



Identification and full-genome sequencing of canine kobuvirus in canine fecal samples collected from Anhui Province, eastern China

Yong Wang¹ · Yongqiu Cui¹ · Yequ Li¹ · Xiaopeng Wang¹ · Kankan Yang¹ · Da Zhang¹ · Liang Zhao¹ · Caixia Bai¹ · Shudong Jiang¹ · Yongdong Li²

Received: 9 January 2020 / Accepted: 9 July 2020 / Published online: 9 August 2020
© Springer-Verlag GmbH Austria, part of Springer Nature 2020

Abstract

Canine kobuvirus (CaKoV), a newly described virus, is the causative agent of gastroenteritis in dogs. In this study, 57 fecal samples from dogs with diarrhea in Anhui Province, eastern China, were collected. Among these, five samples were identified to be infected with CaKoV, by polymerase chain reaction targeting the CaKoV 3D gene. The five CaKoV strains were subjected to phylogenetic analysis. The sequences of VP1 from the five CaKoV strains were 93.6%–96.1% identical to each other and 91.75%–97.95% identical to other reported CaKoV VP1 sequences. In addition, the complete genome of one strain was successfully amplified and sequenced. The genome consisted of 8223 nucleotides and shared 94.6%–97.0% nucleotide and 93.1%–94.0% amino acid sequence identity with other CaKoV isolates. Phylogenetic analysis revealed that the CaKoV strain from Anhui Province was similar to other Chinese strains, and it was more closely related to feline and mouse kobuviruses than to sheep and bovine kobuviruses. Interestingly, all of the CaKoV-positive samples were coinfecting with canine parvovirus. The finding of CaKoV infection in dogs with diarrhea and coinfection with canine parvovirus are a cause for concern and highlight the need for management and preventive measures.

Introduction

Kobuvirus, a genus of the family *Picornaviridae*, is divided into six species, namely *Aichivirus A* (human kobuvirus), *Aichivirus B* (bovine kobuvirus), *Aichivirus C* (porcine kobuvirus), *Aichivirus D* (kagovirus 1), *Aichivirus E* (rabbit picornavirus), and *Aichivirus F* (bat kobuvirus) [3, 16, 20, 21]. Canine kobuvirus (CaKoV) is a member of the species

Aichivirus A [1, 9]. In 2011, the whole genome sequence of a CaKoV isolate was first determined in the United States of America [9]. Thereafter, the complete genomes of CaKoV isolates have been sequenced in various countries, including China, Britain, Italy, South Korea, Tanzania, Kenya, and Japan [2, 6, 7, 10, 14, 19]. The CaKoV genome is 8.1–8.2 kb long, with one open reading frame (ORF) and a 5' untranslated region (UTR). The ORF contains 7332–7341 nucleotides (nt) that encode a polyprotein of 2442–2475 amino acids (aa) [11]. The ORF encodes a leader (L) protein, three structural proteins (VP0, VP3, and VP1), and seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D), and this is followed by a 3'-UTR and a poly(A) tail. The structural proteins form the capsid, which is associated with the adsorption and entry of virus particles into cells. Based on the sequence of the VP1 gene, virus strains can be divided into two groups [12]. The non-structural proteins and intermediates are involved in RNA replication, polyprotein cleavage, and virion assembly [9].

CaKoV is frequently detected in dogs with diarrhea but is also associated with asymptomatic infections [4, 8, 18]. This virus has also been detected in wild carnivores such as jackals, foxes, and hyenas [8, 14]. The virus is found not only in the digestive system but also in the cerebellum, lungs,

Handling Editor: Tim Skern.

Yong Wang and Yongqiu Cui contributed equally to this work and considered to be the co-first authors.

✉ Shudong Jiang
jshudong@163.com

✉ Yongdong Li
liy0551@126.com

¹ Anhui Province Key Laboratory of Veterinary Pathobiology and Disease Control, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, People's Republic of China

² Municipal Key Laboratory of Virology, Ningbo Municipal Center for Disease Control and Prevention, Ningbo 315010, China

tonsils, and liver, indicating that CaKoV might cause serious systemic infections [18].

