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Original article

Emerging of canine kobuvirus in dogs in China, 2015

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Abstract

Canine kobuvirus (CaKoV) is a newly emerging virus in dogs, which relates to the diarrhea of dogs. To investigate the CaKoV infection in dog population, fecal samples of dogs were collected from three provinces of China in 2015. The results of genetic analysis based on the complete VP1 gene showed that six CaKoVs isolates in this study were closely related with the Chinese canine originated isolate CH1 (90.6%-91.9% nucleotide identities). The phylogenetic analysis demonstrated that the Chinese isolates clustered into a unique branch compared with isolates from other countries. The present study suggested that the CaKoVs had established infection in Chinese dog population. The systematic epidemiological investigation should be further carried out to evaluate the prevalence of the CaKoV infection in China.

Key words: canine kobuvirus (CaKoV), dogs, phylogenetic, VP1, China

Introduction

Kobuvirus belongs to the family *Picornaviridae*, a small and non-enveloped virus approximately 27-30 nm in diameter (Reuter et al. 2011), with a single stranded positive sense genomic RNA of 8.2~8.4 kb. Kobuvirus has a very broad host range, which includes humans (Yamashita et al. 1991, Yang et al. 2009), cattle (Yamashita et al. 2003, Khamrin et al. 2008, Jeoung et al. 2011, Candido et al. 2017), pigs (Reuter et al. 2010a, An et al. 2011, Wang et al. 2011, Okitsu et al. 2012, Verma et al. 2013), sheep (Reuter et al. 2010b), goats (Lee et al. 2012), dogs (Di Martino et al.

2013, Li et al. 2016), cats (Choi et al. 2015, Di Martino et al. 2015, Chung et al. 2013) and wild carnivores and wild ruminants (Di Martino et al. 2014, 2015). Based on the genomic organization and sequence similarities, kobuviruses are currently classified into 3 species, *Aichivirus A*, *Aichivirus B* and *Aichivirus C* (<http://www.ictvonline.org/virusTaxonomy.asp?bhcp=1>). Among *Aichivirus A*, three distinct members, including Aichi virus 1 (Aichi virus in human), canine kobuvirus (CaKoV), and murine kobuvirus, have been described (Cho et al. 2015). CaKoV was found to be genetically closely related to mouse kobuvirus and human Aichi virus with 84.0% (Phan et al. 2011)

