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A novel canine influenza H3N2 virus isolated from cats in an animal shelter

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ABSTRACT

The interspecies transmission of avian-origin H3N2 canine influenza virus (CIV) to dogs was first reported in 2007. The present study characterized a novel CIV H3N2 isolated from cats in an animal shelter. A comparative analysis of the deduced amino acid sequences of the A/Canine/Korea/CY009/2010(H3N2) (CY009) and A/Feline/Korea/FY028/2010 (H3N2) (FY028) strains isolated from dogs and cats, respectively, in the animal shelter identified point mutations in 18 amino acid positions within eight viral genes. Interestingly, CY009 and FY028 replicated well in specific pathogen-free embryonated chicken eggs and in mice, respectively. Mice infected with the FY028 strain exhibited significant over expression of IL-10, TNF-α, and IFN-γ ($p < 0.001$) at 3 days postinfection. Thus, an emergency monitoring system should be developed to identify influenza mutations that occur during interspecies transmission in companion animals and for continuous public health surveillance.

1. Introduction

Influenza viruses (H2N2, H7N7, and H7N3) isolated from birds, seals, and humans can all replicate in experimentally-inoculated cats (Hinshaw et al., 1981; Paniker and Nair, 1970, 1972; Romva'ry et al., 1975), while human influenza viruses, H3N2 and H2N2, and influenza B virus can also be transmitted to uninfected cage mates (Hinshaw et al., 1981; Harder and Vahlenkamp, 2010). These observations highlight the need to determine the epidemiological role played by infected cats. However, none of these viruses has been isolated naturally from asymptomatic/symptomatic cats, with the exception of H5N1, which was isolated from cases of avian-to-cat interspecies transmission in Thailand (Songserm et al., 2006), Iraq (Yingst et al., 2006), and Germany (Klopfleisch et al., 2007a, 2007b). Recently, cats have emerged as potential hosts for influenza infections. Several studies have reported the susceptibility of felids to infection by the avian H5N1 and the pandemic H1N1 influenza viruses (Kuiken et al., 2004; Sponseller et al., 2010). Furthermore, there is serological evidence of H3N2 influenza infections in cats (Jeoung et al., 2012; McCullers et al., 2011; Said et al., 2011; Seiler et al., 2010).

The interspecies transmission of avian influenza virus H3N2 to dogs was first reported in South Korea during 2007 (Song et al., 2008a) and the results of an investigation into the systemic transmission of canine influenza virus (CIV) H3N2 between dogs was published in the following year (Song et al., 2009). The pathogenicity of CIV H3N2 was demonstrated in dogs, which had a severe respiratory syndrome (associated with high fever and coughing) and pathogenic lung lesions (Jung et al., 2010). Similarly, sporadic cases of respiratory diseases associated with CIV H3N2 infections were detected in the dog population in southern China (Li et al., 2010) and these results

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corroborated those of a 2007 study that found evidence of CIV H3N2 infections in Korean dogs. Recently, the interspecies transmission of CIV H3N2 to domestic cats was reported in South Korea (Song et al., 2011). The eight viral genes (HA, NA, PB1, PB2, PA, NP, M, and NS) isolated from infected cat were almost identical to those of the canine influenza H3N2 virus, thereby suggesting interspecies transmission from dogs to cats (Song et al., 2011). Thus, there is a public health concern related to the possible emergence of new recombinant feline or canine influenza viruses in companion animals and a threat of zoonotic infections (Song et al., 2011).

The present study aimed to determine whether CIV H3N2 is transmitted naturally from dogs to cats, as well as performing a comparative analysis of CIV H3N2 strains isolated from dogs and cats.