Although the complete genomes of CaKoV isolates from several countries have been sequenced, studies on the characterization of endemic CaKoV strains in mainland China are limited. CaKoV infections have been reported in north-east and southwest China [10, 12]. However, there is a lack of genetic data on circulating CaKoV strains in eastern China. In the present study, CaKoV strains were detected in dogs with diarrhea from Anhui Province, eastern China, and the complete genome of one strain was successfully sequenced. Based on phylogenetic analysis, the prevalence of these CaKoV strains was analyzed, and the genetic diversity of CaKoV was investigated by comparing complete genome and *VPI* gene sequences with those of CaKoV reference strains. To the best of our knowledge, this is the first phylogenetic analysis of CaKoV strains isolated from Anhui Province. The results can help to better understand the epidemiology of CaKoV in eastern China.

Materials and methods

Sample collection

Fecal samples were collected from dogs with diarrhea in several animal hospitals in Hefei, Ma'anshan, Anhui Province from January 2018 to July 2019. All fecal samples were stored at -80°C until further analysis.

RNA extraction and cDNA synthesis

The fecal samples were resuspended in phosphate-buffered saline at approximately 0.1–0.2 g/ml by vortexing and then centrifuged at 12,000 *g* for 10 min. The suspension was then collected in a new centrifuge tube without RNase. Viral RNA was extracted using a TIANamp Virus DNA/RNA Kit (Tiangen Biotech Co., Ltd., China) according to the manufacturer's instructions [10]. The extracted RNA was reverse transcribed into cDNA using a PrimeScript™ First Strand cDNA Synthesis Kit (TaKaRa BIO INC, Japan) according to the manufacturer's instructions [12]. The cDNA was stored at -20 °C until further analysis.

CaKoV detection in samples

Real-time polymerase chain reaction (RT-PCR) was conducted by targeting the CaKoV *3D* gene [7]. The specific primers used for RT-PCR are listed in Table 1. The predicted size of the PCR product was 504 bp. The amplified product was purified and cloned into the pMD-19T vector (TaKaRa). The recombinant plasmid was then sequenced by Sangon Company.

Screening for coinfection with canine enteric pathogens

All CaKoV-positive samples were examined for the presence of canine enteric pathogens. The samples were tested for canine parvovirus (CPV, GenBank accession

Table 1 Specific primers used for detection of CaKoV and other enteroviruses

Primer name	Nucleotide sequence (5'-3')	Target gene	Amplicon size (bp)	Reaction conditions
CaKoV-3D-F CaKoV-3D-R	CCCTGGAACACCCAAGGCCGCT TCTGGTTGCCATAGATGTGGTG	3D	504	94°C for 2 min, followed by 40 cycles at 94°C for 45 s, 48°C for 1 min and 72°C for 50 s and a final extension step at 72°C for 10 min
CaKoV-VPI-F CaKoV-VPI-R	GCGAACTCAGAAGATCTCAATGCGC ATAGGTGGCCTATCTGCACGGACG	VPI	834	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 57°C for 30 s and 72°C for 1 min and a final extension step at 72°C for 10 min
CPV-F CPV-R	GGGGATTCTACGGGTACTTT TGGTAAGCCCAATGCTCTAT	VP2	751	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 50°C for 30 s and 72°C for 50 s and a final extension step at 72°C for 10 min
CCV-F CCV-R	CTTGGTAATCGTGGTGCTAAT TCAATCTGGTCGCCATCTTC	N	530	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 52°C for 30 s and 72°C for 40 s and a final extension step at 72°C for 10 min
CDV-F CDV-R	GAAGGGTCGAAAGCTCAAGG ACACCAACTCCCATAGCATAAC	N	617	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 53°C for 30 s and 72°C for 40 s and a final extension step at 72°C for 10 min
CaAstV-F CaAstV-R	GTAATAACCRCTGATTTAATT AGACCAARGTGTCATAGTTCAG	ORF1b	625	95°C for 5 min, followed by 35 cycles at 95°C for 20 s, 58°C for 30 s and 72°C for 90 s and a final extension step at 72°C for 5 min

CDV, canine distemper virus; CaAstV, canine astrovirus; CaKoV, canine kobuvirus; CPV, canine parvovirus; CCV, canine coronavirus

no. NC001539) by PCR and for canine coronavirus (CCV, GenBank accession no. AB781800), canine distemper virus (CDV, GenBank accession no. JF965338), and canine astrovirus (CaAstV, GenBank accession no. KX599391) by RT-PCR. The specific primers are listed in Table 1. The PCR and RT-PCR products were sequenced by Sangon Company.

