avian/Guangdong/J/2012 (H3N8); CIV-H3N2: A/canine/Guangdong/01/2014(H3N2). Negative control serum was collected from an influenza-negative dog whose serum did not contain antibody against H3N2, H3N8, H9N2, or H1N1 as indicated by HI tests. Positive control sera were prepared from immune rabbits using inactivated viruses. These viruses and control sera were obtained from the Key Laboratory of Comprehensive Prevention and Control for Severe Clinical Animal Diseases of Guangdong Province, the College of Veterinary Medicine, South China Agricultural University.

Detection of influenza virus antibodies

We used a WHO-recommended HI assay [18]. Briefly, the sera were treated with a receptor-destroying enzyme (RDE, Denka Seiken 340016 (370013)) and absorbed with erythrocytes to remove nonspecific inhibitors before the tests. The sera were further diluted to a 1:10 dilution. The samples were two-fold serially diluted in 96-well V bottom microtiter plates, and 4 hemagglutination units (HAU) of the virus were added to each well. The sera and virus mixtures were incubated at room temperature for 30 min. Then, 1% red blood was added to all wells. The plates were incubated at room temperature and read after 30 min. The serum titer was expressed as the reciprocal of the highest dilution of serum at which hemagglutination was inhibited. All assays were conducted twice with triplicate wells each time, and the final titer was only accepted when both replicates yielded matching results.

Sera from dogs with an HI titer ≥ 20 were confirmed with MN recommended by the WHO [18]. Briefly, the sera were treated with RDE, and two-fold serial dilutions were performed in 96-well polystyrene immunoassay plates (Nunclon Delta surface, Nunc, Denmark). Then, equal volumes of virus diluent containing influenza virus at 100 TCID50/50 μl were mixed with the diluted sera. After incubation for an hour, 1.5×10⁴ MDCK cells were added to each well. After incubation for 18–22 hours, the monolayers of MDCK cells were washed with PBS and fixed in cold 80% acetone for 10 minutes. Finally, the viral nucleoprotein (NP) was detected by enzyme-linked immunosorbent assay (ELISA, Immune Technology Company). An HI titer ≥ 20 and an MN titer ≥ 80 is considered positive evidence of previous H3N8 virus infection. Additionally, the specimens with an HI titer ≥ 20 against H3N8 virus were further evaluated for human H3N2 influenza virus (HuIV) antibody using an enzyme-linked
immunosorbing assay (ELISA, H3N2 HA1 Hemagglutinin, HA1 ELISA Kit, Prod. No.: DEIA252, Creative Diagnostics company) according to the manufacturer’s instructions.

Results

After the outbreak of H3N2 CIV in Chicago, US, in April 2015, we sought to evaluate whether subtype H3N8 virus infection had occurred among dogs in China. Thus, from May 2015 to November 2015, we collected sera from 600 pet dogs in Guangzhou, Shanghai, Beijing, and Shenzhen. In total, using a cutoff of HI ≥1:20, 52 (8.66%) of the 600 sera were positive for canine H3N2 virus, 8 (1.33%) of the 600 sera were positive for equine H3N8 virus, and 4 (0.67%) of the 600 sera were positive for avian H3N8 virus (Table 1). Furthermore, the MN assay was conducted to confirm the presence of equine H3N8 and avian H3N8 viruses antibody in the sera (HI ≥1:20). Ultimately, using cutoffs of HI ≥1:20 and MN ≥1:80, 5 specimens were positive for equine H3N8 virus, and 3 specimens were positive for avian H3N8 virus which is also positive for equine H3N8 (Table 2). One of these five H3N8 positive specimens had both subtype H3N2 virus antibody and subtype H3N8 virus antibody (Table 2). Additionally, HI assays were conducted to evaluate the cross-reactivity between these viruses. There was strong cross-reactivity between H3N8 EIV and H3N8 AIV, and no cross-reactivity was observed between H3N8 EIV or H3N8 AIV and H3N2 CIV (Table 3). Additionally, ELISA assays were conducted to eliminate the possibility of H3N2 HuIV cross-reactivity for the subtype H3N8 virus (data not shown).

Discussion

Since CIV was first identified in the US, the H3N8 CIV has mainly been circulating in America, and H3N2 CIV has mainly been circulating in Asia [3, 5, 6]. However, there was an outbreak of the Asian H3N2 CIV in the US in 2015 [17]. Hence, it is urgent to investigate whether there have been previous subtype H3N8 virus infections among dogs in China. Through serological surveillance from May 2015 to November 2015 in Guangzhou, Shanghai, Beijing and Shenzhen, we found that 5 of 600 pet dogs were previously infected with subtype H3N8 virus (H3N8 EIV or H3N8 AIV or H3N8 CIV). Our results were consistent with previous reports, with an 8.66% prevalence of subtype H3N2 virus infection among dogs [7, 11].

