SHORT COMMUNICATION

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Molecular characterization of canine and feline kobuvirus infections in Iran

Ghazal Aftab[1], Farnoosh Arfaee[1], Baharak Akhtardanesh[2]*, Gholamreza Nikbakht Brojeni[3]

[1] Department of Clinical Sciences, Faculty of Specialized Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, Iran; [2] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran; [3] Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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Abstract

Kobuviruses are viral pathogens with broad host range presented in human gastroenteritis cases; but, the pathogenesis of these viruses in companion animals is not well described. In the present study, the presence of canine (CaKVs) and feline kobuviruses (FeKVs) was detected in the 100 fecal samples of diarrhoeic and healthy companion dogs and cats by polymerase chain reaction in Tehran, Iran. The prevalence of infection was estimated as 8.00% and 4.00% in dogs and cats, respectively. All positive samples were belonged to non-diarrhoeic animals except for a feline sample being co-infected with panleukopenia. Sequence analysis showed multiple point mutations in canine and feline Iranian strains and new feline strain was detected in the present study. This is the first detection of CaKVs and FeKVs in Iran; but, the exact role of these enteric viral pathogens and their zoonotic risks are better to be clarified in all endemic regions.

Introduction

Kobuvirus is a newly described genus of family Picornaviridea having three species including *Aichivirus A*, *Aichivirus B* and *Aichivirus C*.[1] Human Aichi virus or *Aichivirus A* was the only member of kobuvirus up to 2003. It was first detected in diarrhoeic children at Aichi prefecture of Japan in 1989.[2] Bovine kobuvirus or *Aichivirus B* was first recognized in healthy cattle fecal samples in 2003; but, porcine kobuvirus or *Aichivirus C* was described in non-diarrhoeic pigs in 2008.[3,4] Canine kobuvirus (CaKV), the first sequenced canine picornavirus, was detected in 2011 from stool samples of diarrhoeic dogs in the United States.[5, 6] It has been reported that CaKV is the closest relative of human Aichi virus. Moreover, the possibility of cross-species transmission and risk of zoonotic infection have been highlighted in dog owners.[7] The CaKV has also been detected in the United Kingdom and South Korea. Immunoglobulin G (IgG) antibody against the Aichi virus was also detected in cat sera, indicating that cats might be susceptible to kobuvirus infection.[8,9] Recently, genetic characterization of feline kobuvirus (FeKV) suggested that it is widespread in cats.[8]

In the present study, molecular characterization of CaKV and FeKV was executed for the first time in Iran.

Materials and Methods

This cross-sectional survey was done to investigate the presence of kobuvirus infection in companion cats and dogs refereed to the Veterinary Hospital of Tehran Azad University, Tehran, Iran.

One hundred fecal samples were collected from 50 young cats and dogs (25 diarrheic and 25 healthy from each species), aged less than one year old. The project underwent ethical review and was approved by an Institutional Animal Care (Approved Nnumber: IR.UK. REC. 1398.02.09) and done by appropriately qualified scientific colleagues.

Complete physical examinations were done, and the health history of each animal was recorded before sampling. All fecal samples were placed in sterilized 2.00 mL microtubes and frozen at –80.00 ˚C for RNA extraction. Fecal samples were suspended in phosphate buffer solution (ATR-MED, Tehran, Iran) in a concentration of approximately 1.00 g mL^{-1}. Suspensions were centrifuged

***Correspondence:**

Baharak Akhtardanesh. DVM, DVSc
Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
E-mail: akhtardanesh@uk.ac.ir

for 10 min. The RNA was extracted using RNA extraction kit according to the manufacturer's instructions (iNtRON Biotechnology, Gyeonggi-do, South Korea). Kobuvirus was detected from fecal samples using reverse transcription-polymerase chain reaction (RT-PCR) with fermentas PCR enzymes. Amplification of the 3D region of kobuvirus was done using DogR2 (5'GTGGGGAAGGTAGAGAAGTAGA GGT), DogF4 (5'-GTCCACACCCCTACCTCCCGCC), DogR1 (5'-CGTGTTTGAGGAAGAGTTGGGTGTC), Nikfeline (TCAC CCTGGGCGAAATTACC) and Nikfeline (TTCCATGGATGGC CTGTTCC) primers. The RT-PCR was performed at 45.00 ˚C for 30 min, and pre-PCR denaturation was performed at 95.00 ˚C for 10 min, followed by 30 cycles of 95.00 ˚C for 30 sec, 55.00 ˚C for 30 sec and 72.00 ˚C for 30 sec. For determination of the full-length sequence of CaKV and FeKV, PCR products were purified and sequenced (Sequencing Service from Bioneer, Daejeon, South Korea). Sequencing results were extracted and corrected in FASTA format by FinchTV as a trace file viewer. The identities of the gene sequences were verified by comparison of the sequences to the GenBank® database through the BLAST algorithm. Finally, the verified sequences were aligned in the MEGA software (version 5.0; Biodesign Institute, Tempe, USA) and the related phylogenetic tree was constructed for sequences.

