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The Pathogenesis of H3N2 Canine Influenza Virus in Beagle Dogs

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Abstract: Canine Influenza Virus (CIV) infection caused severe and acute respiratory symptoms. In recent years, surveys of CIV infection in dogs have been reported worldwide. It has received considerable attention from veterinarians, virologists and epidemiologists. This study shows that CIV infected beagle dogs developed some histopathological changes. These can clarify the prevalence of CIV infection, the CIV mechanism of pathogenesis and provide experimental evidence in beagle dogs.

Key words: Canine influenza virus, H3N2, phylogenetics, influenza A virus, pathogenesis, avian-origin

INTRODUCTION

The influenza A virus, a member of the genus *Orthomyxo virus*, it has a very wide host range, from birds to mammals and it shows varying degrees of host adaptation (Cardona *et al*., 2009; Hadipour, 2011). Canine influenza virus was first identified in racing greyhounds in Florida in January, 2004 (Payungporn *et al*., 2008). Canine influenza is caused by 2 subtypes of influenza A virus: H3N2 and H3N8. In 2005, the H3N8 CIVs is known to an equine-derived H3N8 influenza virus and was first identified in dogs in the United States and in 2007, the H3N2 CIVs are of origin avian and detected in dogs in South Korea and China (Crawford *et al*., 2005; Lee *et al*., 2010; Li *et al*., 2010; Payungporn *et al*., 2008; Song *et al*., 2008). Regardless of subtype, H3N8 or H3N2 CIV could infect nascent individuals and causes clinical signs. The most common sign of canine influenza is a mild respiratory disease that resembles infectious tracheobronchitis. The experimental reproduction of the disease caused by H3N2 CIV induced clinical signs including coughing, sneezing, nasal discharge, fever and shedding of the virus in nasal discharge (Lee *et al*., 2010; Song *et al*., 2008, 2009). In previous pathological findings, the infection produced a distinctively severe and persistent bronchopneumonia with neutrophil infiltration and apoptosis in the tracheal epithelium (Jung *et al*., 2010).

2In view of the CIV may cause great dangerous and safety concerns of influenza virus for public, researchers have to pay more attention to the CIV infection. The present study by histopathological observation and the hemagglutination inhibition test, using the laboratory room isolated from the CIV infected beagle dogs pathological changes of tissues and organs has been systematically studied in order to clear CIV infection in dogs after the pathological changes of tissues and organs. It can clarify CIV infection, the CIV mechanism of disease pathogenesis and provide experimental evidence in beagle dogs.

MATERIALS AND METHODS

Virus: The CIV CGD1 strain A/canine/Guangdong/01/2007 (H3N2), GenBank Accession No GU433369.1. It was isolated in the laboratory and store at -80°C.

Animals: Fifteen CIV-negative beagles, 10 weeks of age were randomly divided to two groups: experimentally Infected (I) and Noninfected (NI). The dogs were maintained in a BSL-2 isolation facilityunder standard husbandry conditions.

Experimental infection with isolated virus: Researchers experimentally reproduced viral infection in 10 weeks old conventional beagle dogs that had been assigned into I and NI groups. Group I dogs (n = 9) were infected intranasally with 2 mL of virus isolate with a titer of 10^6 50% Egg Infectious Dose (EID50)/0.1 mL; group NI dogs (n = 6) were infected intranasally with 2 mL of sterile phosphate buffered saline. Before they were inoculated, the animals were sedated by intramuscular injection of 0.1 mg kg^{-1} acepromazine malate.

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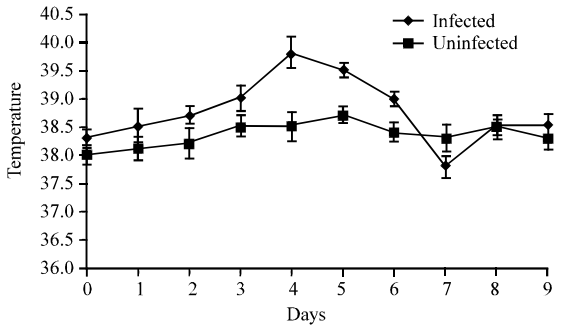

Fig. 1: Clinical body temperature of dogs infected with CIV. They were recorded for 9 dpi. Infected dogs developed a transient clinical fever (≥39.5°C) on day 4th dpi that lasted for only 1 day. The data are expressed as the mean±SE

Clinical observation: The dogs were observed daily for clinical signs of respiratory disease including ocular and nasal discharge, body temperature, sneezing, coughing, dyspnea and depression as described previously (Deshpande *et al*., 2009a, b). The body temperature were tabulated and the average daily scores were calculated as shown in Fig. 1.

