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Comparative analysis of virulence of a novel, avian-origin H3N2 canine influenza virus in various host species

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ABSTRACT

A novel avian-origin H3N2 canine influenza A virus (CIV) that showed high sequence similarities in hemagglutinin and neuraminidase genes with those of non-pathogenic avian influenza viruses was isolated in our routine surveillance program in South Korea. We previously reported that the pathogenicity of this strain could be reproduced in dogs and cats. In the present study, the host tropism of H3N2 CIV was examined by experimental inoculation into several host species, including chickens, pigs, mice, guinea pigs, and ferrets. The CIV infection resulted in no overt symptoms of disease in these host species. However, sero-conversion, virus shedding, and gross and histopathologic lung lesions were observed in guinea pig and ferrets but not in pigs, or mice. Based on the genetic similarity of our H3N2 CIV with currently circulating avian influenza viruses and the presence of α-2,3-linked rather than α-2,6-linked sialic acid receptors in the respiratory tract of dogs, we believed that this strain of CIV would have avian virus-like receptor specificity, but that seems to be contrary to our findings in the present study. Further studies are needed to determine the co-receptors of hemagglutinin or post-attachment factors related to virus internalization or pathogenesis in other animals.

1. Introduction

The first cases of canine influenza virus (CIV) infection with sustained transmission in canine populations were recognized in the United States in 2005 (Crawford et al., 2005). Since the isolation of H3N8 equine influenza virus from dogs showing clinically apparent respiratory signs, outbreaks of equine-origin H3N8 CIV have been reported in the United Kingdom and Australia (Kirkland et al., 2010; Newton et al., 2007). Although the emergence of equine-origin H3N8 CIV has been an important event in the interspecies transmission of influenza A viruses to dogs, the epidemiologic link of H3N8 transmission from horses to dogs remains unclear (Kirkland et al., 2010; Newton et al., 2007). Another case of the susceptibility of dogs to influenza A virus infection through interspecies transmission was reported in Thailand, where a highly pathogenic H5N1 avian influenza virus infection occurred in dogs after they ate a duck carcass harboring the virus (Songserm et al., 2006). Although experimental and epidemiologic studies have been performed to investigate viral transmission among different animal species, the role of dogs in H5N1 virus adaptation to mammals has only been speculated upon (Giese et al., 2008; Maas et al., 2007).

Recently, an H3N2 CIV with more than 95.5% nucleotide sequence homology with currently circulating strains of avian influenza virus was isolated in South Korea (Song et al., 2008). It is notable that the avian-origin H3N2 CIV generated sustained transmission (dog-to-dog) and disease reproduction in experimental challenge study (Lee et al., 2009; Song et al., 2009a) because previously reported CIVs could not reproduce the disease. Furthermore, the virus has been transmitted to different companion

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Table 1
Seroconversion of inoculated or contact animals.

Group	Number of seroconverted animals[a]		
	0 dpi	7 dpi	14 dpi
Ferret – inoculated	0/3	3/3	3/3
Ferret – contacted	0/3	3/3	3/3
Guinea pig – inoculated	0/3	3/3	3/3
Guinea pig – contacted	0/3	0/3	0/3
Chicken	0/9	2/9	4/9
Mouse	0/10	0/10	0/10
Pig	0/3	0/3	0/3

[a] The data are represented by the number of sero-positive animals divided by the number of inoculated or contact animals.

species, specifically domestic cats (Song et al., 2011). A more recent surveillance study in China showed that H3N2 influenza virus isolates from street dogs were similar to those of an H3N2 feline influenza virus isolated from South Korea (Su et al., 2013). These reports suggest that cats may be potential alternate hosts of H3N2 CIV.

Despite intensive global surveillance to monitor further viral evolution, the concern about zoonotic transmission has continuously escalated in public health because of the possibility that a novel pandemic strain could emerge by viral reassortment (Sponseller et al., 2010; Zhu et al., 2011).

Companion animals (e.g., canine, feline, and rodent species) in South Korea and China have many opportunities to encounter H3N2 CIV-infected dogs and cats in outdoor area or in pet shops and veterinary clinics because of the current endemicity of the virus in these countries. Hospitalized animals with certain diseases would be particularly vulnerable to the influenza virus. In addition, influenza can be transmitted between pet animals and livestock (e.g., pigs and chickens) on farms if the livestock are susceptible to H3N2 CIV. Such interspecies transmission of H3N2 CIV may also cause human infections because of close contact. However, the infectivity and pathogenicity of the currently circulating H3N2 CIV in these animal species have not been reported to date. Here, we aimed to determine the comparable infectivity and pathogenicity of the avian-origin H3N2 CIV in different animal species, including chickens (representative avian species), pigs (potential "mixing vessel" species), mice (representative rodent species), guinea pigs (experimental models of influenza in humans), and ferrets (considered the most suitable animal models of influenza in humans).