Results

Four out of the fifty fecal samples of dogs (8.00%) were positive for CaKV. In the cat's population, fecal samples of two kittens (4.00%) were positive for FeKV. All positive samples were seen in non-diarrhoeic animals except one of the feline samples being co-infected with panleukopenia virus. Physical examination results of infected animals were presented in Table 1.

Sequence analysis results showed multiple point mutations in both canine and feline Iranian strains. Some of these mutations changed the amino acid sequences. The CaKV and FeKV sequences generated in this study have been deposited in GenBank® under accession numbers of KT290621 and KT290620, respectively.

Based on the nucleotides encoding for residues 3 and 5, Iranian feline strain is a new strain; but, the canine strain is similar to previously reported data.

A phylogenetic tree was constructed from both samples and additional kobuvirus sequences were retrieved from the GenBank® database. The sequences are shown in Figure 1. Iranian feline and canine isolates separately clustered together with the feline and canine strains.

Iranian dog isolates were segregated together with China, the United States and African strains with full identity. Cat isolate was similar to Italian samples. Both isolates were completely different from mouse kobuvirus and Aichi virus (Fig. 2).

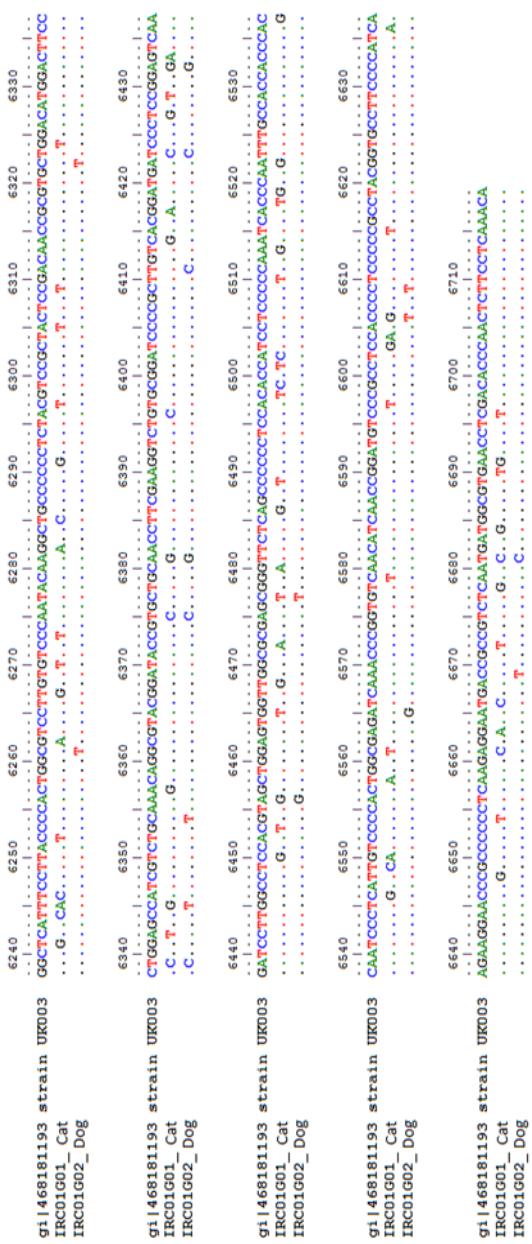

Fig. 1. Alignment of nucleotide sequences of the fragments amplified by polymerase chain reaction from clinical samples. The sequences of two local field variants, one identified as new IRC01G01 and the other as IRC01G01 are shown aligned with sequences of UK003 strains obtained from the GenBank®.

Table 1. Signalement and physical examinations of canine and feline kobuvirus infected dogs and cats.

Species	Animal No.	Sex	Age (month)	Breed	Clinical signs
Dog	11	Male	4	Terrier	Upper respiratory infection, Non-diarrhetic
Dog	18	Male	3	Schitzu	Upper respiratory infection, Non-diarrhetic
Dog	34	Male	5	Golden Retriever	Upper respiratory infection, Non-diarrhetic
Dog	47	Female	12	Bullmastiff	Non-diarrhetic
Cat	23	Male	6	Persian	Diarrhetic, Panleukopenia
Cat	35	Male	5	Persian	Non-diarrhetic