VP1 gene amplification

A specific primer pair targeting CaKoV VP1 was designed based on genome sequences from the GenBank database (accession nos. JQ911763 and MF062158). The PCR-amplified products were purified and cloned into the pMD-19T vector and confirmed by sequencing.

Amplification of the complete CaKoV genome

As shown in Table 2, eight pairs of specific primers were designed based on the sequences of the conserved regions of published CaKoV strains (accession nos. JQ911763 and MF062158). The PCR products were purified and cloned into the pMD-19T vector before sequencing.

Sequence assembly and phylogenetic analysis

The sequences were assembled using SeqMan software (DNASTAR, USA). The nt and aa sequences of the isolates were aligned with those obtained from the GenBank database (Table 3). The sequences were then aligned using the Clustal W method with MegAlign in DNASTAR software. To analyze evolutionary relationships, phylogenetic trees were constructed based on the Kimura 2-parameter model using the neighbor-joining method with 1000 bootstrap replicates with MEGA 6.0 software.

Results

CaKoV detection and coinfection in clinical samples

Fifty-seven fecal samples from dogs with diarrhea were collected to test for CaKoV infection. Among these, five samples were positive for CaKoV, with an incidence rate of approximately 8.7% (5/57) in Anhui Province from January 2018 to July 2019. All CaKoV-positive samples were subjected to further testing to detect other enteroviruses in order to identify possible coinfections. Interestingly, all five CaKoV-positive samples were coinfecting with CPV, and the

Table 2 Specific primers used for RT-PCR amplification and genomic sequencing

Primer name	Nucleotide sequence (5'-3')	Amplification size (bp)	Reaction conditions
CaKoV-1-F CaKoV-1-R	1- TTAAAGTGTGTGCCCCAATCTCTTG 689- CTTGGTAGTCAGTAGCAGTGATGTA	689	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 52°C for 30 s and 72°C for 40 s and a final extension step at 72°C for 10 min
CaKoV-2-F CaKoV-2-R	600- TAATGGCAGGCAGAATGGAATCTCG 1858- TGGGTACGCACGCACGAAGG	1259	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 54°C for 30 s and 72°C for 90 s and a final extension step at 72°C for 10 min
CaKoV-3-F CaKoV-3-R	1745- AACCCCTACATTCCCCATCTCATCAT 2981- CATTCTCAATGTTGGCAGTGTCCCTG	1237	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 53°C for 30 s and 72°C for 90 s and a final extension step at 72°C for 10 min
CaKoV-4-F CaKoV-4-R	2900-TTCATGCAAAACCCCGCCCTCACCT 3937- CTCCACGGTTTGCCGAGTT	1038	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 53°C for 30 s and 72°C for 90 s and a final extension step at 72°C for 10 min
CaKoV-5-F CaKoV-5-R	3803- GGAAGCACAAAGCAAATTTCCCTCT 4784- GGGCCAATATGGAATCCGAGTAA	982	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 53°C for 30 s and 72°C for 60 s and a final extension step at 72°C for 10 min
CaKoV-6-F CaKoV-6-R	4637- AATGTCGAGTGGATCGGAGAAACTG 6649- GGGAGCCGGTTCCTTCTTGAT	2013	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 53°C for 30 s and 72°C for 150 s and a final extension step at 72°C for 10 min
CaKoV-7-F CaKoV-7-R	6568- CGGTGTCAACATCAACCGGATGTCT 7876- CCCAGACTTGAGGCACTGTTCG	1309	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 57°C for 30 s and 72°C for 90 s and a final extension step at 72°C for 10 min
CaKoV-8-F CaKoV-8-R	7641- CGTCCACAATCTATGAAGTCACCT 8216- TTTTTTTTTTTTTTTTTTTT AAGAACAGTTAGAAAAGTT	576	95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 57°C for 30 s and 72°C for 50 s and a final extension step at 72°C for 10 min