H3N8 EIV was first reported to surmount species barriers to cause sustained transmission among dogs in the US in 2004 [3, 4]. Subsequently, this transmission of this virus from horses to dogs appeared in Australia in 2007 [19, 20]. In 1993, H3N8 EIV was transmitted to China [21], and a previous study reported that H3N8 EIV infected pigs and donkeys in China [22, 23]. Therefore, it is possible that this virus crossed species barriers to infect directly to dogs in China. In addition, because Asian H3N2 CIV recently transmitted to US [17], it seems that there are potential transmission routes for CIV between America and Asian countries, including pet dog transportation, army dog introduction to the country, and dog rescue. H3N8 AIV is one of the most common subtype in wild birds [24] and is low pathogenic avian influenza virus (LPAV) for birds. Significantly, H3N8 AIV has established lineages in horses [25] and then transmit to dogs [3], and also have crossed species barriers to infect seals [26]. This suggests that H3N8 AIV could cross species barriers to infect mammals. According to Table 2, these positive sera dogs are no influenza-like symptoms and have no travel (including their house mates), it seems logical that this is the domestic transmission event in China. It is possible that H3N8 AIV crossed species barriers to infect dogs in China, like H3N2 AIV.

Here, we first reported the seroepidemiological evidence of subtype H3N8 virus infection among dogs in China. Further and continuous surveillance needs to be performed for this subtype virus among dogs in China. Currently, dogs are infected with different subtypes of
### Table 1. Prevalence of elevated antibody titers against the subtype H3N2 and H3N8 viruses among dogs by HI assay, China, 2015.

| Cities | CIV-H3N2 HI seroprevalence | No. of HI ≥1:20 (%) | EIV-H3N8 HI seroprevalence | No. of HI ≥1:20 (%) | AIV-H3N8 HI seroprevalence | No. of HI ≥1:20 (%) |
|--------|-----------------------------|---------------------|-----------------------------|---------------------|-----------------------------|---------------------|
|        | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 |
| GZ     | 150  | 3    | 5    | 3     | 1     | 1     | 16(10.67) | 1     | 0     | 1     | 0     | 0     | 2(1.33) | 1     | 0     | 0     | 0     | 0     | 0     | 1(0.67) |
| SH     | 150  | 4    | 3    | 3     | 2     | 2     | 0(6.67)  | 0     | 1     | 0     | 1     | 0     | 2(1.33) | 0     | 1     | 0     | 0     | 0     | 0     | 1(0.67) |
| BJ     | 150  | 2    | 1    | 7     | 1     | 3     | 0(9.33)  | 1     | 0     | 2     | 0     | 0     | 3(2.00) | 0     | 2     | 0     | 0     | 0     | 0     | 2(1.33) |
| SZ     | 150  | 3    | 2    | 4     | 0     | 2     | 1(8.00)  | 0     | 1     | 0     | 0     | 0     | 2(1.33) | 0     | 0     | 0     | 0     | 0     | 0     | 0(0.00) |
| Total  | 600  | 12   | 11   | 17    | 6     | 8     | 2(52.86) | 2     | 2     | 3     | 1     | 0     | 8(1.33) | 1     | 3     | 0     | 0     | 0     | 0     | 4(0.67) |

Note: GZ: Guangzhou; SH: Shanghai; BJ: Beijing; SZ: Shenzhen
EIV-H3N8: A/equine/Heilongjiang/SS1/2013(H3N8); AIV-H3N8: A/avian/Guangdong/J/2012(H3N8)
CIV-H3N2: A/canine/Guangdong/01/2014(H3N2).

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influenza viruses, such as H3N8 [3], H3N2 [6], H1N1 [27], H3N1 [16], H9N2 [28], H5N2 [29] and H5N1 [30], and previous studies have reported that it is possible for dogs to become a “mixing vessel”, similar to pigs [31–33]. Therefore, continuous serological and virological surveillance needs to be performed among dogs.