2. Results

2.1. Serologic responses of different animal species to H3N2 CIV infection

Seropositivity was shown in four of nine infected chickens, in infected guinea pigs and ferrets, and in contact ferrets (Table 1). However, none of the infected mice and pigs or contact guinea pigs showed any evidence of virus-specific antibody responses, and all animals in the negative control group (to which PBS was administered) were seronegative as well.

2.2. Quantification of the shedding of H3N2 CIV in different animal species

To verify the infective and replicative ability of this strain of H3N2 CIV in different animal species, we measured virus titers in the respiratory tract or digestive tract of infected animals. As shown in Fig. 1, virus isolation from nasal swabs obtained at various time points post-inoculation (0–14 dpi) confirmed replication of the virus in the upper respiratory tract of ferrets and guinea pigs. Viral titers in three infected ferrets 1 dpi were $10^{3.1}$, $10^{3.1}$, and $10^{3.5}$ EID_{50}/ml, respectively, and virus was detectable 6 dpi and 7 dpi, while the naïve ferrets in direct contact with infected ferrets shed only low levels of virus on days between 10 and 11 dpi. In the three infected guinea pigs, nasal shedding viral titers were $10^{5.8}$, $10^{6.0}$, and $10^{6.1}$ EID50/ml at 1 dpi, respectively, and the presence of virus was detected between 5 and 7 dpi. However, the virus in the contact guinea pigs was not detectable by the end of the study. No virus shedding was detected in the digestive tract of chickens or in the respiratory tracts of, mice, and pigs (data not shown).

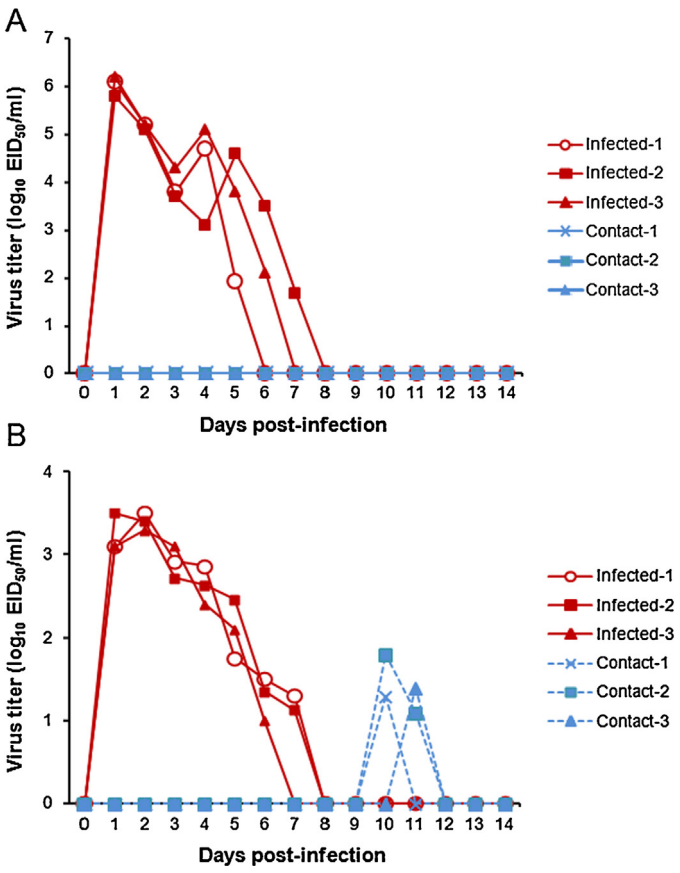

Fig. 1. Nasal shedding of H3N2 CIV from intranasally infected guinea pigs (A) and ferrets (B) and from directly contacted naïve animals. Guinea pigs and ferrets were intranasally inoculated with $10^{7.1}$ EID_{50} (50% egg infectious dose) of virus, and naïve animals were paired with each infected animal species 6 h post-inoculation. Nasal swabs were collected daily until 14 dpi, and relative viral titers were determined by a commercial one-step real-time reverse transcriptase-PCR (RT-PCR) kit. Virus titers were estimated by converting the cycle threshold (C_t) value to EID_{50}/ml by using the coefficient of correlation from the standard curve.

2.3. Clinical signs, gross lesions, and histopathology in different H3N2 CIV-infected animals

No experimental animals either infected or contacted with H3N2 CIV showed clinical signs (including mild respiratory signs) during the study period. However, differences in infection from this strain of H3N2 CIV in the animal species were confirmed by postmortem examination and histopathologic examination of the lungs. On necropsy, primary gross lung lesions were characterized by pulmonary consolidation in ferrets and guinea pigs. Severe reddish consolidation was observed in the lungs of the infected guinea pigs and ferrets 14 dpi (data not shown), whereas no gross lesions were observed in lungs of infected chickens, mice, or pigs. Histopathologic changes were also observed in the lungs of inoculated guinea pigs and ferrets (Fig. 2). In the infected guinea pigs, there was aggregation of lymphoid follicles with clusters of mostly mononuclear