Table 3 Kobuvirus reference strains used in phylogenetic analysis

Strain	Host species	Country	Gene	GenBank accession no.
SMCD-59	Canine	China	Whole genome + VP1	MF062158
CH-1	Canine	China	Whole genome + VP1	JQ911763
US-PC0082	Canine	USA	Whole genome + VP1	JN088541
UK003	Canine	UK	Whole genome + VP1	KC161964
CaKoV-26	Canine	Brazil	Whole genome + VP1	MH747478
12D049	Canine	Korea	Whole genome + VP1	KF924623
WHJ-1	Feline	China	Whole genome	MF598159
12D240	Feline	Korea	Whole genome	KJ958930
FK-13	Feline	Korea	Whole genome	KF831027
TB3	Sheep	Hungary	Whole genome	GU245693
12Q108	Caprine	Korea	Whole genome	NC023422
EGY-1	Bovine	Egypt	Whole genome	KY407744
M-5-1	Mouse	USA	Whole genome	NC015936
HT9	Marmot	China	Whole genome	KY855436
SewKTM	Sewage	Nepal	Whole genome	JQ898342
S-1-HUN	Swine	Hungary	Whole genome	EU787450
XX	Swine	China	Whole genome	KC204684
JS-01-CHN	Swine	China	Whole genome	KP144318

sample with the AH-4 strain was coinfecting with CaAstV (Table 4). There was no coinfection with CCV or CDV.

VP1 gene sequencing and phylogenetic analysis

The VP1 sequences of the five CaKoV strains were 835–838 bp long and encoded polyproteins of 278–279 aa. Phylogenetic analysis demonstrated that the CaKoV isolates were divided into two separate groups: one including CaKoV isolates from China, and the other including CaKoV isolates from the USA, England, Korea, and Brazil. As shown in Fig. 1a, the VP1 sequences of the five CaKoV strains detected in this study clustered into a single group, with 100% bootstrap support.

Complete genome analysis of CaKoV strain AH-1/CHN/2019

In this study, the complete genome sequence of one CaKoV isolate was successfully amplified. The complete

genome was 8223 nt long; it contained an open reading frame (ORF) of 7335 nt excluding the poly(A) tail, encoding a partial 5'-UTR of 601 nt and a 248-nt 3'-UTR. The whole sequence was uploaded to the GenBank database and named “CaKoV AH-1/CHN/2019” (accession no. MN449341). The complete genome of CaKoV AH-1/CHN/2019 has 20.3% A, 20.9% G, 21.4% T, and 37.4% C and thus has a high G+C content (58.29%), like other kobuviruses (52%–59%).