In this study, there are two potential limitations should be kept in mind. First, although no cross-reactivity between subtype H3N8 virus and H3N2 CIV or H3N2 HuIV, the strong cross-reactivity between H3N8 EIV and H3N8 AIV can not be eliminated. Nonetheless, the subtype H3N8 virus infects in dogs is been verified in this study. Second, no H3N8 CIV that is known as an equine-origin was used in this study to testify the results, but the origin H3N8 EIV was used as a substitute.

**Ethical approval**

This study protocol was reviewed and approved by the Institutional Review Board of South China Agricultural University.

**Table 2. Characteristics of the study subjects whose sera were reactive against subtype H3N8 viruses.**

| Number | Age       | Traveleda | Influenza vaccinated | Collect city | Collect day     | Influenza-like symptoms | EIV-H3N8 | AIV-H3N8 | CIV-H3N2 |
|--------|-----------|-----------|---------------------|--------------|----------------|------------------------|----------|----------|----------|
|        |           |           |                     |              |                |                        | Titer by HI | Titer by MN | Titer by HI | Titer by MN | Titer by HI | Titer by MN |
| 1      | 7 years   | No        | No                  | Guangzhou    | 2015.6.04      | No                     | 1:20      | 1:20      | 0        | 0        | 0        | 0        |
| 2      | 2 months  | No        | No                  | Guangzhou    | 2015.6.04      | No                     | 1:80°     | 1:160     | 1:20     | 1:80     | 0        | 0        |
| 3      | 3 months  | No        | No                  | Shanghai     | 2015.7.13      | No                     | 1:40      | 0         | 0        | 0        | 1:20     | 0        |
| 4      | 9 months  | No        | No                  | Shanghai     | 2015.7.13      | No                     | 1:160     | 1:320     | 1:40     | 1:80     | 0        | 0        |
| 5      | 4 years   | No        | No                  | Beijing      | 2015.7.24      | No                     | 1:20      | 0         | 0        | 0        | 0        | 0        |
| 6      | 3 years   | No        | No                  | Beijing      | 2015.7.24      | No                     | 1:80      | 1:160     | 1:40     | 1:80     | 0        | 0        |
| 7      | 2 years   | No        | No                  | Beijing      | 2015.6.24      | No                     | 1:80      | 1:80      | 1:40     | 1:40     | 1:40     | 1:80     |
| 8      | 5 months  | No        | No                  | Shenzhen     | 2015.10.07     | No                     | 1:40      | 1:80      | 0        | 0        | 0        | 0        |

Note: EIV-H3N8, A/equine/Heilongjiang/SS1/2013(H3N8); AIV-H3N8, A/avian/Guangdong/J/2012(H3N8); CIV-H3N2, A/canine/Guangdong/01/2014(H3N2).

a include dogs and their house mates.

b Bold indicates positive specimen by HI titer ≥ 20 and MN titer ≥ 80.

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**Table 3. HI titers of control sera against reference virus strains.**

| Reference strains | EIV-H3N8 | AIV-H3N8 | CIV-H3N2 | Negative serum |
|-------------------|----------|----------|----------|----------------|
| EIV-H3N8          | >1280    | 640      | /        | /              |
| AIV-H3N8          | 1280     | >1280    | /        | /              |
| CIV-H3N2          | /        | /        | >1280    | /              |

Note: EIV-H3N8, A/equine/Heilongjiang/SS1/2013(H3N8)
AIV-H3N8, A/avian/Guangdong/J/2012(H3N8)
CIV-H3N2, A/canine/Guangdong/01/2014(H3N2).

Positive sera were prepared from immune rabbits using inactivated viruses. / indicates HI titers ≤ 10.

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Author Contributions
Conceived and designed the experiments: SL PZ. Performed the experiments: LW XF SH WZ XZ. Analyzed the data: PZ. Contributed reagents/materials/analysis tools: PZ. Wrote the paper: SL PZ.

