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Short Communication

Interspecies transmission of canine influenza virus H5N2 to cats and chickens by close contact with experimentally infected dogs

Feng Hai-xia [a,b,1], Liu Yuan-yuan [a,b,1], Song Qian-qian [a,b], Ling Zong-shuai [c], Zhang Feng-xia [a,b], Zhu Yan-li [b], Jiang Shi-jin [a,b], Xie Zhi-jing [a,b,*]

[a] Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, Taian, Shandong 271018, China
[b] College of Veterinary Medicine, Shandong Agricultural University, Taian, Shandong 271018, China
[c] Poultry Disease Lab of Shandong Entry-exit Inspection, Jinan, Shandong 250014, China

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ABSTRACT

The novel H5N2 influenza virus, CA/SD/JT01/09, was isolated from the dog exhibiting respiratory signs in China in 2009. Dog to dog transmission of the novel H5N2 was previously confirmed. But interspecies transmission of the virus between dogs and the other animals has still remained unclear. To determine whether the virus can be transmitted directly from dogs to cats and chickens, we conducted contact exposure experiments. Susceptible cats and chickens were housed in the room which the novel H5N2 infected dogs were housed in, respectively. As a result, only one cat showed clear manifestations of H5N2 infection, but susceptibility of the other cats to H5N2 was confirmed by seroconversion. Eight of the exposure chickens showed clear manifestations of illness and 2 chickens died, and it demonstrates that chickens are susceptible to the recombinant H5N2. It implied that close contact between the H5N2-infected dogs and the cats and chickens resulted in spread of the virus to the sentinel animals.

1. Introduction

Influenza has been long considered as having no infectious possibilities in dogs (Beeler, 2009). But in the last several years, respiratory diseases in dogs caused by more and more influenza virus (IV) subtypes were reported, such as H3N8 (Crawford et al., 2005; Yamanaka et al., 2009), H3N2 (Song et al., 2008), H5N1 (Songserm et al., 2006; Tiensin et al., 2005) and H1N1 (Lin et al., 2012).

H5N2 IV is present in wild birds usually with low pathogenicity. After introduction into domestic poultry, however, the virus may mutate into a highly pathogenic avian influenza virus (García et al., 1996; Snoeck et al., 2011). Many H5N2 IVs have been recovered from avian species (García et al., 1996; Snoeck et al., 2011; Zhao et al., 2012), pigs (Lee et al., 2009) and humans (Ogata et al., 2008). Canine influenza virus (CIV) H5N2, CA/SD/JT01/09, has recently emerged as a respiratory pathogen in dogs, which resulted from the reassortment of swine influenza virus H5N1 and avian influenza virus H9N2 (Zhan et al., 2012).

Close contact between CA/SD/JT01/09-infected and noninfected dogs resulted in intraspecies transmission (Song et al., 2013). But interspecies transmission of the virus between dogs and the other animals has still

* Corresponding author at: College of Veterinary Medicine, Shandong Agricultural University, Taian, Shandong 271018, China.
Tel.: +86 538 8242673; fax: +86 538 8241419.
E-mail addresses: xiezhijing@126.com, xiezhj@sdau.edu.cn (X. Zhi-jing).
[1] Both the authors contributed equally to this work.

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remained unclear. The objectives of the study are to clarify whether close contact between experimentally CIV H5N2 infected dogs and healthy cats and chickens could lead to interspecies transmission.

2. Materials and methods

The virus, CA/SD/JT01/09, was received in allantoic fluid after a single passage in ten-day-old specific-pathogen-free embryonated chicken eggs. To clarify whether the virus can be transmitted directly from dogs to cats and chickens, experiments were performed on four groups housed in different rooms of the isolation facility at Poultry Disease Lab of Shandong Entry-exit Inspection, Jinan, Shandong. Animals in the study comprised 12 beagles (9–11 weeks of age), 10 domestic cats (9–10 weeks of age) and 20 commercial layers (8–9 weeks of age), which were divided into four groups. All the animals were healthy and negative for IV antigen and anti-IV antibody. The animals of each group were housed in adjacent cages in the room. In the first group, 3 dogs were inoculated intranasally with a 10^6 50% egg infectious dose (EID50), using CA/SD/JT01/09 respectively, and 5 domestic cats were housed in the same containment room to test for dog-to-cat interspecies transmission of virus from day 1 postinfection (p.i.) onwards. In the second group, 3 dogs were inoculated intranasally with a 10^6 EID50, using CA/SD/JT01/09 respectively, and 10 chickens, serving as sentinel animals, were housed in the same containment room to test for dog-to-chicken transmission of virus from day 1 p.i. onwards. The third and the fourth groups served as negative controls, respectively. In the third group, 3 dogs were inoculated intranasally with 1 mL of sterile phosphate buffered saline, 5 domestic cats were housed in the same room from day 1 p.i. onwards. In the fourth group, 3 dogs were inoculated intranasally with 1 mL of sterile phosphate buffered saline, 10 chickens were housed in the same room from day 1 p.i. onwards.