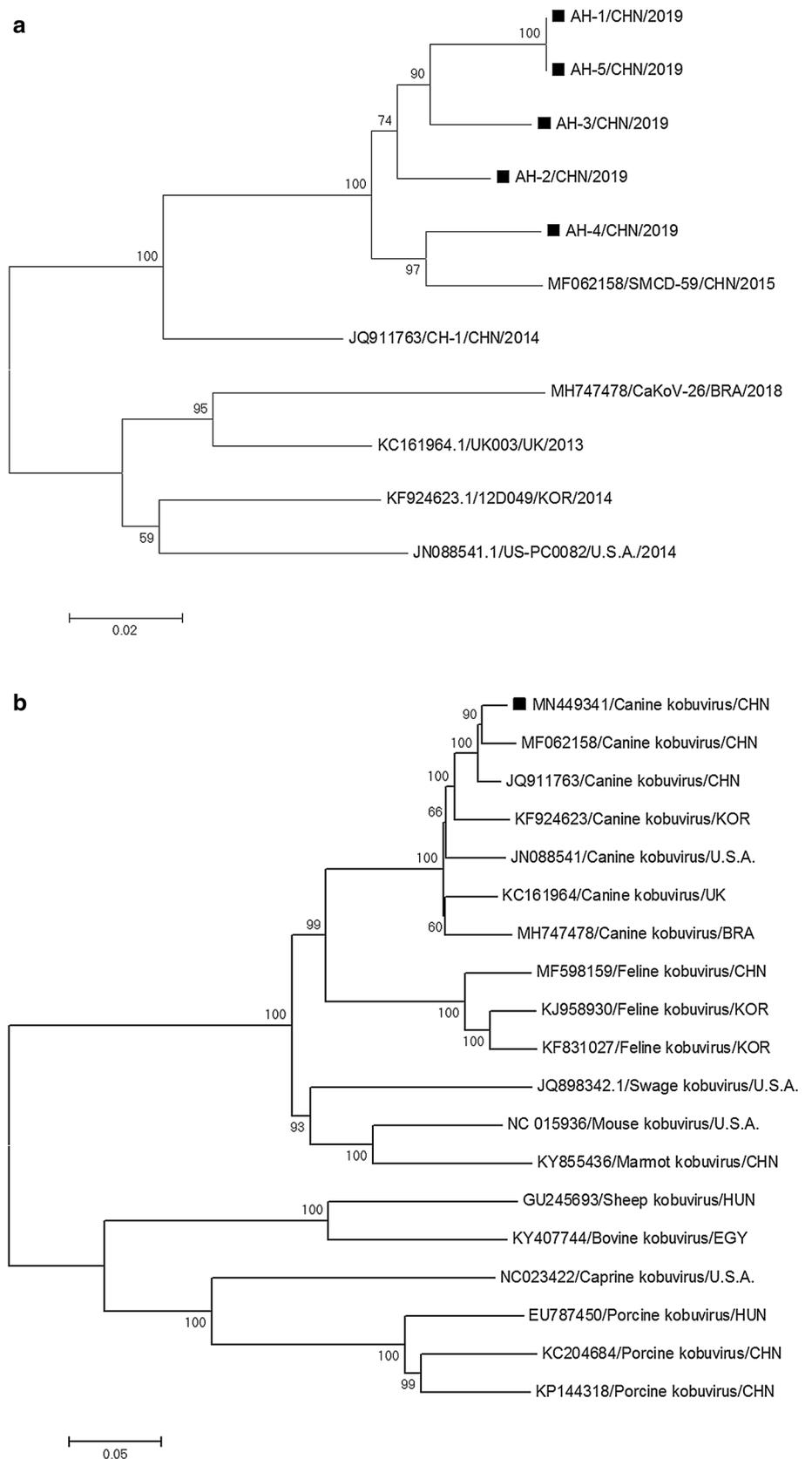
The CaKoV AH-1/CHN/2019 strain showed 94.6%–97% nucleotide sequence identity and 93.1%–94% aa sequence identity with CaKoV strains CH-1 (JQ911763), SMCD-59 (MF062158), CU101 (MK201777), and 12D049 (KF924623). We performed a phylogenetic analysis based on the complete genome of CaKoV AH-1/CHN/2019 and other representative kobuviruses (Fig. 1b).

Table 4 Coinfection status of CaKoV-positive samples

Strain	Feeding model	Diarrhea	CPV	CaAstV	CCV	CDV
AH-1	Domesticated	+	+	-	-	-
AH-2	Domesticated	+	+	-	-	-
AH-3	Domesticated	+	+	-	-	-
AH-4	Domesticated	+	+	+	-	-
AH-5	Domesticated	+	+	-	-	-

CaKoV, canine kobuvirus; CPV, canine parvovirus; CCV, canine coronavirus; CDV, canine distemper virus; CaAstV, canine astrovirus

Fig. 1 a. Neighbor-joining phylogenetic tree based on the *VPI* sequences of the CaKoV strains. Isolates from this study are indicated by black boxes. The tree was constructed using the neighbor-joining method with the Kimura 2-parameter model and 1000 bootstrap replicates using MEGA6.0 software. **1**
b. Phylogenetic tree based on an alignment of the complete genome sequences of CaKoV and other members of the genus *Kobuvirus*. The tree was constructed using the neighbor-joining method with the Kimura 2-parameter model and 1000 bootstrap replicates using MEGA6.0 software. Isolates from this study are indicated by black boxes



Discussion

Viruses are the main cause of gastroenteritis in animals, especially in puppies under 1 year of age [15]. The viruses frequently associated with gastroenteritis are CPV-2, CDV, CCV, rotaviruses, adenoviruses, and some newly identified viruses such as CaKoV and CaAstV [11]. Animal kobuviruses have been detected in pigs, cattle, sheep, and dogs in several countries.