References
1. Kuiken T, Holmes EC, McCauley J, Rimmelzwaan GF, Williams CS, Grenfell BT. Host species barriers to influenza virus infections. Science. 2006; 312(5772):394–7. PMID:16627737
2. Yoon SW, Webby RJ, Webster RG. Evolution and ecology of influenza A viruses. Curr Top Microbiol Immunol. 2014; 385:359–75. doi:10.1007/82_2014_396 PMID:24990620
3. Crawford PC, Dubovi EJ, Casteleman WL, Stephenson I, Gibbs EP, Chen L, et al. Transmission of equine influenza virus to dogs. Science. 2005; 310(5747):482–5. PMID:16186182
4. Payungporn S, Crawford PC, Kouo TS, Chen LM, Pompey J, Casteleman WL, et al. Influenza A virus (H3N8) in dogs with respiratory disease, Florida. Emerging infectious diseases. 2008; 14(6):902–8. doi:10.3201/eid1406.071270 PMID: 18507900
5. Li S, Shi Z, Jiao P, Zhang G, Zhong Z, Tian W, et al. Avian-origin H3N2 canine influenza A viruses in Southern China. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2010; 10(8):1286–8. doi: 10.1016/j.meegid.2010.08.010 PMID: 20732458
6. Sun Y, Sun S, Ma J, Tan Y, Du L, Shen Y, et al. Identification and characterization of avian-origin H3N2 canine influenza viruses in northern China during 2009–2010. Virology. 2013; 435(2):301–7. doi: 10.1016/j.virology.2012.09.037 PMID: 23063406
7. Su S, Li HT, Zhao FR, Chen JD, Xie JX, Chen ZM, et al. Avian-origin H3N2 canine influenza virus circulating in farmed dogs in Guangdong, China. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2010; 10(8):1286–8. doi: 10.1016/j.meegid.2010.08.010 PMID: 20732458
8. Su S, Chen JD, Jia K, Khan SU, He SY, Fu XL, et al. Evidence for Subclinical Influenza A(H1N1)pdm09 Virus Infection among Dogs in Guangdong Province, China. Journal of clinical microbiology. 2014; 52(5):1762–5. doi: 10.1128/JCM.03522-13 PMID: 24599880
9. Su S, Zhou P, Fu XL, Wang LF, Hong ML, Lu G, et al. Virological and Epidemiological Evidence of Avian Influenza Virus Infections Among Feral Dogs in Live Poultry Markets, China: A Threat to Human Health? Clinical Infectious Diseases. 2014; 58(11):1644–6. doi: 10.1093/cid/ciu154 PMID: 24621952
10. Su S, Qi W, Zhou P, Xiao C, Yan Z, Cui J, et al. First Evidence of H1N8 Avian Influenza Virus Infections among Feral Dogs in Live Poultry Markets in Guangdong Province, China. Clinical Infectious Diseases. 2014; 59(5):748–50. doi: 10.1093/cid/ciu345 PMID: 24812294
11. Su S, Chen JD, Jia K, Khan SU, He SY, Fu XL, et al. Evidence for Subclinical Influenza A(H1N1)pdm09 Virus Infection among Dogs in Guangdong Province, China. Journal of clinical microbiology. 2014; 52(5):1762–5. doi: 10.1128/JCM.03522-13 PMID: 24599880
12. Sun Y, Sun S, Ma J, Tan Y, Du L, Shen Y, et al. Identification and characterization of avian-origin H3N2 canine influenza viruses in northern China during 2009–2010. Virology. 2013; 435(2):301–7. doi: 10.1016/j.virology.2012.09.037 PMID: 23063406
13. Su S, Chen JD, Jia K, Khan SU, He SY, Fu XL, et al. Evidence for Subclinical Influenza A(H1N1)pdm09 Virus Infection among Dogs in Guangdong Province, China. Journal of clinical microbiology. 2014; 52(5):1762–5. doi: 10.1128/JCM.03522-13 PMID: 24599880
14. Su S, Zhou P, Fu XL, Wang LF, Hong ML, Lu G, et al. Virological and Epidemiological Evidence of Avian Influenza Virus Infections Among Feral Dogs in Live Poultry Markets, China: A Threat to Human Health? Clinical Infectious Diseases. 2014; 58(11):1644–6. doi: 10.1093/cid/ciu154 PMID: 24621952
15. Su S, Qi W, Zhou P, Xiao C, Yan Z, Cui J, et al. First Evidence of H1N8 Avian Influenza Virus Infections among Feral Dogs in Live Poultry Markets in Guangdong Province, China. Clinical Infectious Diseases. 2014; 59(5):748–50. doi: 10.1093/cid/ciu345 PMID: 24812294
16. Song D, Moon HJ, An DJ, Jeoung HY, Kim H, Yeom MJ, et al. A novel reassortant canine H3N1 influenza virus between pandemic H1N1 and canine H3N2 influenza viruses in Korea. The Journal of general virology. 2012; 93(Pt 3):551–4. doi: 10.1099/vir.0.037739-0 PMID: 22131311
17. Centers for Disease Control and Prevention(CDC). Update on H3N2 Canine Influenza (Dog Flu) Virus. 2015. Available: http://www.cdc.gov/flu/news/canine-influenza-Sequencing.htm.
18. WHO. Manual for the laboratory diagnosis and virological surveillance of influenza. 2011; Available: http://www.aitoolkit.org/site/DefaultSite/filesystem/documents/WHO%20lab%20 Manual.pdf.
19. Crispe E, Finlaison DS, Hurt AC, Kirkland PD. Infection of dogs with equine influenza virus: evidence for transmission from horses during the Australian outbreak. Aust Vet J. 2011; 89 Suppl 1:27–8. doi: 10.1111/j.1751-0813.2011.00734.x PMID: 21711279