To be assured that the experimenters were not the means for the transmission, the experimenters were required to wear separate personal protection equipment to enter each room, and new needles, syringes and sterilized swabs were used for each animal and each procedure. Clinical signs of infection were daily monitored p.i. To determine virus shedding, nasal, rectal and cloacal swabs were collected from the animals for 14 days. The swab elutes were firstly tested by A single-step quantitative real-time reverse transcription-PCR (qRT-PCR), and the primer-probe set targeted a highly conserved region of the matrix (M) gene of type A influenza virus (Payungporn et al., 2008). If positive for the matrix (M) gene of IV, the swab elutes were further titrated in ten-day-old specific-pathogen-free embryonated chicken eggs, respectively. The HA and NA gene segments from shed viruses were obtained by RT-PCR and sequenced as previously described (Zhan et al., 2012). Two sentinel cats, two sentinel chickens, two control cats and two control chickens were euthanized on day 4 p.i. and day 14 p.i., respectively. If they died of infection during the study, the animals were necropsied. The tissue samples were collected, including nasal vestibule, upper respiratory tract, lungs, heart, liver, spleen, brain, kidneys, pancreas and intestines. A part of the samples were rapidly immersed in 10% neutral formalin buffer to prevent autolysis, and then processed into paraffin, sectioned at 4 μm using the microtome Leica RM2235 (Leica Microsystems Ltd.), and stained with hematoxylin and eosin (HE) for the detection of histological lesions by light microscopy. The other samples were kept at −80 °C until used for virological examinations, using qRT-PCR. On days 14 p.i., serum samples were collected from surviving animals, and stored at −80 °C until used. Serum antibodies against IV were tested using ID Screen® Influenza A Antibody Competition ELISA kit (IDvet) and hemagglutination inhibition tests (HI) according to World Health Organization manual on animal influenza diagnosis and surveillance. The study was carried out according to the European Union Animal Welfare legislation, and also complied with the current laws of China.

3. Results and discussion

Six dogs of the two experimental groups developed clinical signs, including conjunctivitis, sneezing, nasal discharge, mild coughing and seroconversion, and finally recovered from the disease, as previously described (Song et al., 2013). Virus shedding from the inoculated dogs was confirmed by qRT-PCR from day 1 to 5 p.i. The peak titers of the nasal swab elutes were $10^{3.0}$–$10^{4.9}$ EID50/mL at day 1–3 p.i., by titration in 10-day-SPF-eggs. The HA and NA gene segments from shed viruses were identical to the inoculated virus. The rectal swab elutes from the dogs were negative for IV. The control dogs showed no clinic signs, virus shedding and seroconversion.

During the study, one of the exposure cats showed clear manifestations of illness with conjunctivitis, sneezing, nasal discharge and depression from day 3 to 4 p.i. qRT-PCR data showed that the M gene of IV was present in the nasal swab elutes from the ill cat and another cat (Table 1). The peak titers were $10^{3.1}$–$10^{4.3}$ EID50/mL at day 3–4 p.i. The HA and NA gene segments from shed viruses were identical to the inoculated virus. The rectal swab elutes from the two cats were negative for IV. The swab elutes from the rest of the sentinel cats were negative for IV. Necropsy examination found gross lesions in the lung of the ill cat, including multifocal to coalescing reddish consolidations. The ill cat showed the histopathologic features (Fig. 1), including suppurative tracheitis, multilobular or diffuse necrotizing tracheobronchitis, multilobular bronchiolitis and alveolitis. The nasal, trachea and lung tissues from the ill cat were positive for the M gene of IV by qRT-PCR (Table 1), the other tissue samples negative. The tissue samples collected from the other cats on days 4 and 14 p.i. were negative for IV. Seroconversion was confirmed in the surviving cats (Table 1). And the control cats showed no clinic signs, virus shedding and seroconversion. During the study, none of the cats died of the disease.

Eight of the exposure chickens showed clear manifestations of illness with conjunctivitis, sneezing, nasal discharge, depression and ruffled feathers from day 3 to 5 p.i., and 2 chickens progressed to loss of consciousness and then died quickly at day 4 p.i. The M gene of IV was