In this study, five strains of CaKoV were detected in fecal samples from 57 dogs with diarrhea in Anhui Province. The results showed that the CaKoV infection rate was approximately 8.7% (5/57). According to a previous study, the CaKoV infection rate in southwest and northeast China was 50.46% and 17.91%, respectively [10, 12]. Thus, the CaKoV infection rate in Anhui Province may be lower than that in southwest and northeast China. We are continuing to collect samples from animal hospitals and animal shelters in Anhui Province for further study. Furthermore, according to previous studies, enteroviruses are frequently found in coinfections with other pathogens. Therefore, we simultaneously tested for some common pathogens. It has been reported that the CPV coinfection rate ranges from 41.67% to 100% [10, 13]. Interestingly, in this study, all of the CaKoV-positive samples showed the co-occurrence of CPV infection (Table 4), indicating the CPV co-infection rate in eastern China was higher than that in northeast and southwest China. Further study is required to identify synergy with parvoviruses [10, 12]. Only one sample was coinfecting with CaAstV, and there was no coinfection with CCV or CDV. It has been reported that CaKoV can cause serious systemic infections [18]. Together, these findings imply that the high coinfection rate of CaKoV with other enteroviruses should be considered an important risk factor that could lead to gastroenteritis. The infection mechanism of CaKoV and the role of coinfection with other enteroviruses should be further investigated. We will expand the number and scope of samples collected in the future to compare diarrheic and asymptomatic infections.

The VP1 protein of aichivirus is the most exposed and immunodominant portion of the kobuvirus capsid, and it is the most variable structural protein [5, 17, 21]. Phylogenetic analyses of the VP1 sequence and the complete genome sequence of CaKoV allowed us to obtain information on the genetic relationships among the identified strains and other CaKoV strains reported worldwide, as well as other members of the genus *Kobuvirus* [13]. The five amplified VP1 sequences showed significant clustering and a high degree of similarity to previously reported Chinese strains. This result was consistent with that of a previous phylogenetic analysis based on VP1 of strains from China [12]. In this study, compared with other

published CaKoV VP1 aa sequences, the five CaKoV VP1 aa sequences contained substitutions. Whether these differences affect protein function still needs to be clarified. Notably, the phylogenetic analysis showed that the Chinese strains clustered separately from other published VP1 sequences of strains from Europe and South America, suggesting that the CaKoV strains from China may have a similar evolutionary background. In addition to this, we found that strains from Anhui and southwest China were more closely associated with strains from Asia, and according to a previous study, strains from northeast China showed similarity to strains from Europe, suggesting that there may be two different CaKoV lineages in mainland China.

To investigate the genetic characteristics of CaKoV strains circulating in dogs in Anhui Province, we successfully amplified the complete genome of one CaKoV strain, which was named CaKoV AH-1/CHN/2019. Our study is the first to report the complete genome sequence of a CaKoV strain from Anhui Province, and this will be helpful for further understanding CaKoV epidemiology in China. A phylogenetic analysis based on the complete genome sequence also indicated that the CaKoV strain was more closely related to strains from China than to those from Europe, Australia, and America. This is in agreement with previously published results [10, 12]. Furthermore, a phylogenetic analysis of CaKoV and members of different kobuvirus species revealed that CaKoV AH-1/CHN/2019 was more closely related to feline kobuviruses than to sheep and bovine kobuviruses. One of the most likely reasons for this is that cats and dogs are often raised together in modern families; once a cat or dog is infected with a kobuvirus, cross-species cross-infection can easily occur. We will focus on this aspect in future research. The links between different kobuvirus strains need to be confirmed by extensive epidemiological surveys.

In conclusion, CaKoV showed widespread circulation in dogs with diarrhea in Anhui Province. The results of the present study provide phylogenetic information on the molecular epidemiology of CaKoV and indicate that the high coinfection rate of CaKoV and CPV deserves more attention.