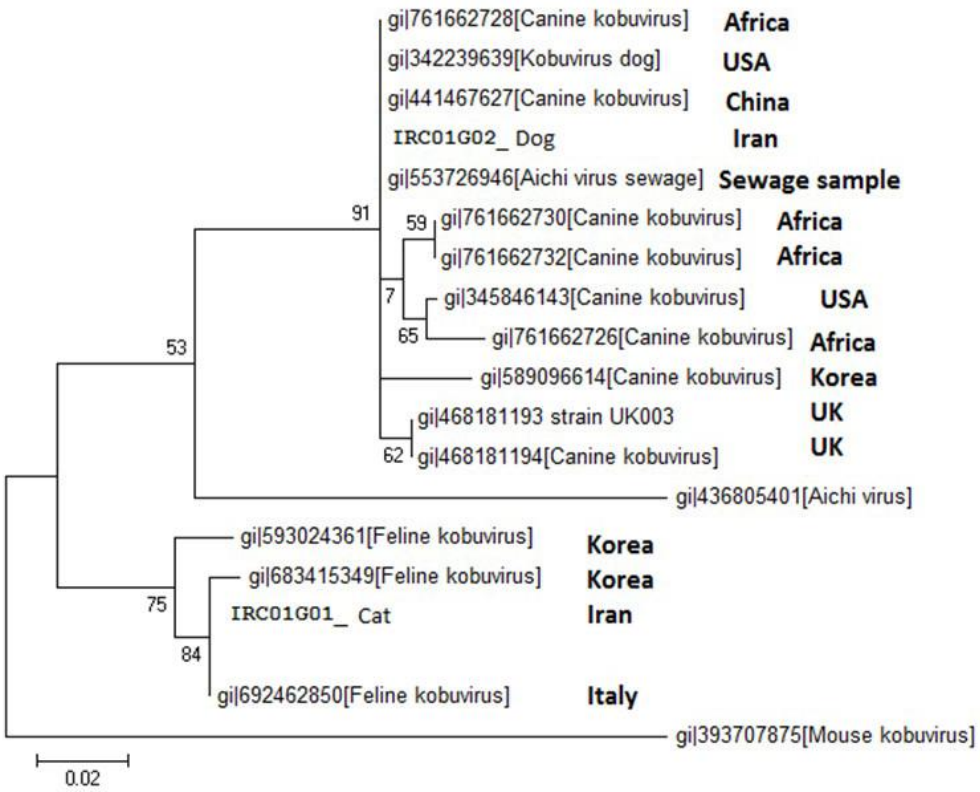

Fig. 2. Maximum likelihood tree based on the partial canine and feline kobuvirus amino acid sequences (6238-6719 nt). Iranian isolates are indicated by IRC01G01-2. The sequences from regions outside Iran were retrieved from GenBank®. A statistical support was provided by bootstrapping over 1,000 replicates.

Discussion

Despite the high seroprevalence of *Aichivirus A* infection in humans, (up to 80.00 - 95.00% at the age of 30 - 40), RT-PCR showed low prevalence of virus (about 0.50 - 3.00%) in human gastroenteritis cases suggesting that the virus might circulate without causing any symptoms.[10-12] Studies on the other members of genus kobuvirus, showed higher RT-PCR prevalence of these viruses including 1.00 to 34.50%, for *Aichivirus B* or bovine Aichi virus and 3.90% up to 100% for *Aichivirus C* or porcine Aichi virus.[1,13-15] Following the first identification of CaKVs in USA, a sero-prevalence study in UK showed that 37.40% and 69.90% of the studied dogs and cats had specific IgG antibodies against the Aichi virus, respectively.[9] The CaKVs and FeKVs prevalence rates were 15.25% and 14.50% in South Korea, respectively. The FeKVs were reported from South Korea for the first time.[8,16,17]

Our results provide the first detection of CaKV and FeKV from Iran. In this study, the CaKV and FeKV infections were detected in 6.00% and 4.00% of the studied dogs and cats, respectively. Our observations revealed that there is no significant association between kobuvirus infection and gastroenteritis.

Even though there is a close relation between Aichi virus infection with a wide range of clinical signs such as fever, conjunctivitis, respiratory symptoms and acute gastroenteritis in pigs and cows, controversial results have been observed between other members of kobuviruses and diarrhea.[18]

The high co-infection rate of CaKVs with the enteric disease was reported, and it was presented as an etiological factor of canine viral enteritis.[7] Also, Di Martino *et al.* have reported co-infection of FeKVs with other feline pathogens.[19] In this study, one of the FeKVs positive cats was diarrhoeic and co-infected by panleukopenia virus. Iranian cat isolated kobuvirus was similar to the Italian strain; but, it was completely different from mouse kobuvirus and Aichi virus. However, Chung *et al.* have reported that FeKV, CaKV, mouse kobuvirus and human Aichi virus are closely clustered in a phylogenetic tree.[8] In agreement with our results, the difference between FeKV, CaKV, mouse kobuvirus and human Aichi virus strains was reported in Italy formerly.[19]

In the phylogenetic analysis based on a portion of the 3D region, isolated Iranian CaKV was in the same lineage with Chinese, American and African strains. Our data support that CaKVs from different countries are not forming a single lineage. It has been reported that based on phylogenetic tree derived from the partial 3D gene of human and various animal species, CaKVs cluster into a single lineage.[7]

According to the close relationship between CaKVs and FeKVs with human Aichi virus and a report of inter-species transmission of *Aichivirus B* (bovine kobuvirus), there is a considerable transmission risk of CaKVs and FeKVs from pets to human.[20]

In conclusion, the detection of CaKV and FeKV strains in Iranian pets showed the worldwide distribution of these viruses. Sequence analysis results showed multiple point mutations in canine and feline Iranian strains and new feline strain was also detected. Still, the exact role of these enteric viral pathogens and their zoonotic risks are better to be clarified in all endemic regions.

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Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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