Detection of serum responses: To detect antibodies against nucleoprotein and HI for CIV-specific antibodies, researchers analyzed convalescent-phase serum samples from 3 dogs in each group for virus-specific antibodies by ELISA (Animal Genetics, Inc.). The use of this ELISA for CIV detection has been previously validated (Lee *et al*., 2009; An *et al*., 2010). Serum samples were treated with Receptor-Destroying Enzyme (RDE) and evaluated for antibody titers by HI assay, HI assays were performed according to World Organization for Animal Health-recommended procedures (OIE World Organization for Animal Health, 2005).

Anatomy pathogenesis and histopathological changes of infected dogs: At 3, 6 and 9 days post inoculation (dpi), 3 group I dogs and 2 group NI dogs were humanely euthanized for gross and histopathologic examination. All necropsy procedures were performed by veterinary pathologists. All organs were rapidly immersed in 10% neutral formalin buffer to prevent autolysis and stored overnight.

RESULTS AND DISCUSSION

Clinical responses to challenge: Clinical signs including sneezing and nasal discharge were observed in group I animals at dpi 3-9. At 24th h after inoculation, fever developed in group I puppies (meanrectal temperature = 39.80°C) (Fig. 1) and lasted only 1 day. The rectal temperatures of groups NI dogs remained below 39.5°C and no clinical signs were observed throughout the experiment.

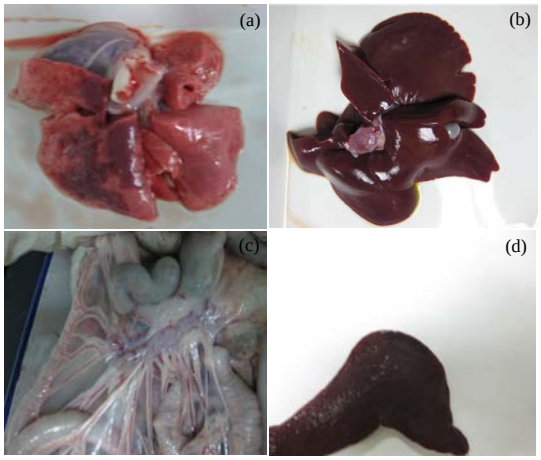

Fig. 2: Anatomy pathogenesis changes of a dog with H3N2 canine influenza virus; a) macroscopic lung lesion; b) liver lesion; c) lymph nodes lesion and d) spleen lesion

Antibody titer: There was no detectable antibody before challenge in all groups. The ELISA assay started detecting positive anti-NP antibody response (>50 of PI value). In group I dogs 4 days after challege, the HI titers of all group I animals indicated >1:64 while groups NI was no detectable antibody. The anti-influenza H3N2 virus antibody titers of group I remained high (>1:64) for the duration of testing (until DPI 7). For the HI test, all samples were also tested with other antigens (H1, H3, H5, H7 and H9) they were all negative.

Anatomy pathogenesis changes: The gross lung lesions induced by experimental H3N2 CIV infection were characterized by severe reddish-tan consolidation, especially involving the intermediate lobes (Fig. 2a) and were of similar severity on dpi 3, 6 and 9. Furthermore, there were necrotic points in the liver (Fig. 2b), lymph nodes makes large and bleed (Fig. 2c). The edge of spleen appear bleeding and necrosis (Fig. 2d).

Histopathologic findings: Histopathologic lesions were observed in the trachea and lungs, liver and extrapulmonary lesions were absent in dogs infected with the isolate (A/canine/Guangzhou/01/2007 [H3N2]).

