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Beagle dogs have low susceptibility to BJ94-like H9N2 avian influenza virus

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ABSTRACT

In China, dogs are considered significant intermediate hosts of influenza viruses and have been reported to be infected with H9N2; additionally, a reassortant H9N2 virus has been isolated in dogs. Currently, there are three different lineages of H9N2, including BJ94-like, G1-like, and Y439-like lineages; BJ94-like H9N2 has been circulating in various types of poultry in southern China. Additionally, a number of studies have reported that H9N2 evolves rapidly and is frequently reassorted with H5N1, H7N9, or H10N8 to generate novel reassortants, which is significant for poultry and humans. In this study, two groups of beagles were inoculated either intranasally or intratracheally with the BJ94-like H9N2 virus. However, only four of the seven beagles in the intranasal group and five of the seven beagles in the intratracheal group displayed a mild fever; similarly, only two of the five beagles in the intranasal group and three of the five beagles in the intratracheal group underwent seroconversion. However, no viruses were detected from nasal swabs or rectal swabs or in the lungs of any of the inoculated beagles. Our results demonstrated that beagles have low susceptibility to the BJ94-like H9N2 avian influenza virus, which is the main virus circulating in southern China, indicating that the BJ94-like H9N2 virus does not currently threaten the health of dogs.

1. Introduction

The H9N2 influenza virus is one of the widely distributed viruses present in the world (Alexander, 2007; Guan et al., 2000; Xu et al., 2007). Although the H9N2 influenza virus does not cause severe morbidity or mortality in poultry, the H9N2 influenza virus can enhance its pathogenicity towards mammals and poultry by rapid evolution or recombination with other influenza viruses. In 1999, the mild H9N2 influenza virus infected individuals in Hong Kong and was shown to be similar to another H9N2 virus isolated from a quail in Hong Kong in late 1997 (Lin et al., 2000; Peiris et al., 1999; Saito et al., 2001). Previous studies have demonstrated that some internal segments of H5N1 were introduced into the H9N2 virus, which indicated that the novel influenza virus could form potential pandemics (Guan et al., 2000; Xu et al., 2007). In 2013 and 2014, the avian influenza A H7N9 virus infected humans and then quickly spread, causing severe mortality in China (Gao et al., 2013; Liu et al., 2014). Additionally, numerous studies have revealed that six internal segments of the H7N9 virus originated from different H9N2 viral groups (Liu et al., 2013, 2014; Qi et al., 2014). On November 30, 2013, a novel reassortant avian influenza A H10N8 virus caused the death of a 73-year-old woman, and sequence analyses showed that the six internal segments were also from the H9N2 virus (Chen et al., 2014). Therefore, reassortment of the novel influenza virus with the H9N2 virus could create a serious threat to public health and requires more attention.

Dogs were considered unsusceptible to influenza viruses for many years, until the equine-origin H3N8 canine influenza virus (CIV) was first isolated in the United States in 2004 (Crawford et al., 2005). In China, Li and colleagues first isolated the CIV H3N2 virus, which derived from the avian influenza virus (AIV) in 2006 and 2007 (Li et al., 2010). Additionally, in recent years, different influenza viruses were isolated from dogs, such as the 2009 pandemic H1N1 virus, the reassortant H3N1 virus, the avian highly pathogenic H5N1 virus, and the avian low pathogenic H9N2 virus (Lin et al., 2012; Song et al., 2012; Songserm et al., 2006; Sun et al., 2013). Furthermore, a number of serology studies revealed that dogs could become infected with H3, H5, H1 and H9 (Su et al., 2014b,c; Sun et al., 2013; Yin et al., 2014). These finding indicated that dogs function as "mixing vessels", similar to pigs, by infecting and reassorting different influenza subtype viruses.

The three different lineages of H9N2 are the BJ94-like, the G1-like, and the Y439-like or Korean-like lineages, and the prototypes viruses are A/Chicken/Beijing/1/94, A/Quail/HongKong/G1/

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97, and A/Duck/Hong Kong/Y439/97, respectively (Guan et al., 1999; Xu et al., 2007). In southern China, BJ94-like has recently been circulating in various types of poultry and has become the primary lineage in poultry (Choi et al., 2004; Chu et al., 2011; Xu et al., 2007).

The avian influenza A reassortant H9N2 virus has been isolated, and a serology study in dogs reported that 44.85% of dogs were positive against H9N2 virus in Guangxi (a province in southern China) by a hemagglutination inhibition (HI) test according to standard protocols with $\geqslant$40 titer for positive (Sun et al., 2013). However, our previous studies revealed that the serology prevalence of dogs infected with H9N2 were 0.0% in pet dogs by HI $\geqslant$ 40 titer and 2.9% in feral dogs by HI $\geqslant$ 20 titer (Su et al., 2014a,c). Currently, the BJ94-like virus is circulating in various types of poultry in southern China. Additionally, H9N2 has frequently reassorted with H5N1, H7N9, or H10N8 to generate novel reassortants, which is significant for poultry and humans (Chen et al., 2014; Guan et al., 2000; Liu et al., 2013, 2014; Xu et al., 2007). Therefore, we evaluated the BJ94-like H9N2 influenza virus infection in dogs to further understand the susceptibility of dogs to the BJ94-like H9N2 virus and to understand whether this virus has a potential threat towards dogs in southern China.