20. Kirkland PD, Finlaison DS, Crispe E, Hurt AC. Influenza virus transmission from horses to dogs, Australia. Emerging infectious diseases. 2010; 16(4):699–702. doi: 10.3201/eid1604.091489 PMID: 20350392

21. Guo Y, Wang M, Zheng GS, Li WK, Kawaoka Y, Webster RG. Seroepidemiological and molecular evidence for the presence of two H3N8 equine influenza viruses in China in 1993–94. The Journal of general virology. 1995; 76 (Pt 8):2009–14. PMID: 7636481

22. Tu J, Zhou H, Jiang T, Li C, Zhang A, Guo X, et al. Isolation and molecular characterization of equine H3N8 influenza viruses from pigs in China. Archives of virology. 2009; 154(5):887–90. doi: 10.1007/s00705-009-0381-1 PMID: 19396578.

23. Qi T, Guo W, Huang W, Dai L, Zhao L, Li H, et al. Isolation and genetic characterization of H3N8 equine influenza virus from donkeys in China. Veterinary microbiology. 2010; 144(3–4):455–60. doi: 10.1016/j.vetmic.2010.01.006 PMID: 20153940

24. Henaux V, Samuel MD, Dusek RJ, Fleskes JP, Ip HS. Presence of avian influenza viruses in waterfowl and wetlands during summer 2010 in California: are resident birds a potential reservoir? PLOS ONE. 2012; 7(2):e31471. doi: 10.1371/journal.pone.0031471 PMID: 22328934

25. Gibbs EP, Anderson TC. Equine and canine influenza: a review of current events. Animal health research reviews / Conference of Research Workers in Animal Diseases. 2010; 11(1):43–51. doi: 10.1017/S1466252310000046 PMID: 20426896

26. Anthony SJ, St Leger JA, Pugliares K, Ip HS, Chan JM, Carpenter ZW, et al. Emergence of fatal avian influenza in New England harbor seals. MBio. 2012; 3(4):e00166–12.

27. Lin D, Sun S, Du L, Ma J, Fan L, Pu J, et al. Natural and experimental infection of dogs with pandemic H1N1/2009 influenza virus. The Journal of general virology. 2012; 93(1):119–23. doi: 10.1099/vir.0.037358-0 PMID: 21976611

28. Sun XX, Xu XK, Liu Q, Liang DJ, Li CY, He QS, et al. Evidence of avian-like H9N2 influenza A virus among dogs in Guangxi, China. Infection Genetics And Evolution. 2013; 20:471–5.

29. Hai-xia F, Yuan-yuan L, Qian-qian S, Zong-shuai L, Feng-xia Z, Yan-li Z, et al. Interspecies transmission of canine influenza virus H5N2 to cats and chickens by close contact with experimentally infected dogs. Veterinary microbiology. 2014; 170(3–4):414–7. doi: 10.1016/j.vetmic.2014.02.040 PMID: 24856135

30. Songserm T, Aomsin A, Jam-on R, Sae-Heng N, Pariyothorn N, Payungporn S, et al. Fatal avian influenza A H5N1 in a dog. Emerging infectious diseases. 2006; 12(11):1744–7. PMID: 17283627

31. Gonzalez G, Marshall JF, Morrell J, Robb D, McCauley JW, Perez DR, et al. Infection and pathogenesis of canine, equine, and human influenza viruses in canine tracheas. Journal of virology. 2014; 88(19):9206–19. doi: 10.1128/JVI.00887-14 PMID: 24899186

32. Su S, Zhou P, Fu X, Wang L, Hong M, Lu G, et al. Virological and epidemiological evidence of avian influenza virus infections among feral dogs in live poultry markets, China: a threat to human health? Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2014; 58(11):1644–6.

33. Su S, Qi W, Zhou P, Xiao C, Yan Z, Cui J, et al. First evidence of H10N8 Avian influenza virus infections among feral dogs in live poultry markets in Guangdong province, China. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2014; 59(5):748–50.