2. Material and methods

2.1. The animal shelter and the disease outbreak history

The interspecies spread of CIV H3N2 infection was investigated in an animal shelter in Gyunggido, which had an average population of 400 dogs and 60 cats. The shelter had four buildings for housing animals (three for dogs and one for cats). Each room in the animal houses could accommodate approximately 20–40 individuals. Most animals were kept in individual cages, although dogs were kept together in a floor-type animal room in one building. During October 2010, mild to severe symptoms of respiratory disease, including dyspnea, coughing, and high fever, were observed in 25 street dogs at the shelter. Three weeks later, over 300 dogs were infected with avian-origin influenza virus H3N2, while four cats exhibited similar clinical signs (dyspnea and coughing) at the same time. Two weeks later, 28 cats began to present significant signs of respiratory disease. The affected dogs experienced 77% morbidity and 23% mortality within the first 3 weeks of CIV infection, while the affected cats experienced 46.6% morbidity and 21.7% mortality 2 weeks after the disease onset in cats.

2.2. Antigen detection

The influenza viral antigens in lung specimens from dead animals and nasal swabs from infected animals were detected rapidly using a commercially available Antigen Rapid CIV Ag Test Kit (Bionote Cat No. RG 11-07, South Korea). This test kit was used to detect influenza A virus nucleocapsid protein (NP) in the tissue and/or swab samples, according to the manufacturer's instructions.

2.3. Genome sequencing

Virus isolation and propagation were performed in 10-day-old specific pathogen-free (SPF) embryonated chicken eggs (ECEs) and the virus subtypes were determined by RT-PCR (Song et al., 2008a). The nucleotide sequencing of the eight gene segments from the virus isolates was performed as described previously, with some modifications (Song et al., 2008a). The sequences of the isolated viral genes were analyzed and aligned using the Bioedit program (www.mbio.ncsu.edu/BioEdit/bioedit.html). The HA gene nucleotide sequence similarities of the influenza viruses isolated from five species (pig, chicken, duck, dog, and cat) were compared using SimPlot version 3.5.1 (http://sray.med.som.jhmi.edu/SCRoftware/simplot). The sequences of the complete genes for CIV H3N2 strains was deposited at the GenBank under accession numbers KC755899 and KC755926.

2.4. Passages in embryonated chicken eggs and mice

The swab and homogenized tissue samples (10% w/v) were prepared in 1 ml of PBS containing 1% gentamycin. The debris was pelleted by centrifugation ($2000 \times g$, 10 min) and the supernatant was collected. The supernatant samples were serially diluted 10-fold and inoculated into 10-day-old ECEs. After 3 days' incubation, the allantoic fluid was collected and the HA activity was tested. The virus titer of each sample was calculated using the Reed–Muench method (Reed and Muench, 1938).

Four female BALB/c mice (8 weeks old) were inoculated intranasally with 30 μl of the supernatant sample. Five days postinoculation (p.i.), blood and lung tissue samples were collected from all of the mice by necropsy and analyzed to detect CIV antigen using the rapid test kit.

2.5. Cytokine profiling

Mice were injected intranasally with 30 μl inocula of the A/Feline/Korea/FY028/2010 (H3N2) (FY028) and A/Canine/Korea/CY009/2010(H3N2) (CY009) strains and PBS (control), and blood samples were taken at 3 and 9 days p.i. The blood samples were collected using sodium citrate tubes and were centrifuged at $1000 \times g$ at 4 °C for 10 min. The plasma samples were harvested and filtered through a sterile 0.22 μm filter system and stored immediately at −20 °C until use. Next, cytokine assays were performed using a Bio-Plex™ Mouse Cytokine 8-plex panel (BioRad, USA) according to the manufacturer's instructions.

2.6. Statistical methods

One-way ANOVA was used to analyze the data. Data from at least three independent experiments were expressed as the mean ± S.E.M. The main effects were compared using a Newman-Keuls post-test. $p < 0.001$ was considered significant.

3. Results

3.1. Analysis of the HA, NA, and M genes in CIV H3N2

The divergence and percentage identity between the HA protein from CY009 and FY028 strains were 0.4% and 99.6%, respectively. For the NA protein and the M protein, the results were 0.4% and 99.1%, and 0.3% and 99.7%, respectively (data not shown). Compared with the A/canine/Korea/GCVP01/2007 strain (the first strain isolated in 2007), the deduced amino acid sequences of the FY028 and CY009 strains shared 98.8% and 98.8% identity with