2. Results

2.1. Animal experiment

Three control beagles showed no fever or other clinical signals, no viral shedding, and no seroconversion (Fig. 1A–F). No hemagglutination occurred in the allantoic fluids from the 9- to 12-day-old embryonated specific-pathogen-free (SPF) chicken eggs, which were incubated at 37 °C for 72 h and injected with water washings from the nasal and rectal areas (Fig. 1E and F). The real-time qRCR were performed to confirm that no viral RNA in nasal and rectal swabs (data not shown). These findings indicated that the inoculated dogs did not shed any viruses. No viral replication was detected by EID_{50} and real-time qPCR in the lungs, tracheas, and turbinates from the beagles that were euthanized on day 4 p.i., and these tissues showed no pathology in the hematoxylin-eosin staining (H&E), and no viral antigens were detected by the immunohistochemical experiment (Fig. 1G). Additionally, only four of seven beagles in the intranasal group and five of seven beagles in the intratracheal group displayed a mild fever, and none of the inoculated animals showed any notable symptoms of disease (Fig. 1A, B and Table 1). Serum antibody levels were measured using hemagglutination inhibition (HI), and two of five remaining beagles in the intranasal group and three of five remaining beagles in the intratracheal group underwent seroconversion (Fig. 1C, D and Table 1). The *P* value between intranasal group and intratracheal group for mild fever and seroconversion were more than 0.05 which indicated that there are no significant different between intranasal group and intratracheal group for slight fever and seroconversion (Table 1).

3. Discussion

Two different H9N2 viral lineages have been circulating in southern China (the BJ94-like and G1-like lineages): BJ94-like viruses have been circulating in various types of poultry, whereas G1-like is predominant in quail (Guan et al., 2000, 1999; Xu et al., 2007). The long-term cocirculation of H9N2 with other influenza viruses in intermediate hosts could undergo potential genetic reassortments to create novel pandemic viruses (Peiris et al., 2001; Xu et al., 2007). Additionally, during 2013–2014, the novel influenza viruses H7N9 and H10N8 fatally infected individuals (Chen et al., 2014; Gao et al., 2013; Liu et al., 2014). Interestingly, all six internal genes of H7N9 and H10N8 were closely related to avian influenza H9N2 (Chen et al., 2014; Liu et al., 2014; Qi et al., 2014).

In this study, beagles were inoculated with the BJ94-like GD/V H9N2 virus either intranasally or intratracheally, and the results indicated that the beagles showed low susceptibility to this virus: only two of five beagles in intranasal group and three of five beagles in intracheal group underwent seroconversion, and four of seven beagles in intranasal group and five of seven beagles in intracheal group displayed slight clinical signals.

Our results did not match some previous reports, which showed that the AIV H9N2 virus could cause relative severe infection in dogs and cats when inoculated intranasally and orally (Amirsalehy et al., 2012; Zhang et al., 2013). The host and virus are the factors for the virus infection. For host, there are natural species barriers between avian and mammal for influenza viruses, and overcoming the natural species barriers through mutation, adaptation, or reassortment with other influenza viruses remains the greatest concern (Jackson et al., 2009; Katz et al., 2009). BJ94-like GD/V H9N2 virus is avian-origin influenza and the H9N2 virus can robustly stimulate Type I and Type III interferon (IFN) expression(Sutejo et al., 2012), which may relate to the mild effect in inoculated beagles. For virus, the distinct characterization of the different H9N2 strains could cause the varying replication in dogs, as seen in previous studies on H5N1in dogs (Chen et al., 2010; Giese et al., 2008; Maas et al., 2007). Influenza A virus has a RNA-dependent RNA polymerase constituted from PA (polymerase acidic), PB1 (polymerase basic 1) and PB2 (polymerase basic 2) subunits and assembled with nucleoproteins (NP) and a viral RNA (vRNA), forming a viral ribonucleoprotein (RNP) complex in the host nucleus (Neumann et al., 2004). The RNP is significant for the viral replication, viral infection, activity and pathogenesis (Li et al., 2014; Naffakh et al., 2008; Paterson et al., 2014). The RNP subunits of the virus that used in the study of Zhang et al. is not the BJ94-like lineage, which is different from the BJ94-like GD/V H9N2 virus. The different RNP subunits of viruses may be the significant reason for the vary results.

Besides, the different animal species used in the studies, such as SPF beagles in our study and undefined animal species in the study of Zhang et al. and rural dogs in study of Amirsalehy et al., could have caused the distinct results. As well, although antibodies against H9, H5, and H7 influenza A subtypes in the study of Zhang et al. were not detected in the animals, the undefined species of dog and rural dogs might have been infected by other viruses that could potentially promote influenza virus infection in the dogs, thus affecting the results of the experiments.

The viral dose used to infect the dogs were the same in our study ($10^{6.3}$ EID_{50}) and in the study of Zhang et al. (2×10^6 EID_{50}), which demonstrated that the dose is not the reason for the different results. However, the different doses were used between our study and the study of Amirsalehy et al. ($10^{7.5}$ EID_{50}). Approximately 10^6 EID_{50} were used to infect dogs in most previous research (Chen et al., 2010; Crawford et al., 2005; Kim et al., 2013), so $10^{6.3}$ EID_{50} virus is appropriate dose to inoculate beagles to evaluate the infection.

BJ94-like H9N2 has been circulating in various types of poultry in southern China, and A/chicken/Guangdong/V/2008(H9N2) belongs to the BJ94-like lineage. A genetic analysis showed that a glutamine mutation to leucine at the receptor binding position 226 of the HA gene could promote viral infection in mammals (Suzuki et al., 2000). Additionally, a previous researcher reported that the replicative efficiency and virulence of A/chicken/Guangdong/V/2008(H9N2) was significantly enhanced in mice with lysine in position 627 of PB2 instead of glutamic acid in that position (Tian et al., 2012). These findings revealed that A/chicken/