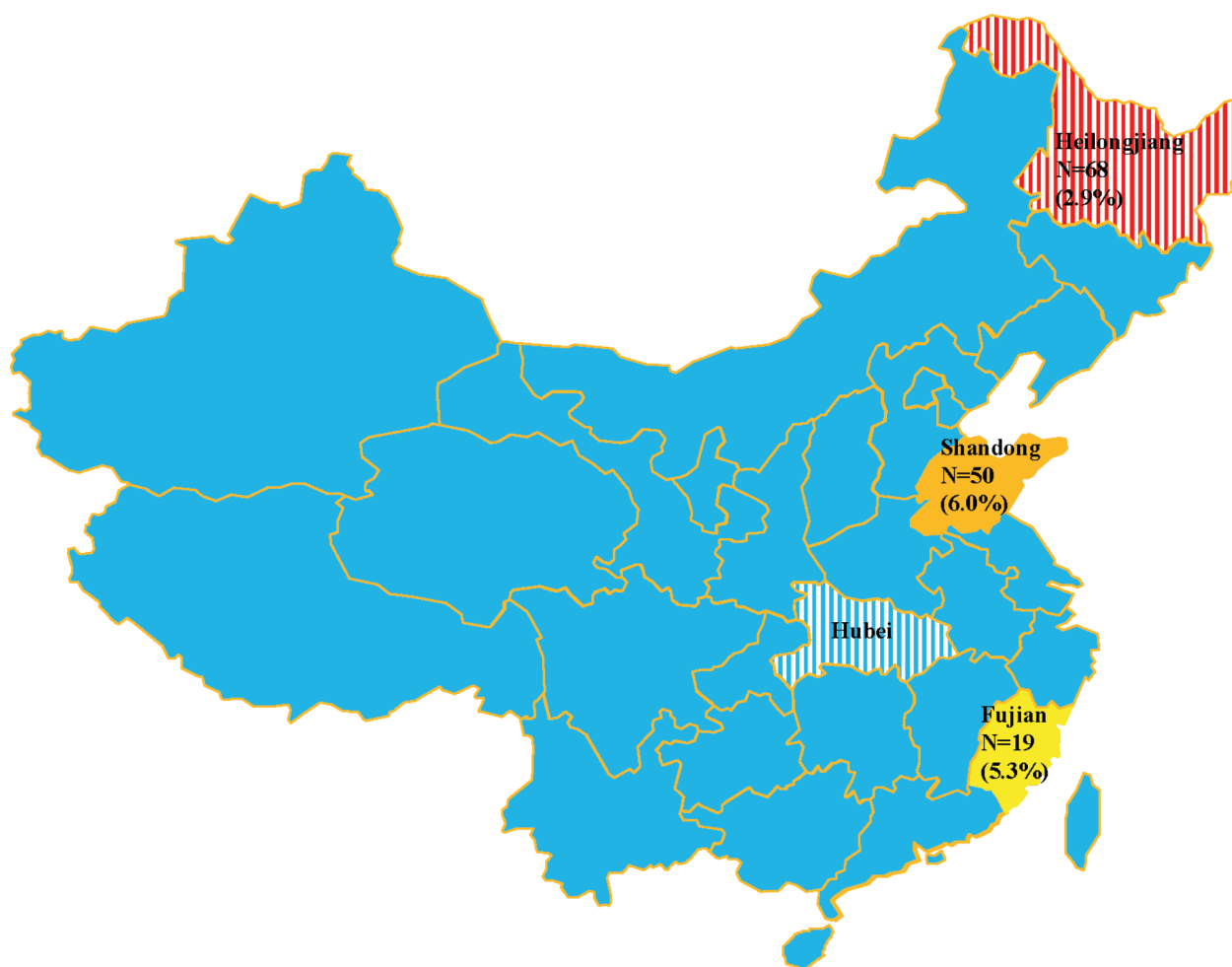


Fig. 1. The map of the sampling regions. The Heilongjiang province, Shandong province, and Fujian province are marked with color of red, orange and yellow, respectively. The number indicates the number of samples and the rate indicates the positive samples ratio. Provinces in which kobuviruses were previously reported in dogs are shown in white stripes.

and 80.0% (Carmona-Vicente et al. 2013) amino acid identity in the polyprotein, respectively. CaKoV was firstly described in 2011 (Kapoor et al. 2011, Li et al. 2011), so it is a newly emerging virus in dogs. Since then, it has been detected in different countries worldwide (Oem et al. 2014, Olarte-Castillo et al. 2015, Li et al. 2016). CaKoV could cause the diarrhea and threaten the health of the dogs. In China, Li et al. first reported the CaKoV infection in dogs in certain regions of Heilongjiang province in 2016 (Li et al. 2016). Kong et al. also documented the CaKoV infection in diarrhea dogs in the same year (Kong et al. 2016). So far, there are only two documents to record the CaKoV infection in dogs in China. The detail information of CaKoV epidemics among dogs in China remains unknown. Therefore, the purpose of this study was to investigate the prevalence and infection of CaKoV in diarrheic or asymptomatic dogs in the part of the territory of China.

Materials and Methods

In the present study, a total of 127 fecal samples of dogs with diarrhea or no clinical syndrome were collected from Heilongjiang, Shandong and Fujian provinces of China from March 12 to December 28, 2015 (Fig. 1), and were stored at -80°C . Viral gene sequencing and analysis were conducted as follows. In brief, viral RNA was directly extracted from fecal samples using RNeasy Mini Kit (Qiagen, Hilden, Germany) and reverse transcription (RT) were carried out under standard conditions using random hexamer. PCR was performed using specific primers (CaKoV-VP1F: 5'-CGAACTCAGAAGATCTCAAT-3' and CaKoV-VP1-R: 5'-ATAGGTGGGCCTATC-TGCAC-3') for VP1 gene segments. PCR products were purified with the QIA quick PCR purification Kit (Qiagen, Hilden, Germany) and cloned into pMD18-T vector (TaKaRa, Dalian, China), then

Table 1. Detection of canine kobuviruses in diseased and healthy dogs.