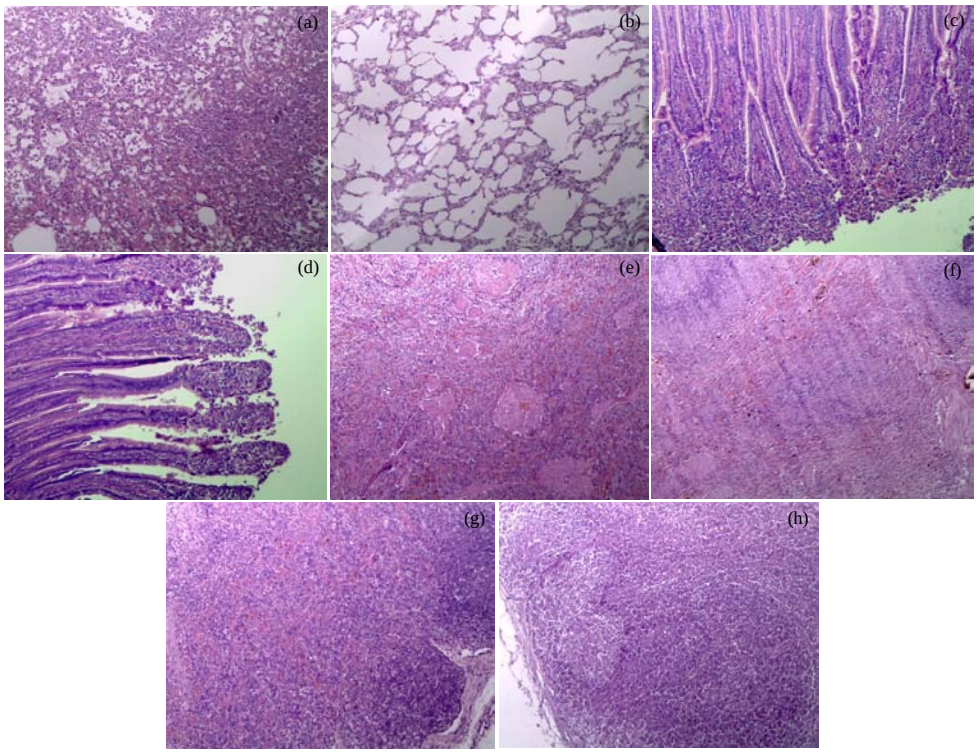

Fig. 3: a, b) Histopathological changes of a dog with H3N2 canine influenza virus infected lung and control; c, d) Infected duodenum and control; e, f) Infected spleen and control and g, h) Infected lymph nodes and control

Although, minor differences in the severity of the histologic findings were observed among the 9 infected dogs, all infected dogs shared the following histopathologic features regardless how long after inoculation tissues were collected.

Lung: The lung of severe virus-infected appeared congestion, alveolar filled with exudates and infiltration of lymphocytes, consolidation and intra-alveolar septa became slightly widened with exudates and erythrocytes (Fig. 3a and b).

Duodenum: Diffuse bleeding, there are inflammation lesions in the end of duodenal. Moreover, a large number of lymphocytes infiltration and lymphoid hyperplasia in the submucosa (Fig. 3c and d).

Spleen: Blood vessels had been seen congestion, hemorrhage and necrosis (Fig. 3e and f).

Lymph nodes: Capillary have been seen the congestion, bleeding, lymphocyte degeneration and necrosis (Fig. 3g and h).

The H3N2 CIV infected dogs exhibited clinical signs including sneezing, loss of appetite, coughing, depression, breathing difficulty, nasal discharge, pneumonia, myositis, cardiac dysfunctions and central nervous syndrome which were consistent with previous studies (Song *et al*., 2008; Li *et al*., 2010; Lee *et al*., 2010). Coughing and nasal discharge was the predominant displayed by the majority of the infected dogs and they gradually developed severe respiratory disease until 9 dpi. However, fever is the most significant clinical feature in influenza virus infection.

It was predicted that the cause of fever would be neutrophils along with the related chemoattractant cytokines including TNF-a, IL-1, IL-8 and interferon (Kim *et al*., 2009). They may play a important role in the pathogenesis of H3N2 CIV in dogs. The H3N2 CIV infected tests prove that the dog has spread already. The researchers believe that this virus may be become the second canine influenza virus (Harder and Vahlenkamp, 2010).

CONCLUSION

It is worthy of attention with the national police dogs, military dogs cited kinds of work become more frequent, dogs will present regional expansion of an influenza pandemic trend, good knowledge of canine influenza virus spread and the associated rapid detection technology and research and development of effective vaccines for the prevention of canine influenza positive the introduction of the virus and the prevalence of far-reaching.

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