Health status	Province	No. samples	No. positive
Diseased	Heilongjiang	28	2(7.1%)
	Shandong	16	2(12.5%)
	Fujian	5	1(20.0%)
	Total	49	5(10.2%)
Healthy	Heilongjiang	40	0
	Shandong	34	1(2.9%)
	Fujian	14	0
	Total	88	1(1.1%)
Total	Heilongjiang	68	2(2.9%)
	Shandong	50	3(6.0%)
	Fujian	19	1(5.3%)
	Total	127	6 (4.7%)

sequenced. Sequences were edited by using SeqMan program and the homology was analyzed by using MegAlign program (DNASTAR, Madison, WI). The phylogenetic tree was generated with the MEGA 5 software by using the Clustal W alignment algorithm and the neighbor joining method, and bootstrap analysis was performed with 1000 replicates. The kobuviruses complete VP1 nucleotide sequences available in GenBank were selected as reference viruses.

Results

The results showed that CaKoVs were identified in six specimens with an overall positive rate of 4.7% (6/127). In the diseased dogs, the average positive rate was 10.2% (5/49), and in the healthy dogs, the average positive rate was 1.1% (1/88) (Table 1). The isolates were named SD2015-1, SD2015-2, SD2015-3, FJ2015-4, HLJ2015-5, and HLJ2015-7 (GenBank accession numbers: KU513555-KU513560).

The nucleotide sequence analysis of VP1 genes showed that the homology between the six CaKoVs isolated were 94.4%-98.3%, which closely related to the Chinese canine-originated isolate CH1 (90.6%-91.9%). And the homology of isolated CaKoVs was 83.0%-86.7% with the isolates from other countries including Italy, United Kingdom, Tanzania, South Korea, USA, and Japan, among which the homology was 87.0%-98.6%. The homology of CH1 VP1 gene was 86.0%-88.9% with the isolates from other countries, as above. The six isolates showed a lower than 75.2% and 69.0% homology with feline kobuviruses and human Aichi viruses, respectively.

The homology of amino acid sequences of VP1 between the six CaKoVs isolates were 97.8%-100%, which were closely related with the CH1 (86.2%-87.6%). The homology of the six CaKoVs

isolates was 88.1%-92.4% with the foreign CaKoVs isolated in Italy, United Kingdom, Tanzania, South Korea, USA, and Japan, and the homology was 92.0%-98.9% between the foreign isolates, and was 82.8%-88.0% between the foreign isolates and CH1. The six CaKoVs isolates showed a lower than 88.0% homology with human Aichi viruses and 74.4% with rat kobuvirus.

The phylogenetic analysis of the six isolates in this study demonstrated that the VP1 genes located in the same cluster with Chinese canine kobuvirus CH1, which co-clustered in the branch of canine/fox clade (Fig. 2). But the Chinese isolates including the CH1 and the six isolates in this study formed a unique cluster (Fig. 2).

The molecular character of VP1 protein indicated that the Chinese isolate CH1 had a deletion of G at site of 174, and had two insertions of LV and P between site of 180-181 and 193-194, respectively. The American strain dog/AN211D/USA/2009 had a deletion of L at site of 118. While the six Chinese isolates in this study kept the same pattern with other countries reference strains except dog/AN211D/USA/2009.

Discussion

In the present study on the 127 fecal samples, five CaKoV-positive samples were identified in diarrheic dogs, and one CaKoV-positive samples was detected in an asymptomatic dog, which suggested that the CaKoVs may be one of the important factors to cause the diarrhea in dogs. However, the real role of CaKoV in diarrheic dogs is unclear, because the virus cannot be isolated from the clinical samples of dogs so far. Our study also showed that co-infection of the CaKoVs and other canine viruses causing diarrhea was common event in some cases (data not shown). The homology and phylogenetic analysis of VP1 indicated

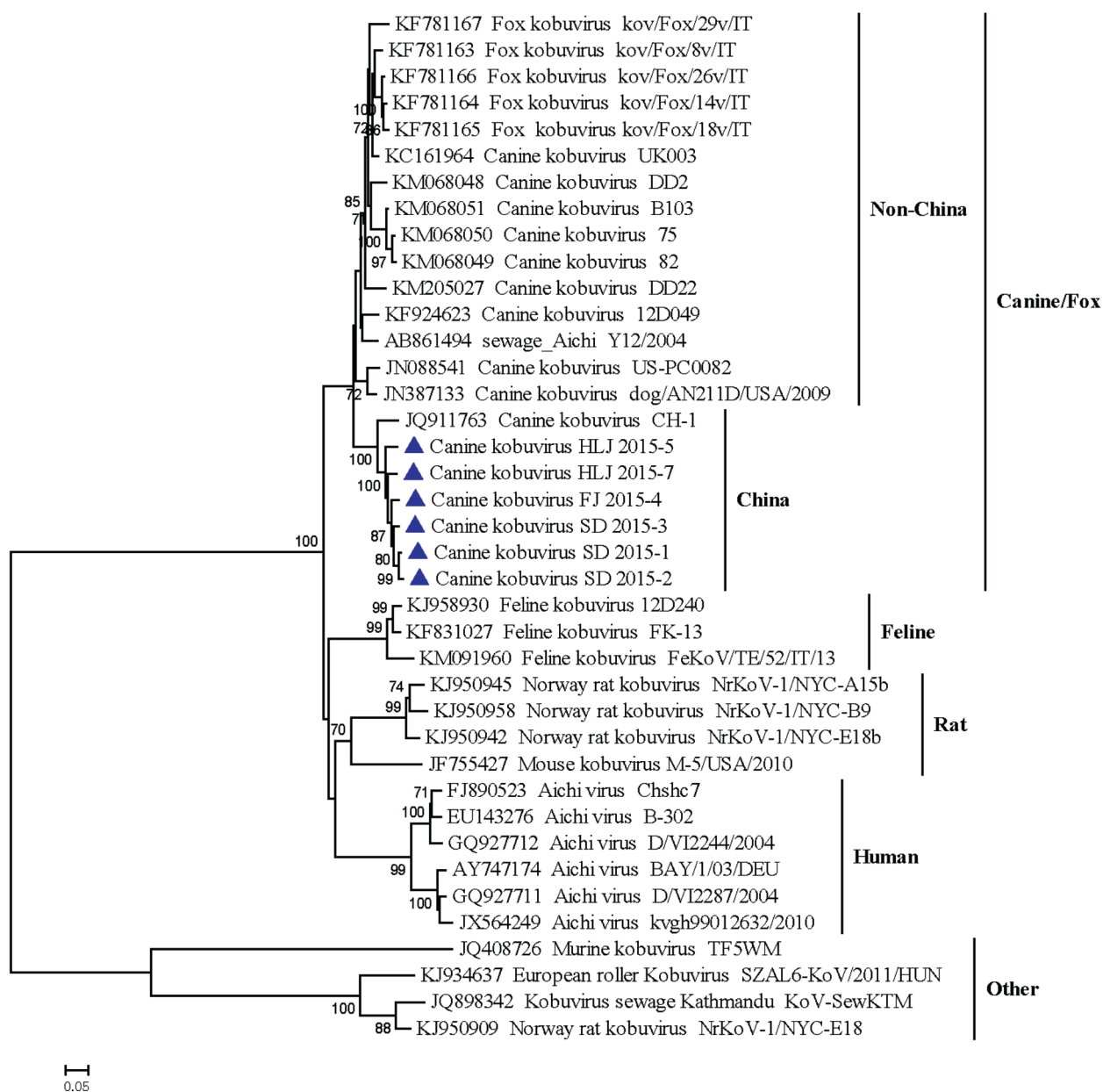


Fig. 2. Phylogenetic analysis of VP1 genes of canine kobuviruses. Phylogenetic tree of the entire VP1 genes of the six canine kobuviruses isolated in this study (marked with black triangle) and the strains with high similarity available in GenBank was constructed using the neighbor-joining clustering method in MEGA version 5 with a p-distance. Bootstrap analysis was performed with 1000 replicates and the bootstrap values for each node are given if >70%. Scale bar indicates nucleotide substitutions per site.

that the six CaKoV isolated in this study were closely related with CaKoV isolates and clustered in the same clade. But the Chinese isolates formed a unique branch, suggesting that the genetic origination of Chinese isolates may be different from other countries isolates and the genetic evolution of Chinese CaKoVs was restricted by geography. The CaKoV positive samples from dogs in this study were detected from three provinces including Heilongjiang, Shandong, and Fujian in China in 2015. A previous study

also demonstrated that the CaKoV had existed in Hubei province in China, the sequence of which was submitted to GenBank in 2012. Moreover, the CaKoV had been found in different regions of Heilongjiang province, including the city of Mudanjiang, Daqing, and Harbin (Li et al. 2016). These data showed that the CaKoVs in dogs were detected in at least four provinces in China currently, suggesting that CaKoV infection was relatively common in the dog population. However, the prevalence state of CaKoVs in dogs in

whole country of China still remains unknown. Thus, the systematic epidemiology and molecular genetics of CaKoV needs to be studied further.

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