Acknowledgements This work was supported financially by the Anhui Provincial Primary Research & Development Plan (201904f06020030).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ambert-Balay K, Lorrot M, Bon F, Giraudon H, Kaplon J, Wolfer M, Lebon P, Gendrel D, Pothier P (2008) Prevalence and genetic

- diversity of Aichi virus strains in stool samples from community and hospitalized patients. *J Clin Microbiol* 46:1252–1258
2. Carmona-Vicente N, Buesa J, Brown PA, Merga JY, Darby AC, Stavisky J, Sadler L, Gaskell RM, Dawson S, Radford AD (2013) Phylogeny and prevalence of kobuviruses in dogs and cats in the UK. *Vet Microbiol* 164:246–252
 3. Chang JT, Chen YS, Chen BC, Chao D, Chang TH (2013) Complete genome sequence of the first Aichi virus isolated in Taiwan. *Genome Announc* 1(1):e00107–12
 4. Charoenkul K, Janetanakit T, Chaiyawong S, Bunpapong N, Boonyapisitsopa S, Tangwangvivat R, Amonsin A (2019) First detection and genetic characterization of canine Kobuvirus in domestic dogs in Thailand. *BMC Vet Res* 15:254
 5. Chen L, Zhu L, Zhou YC, Xu ZW, Guo WZ, Yang WY (2013) Molecular and phylogenetic analysis of the porcine kobuvirus VP1 region using infected pigs from Sichuan Province, China. *Virol J* 10:281
 6. Choi S, Lim SI, Kim YK, Cho YY, Song JY, An DJ (2014) Phylogenetic analysis of astrovirus and kobuvirus in Korean dogs. *J Vet Med Sci* 76:1141–1145
 7. Di Martino B, Di Felice E, Ceci C, Di Profio F, Marsilio F (2013) Canine kobuviruses in diarrhoeic dogs in Italy. *Vet Microbiol* 166:246–249
 8. Di Martino B, Di Profio F, Melegari I, Robetto S, Di Felice E, Orusa R, Marsilio F (2014) Molecular evidence of kobuviruses in free-ranging red foxes (*Vulpes vulpes*). *Arch Virol* 159:1803–1806
 9. Khamrin P, Maneeakarn N, Okitsu S, Ushijima H (2014) Epidemiology of human and animal kobuviruses. *Virusdisease* 25:195–200
 10. Li C, Wei S, Guo D, Wang Z, Geng Y, Wang E, Zhao X, Su M, Wang X, Sun D (2016) Prevalence and phylogenetic analysis of canine kobuviruses in diarrhoeic dogs in northeast China. *J Vet Med Sci* 78:7–11
 11. Li L, Pesavento PA, Shan T, Leutenegger CM, Wang C, Delwart E (2011) Viruses in diarrhoeic dogs include novel kobuviruses and sapoviruses. *J Gen Virol* 92:2534–2541
 12. Li M, Yan N, Wang M, Zhang B, Yue H, Tang C (2018) Prevalence and genomic characteristics of canine kobuvirus in southwest China. *Arch Virol* 163:459–466
 13. Miyabe FM, Ribeiro J, Alfieri AF, Alfieri AA (2019) Detection of canine kobuvirus RNA in diarrheic fecal samples of dogs with parvoviruses. *Braz J Microbiol* 50:871–874
 14. Olarte-Castillo XA, Heeger F, Mazzoni CJ, Greenwood AD, Fyumagwa R, Moehlman PD, Hofer H, East ML (2015) Molecular characterization of canine kobuvirus in wild carnivores and the domestic dog in Africa. *Virology* 477:89–97
 15. Ortega AF, Martinez-Castaneda JS, Bautista-Gomez LG, Munoz RF, Hernandez IQ (2017) Identification of co-infection by rotavirus and parvovirus in dogs with gastroenteritis in Mexico. *Braz J Microbiol* 48:769–773
 16. Reuter G, Boldizsar A, Pankovics P (2009) Complete nucleotide and amino acid sequences and genetic organization of porcine kobuvirus, a member of a new species in the genus Kobuvirus, family Picornaviridae. *Arch Virol* 154:101–108
 17. Reuter G, Boros A, Pankovics P (2011) Kobuviruses—a comprehensive review. *Rev Med Virol* 21:32–41
 18. Ribeiro J, Headley SA, Diniz JA, Pereira AH, Lorenzetti E, Alfieri AA, Alfieri AF (2017) Extra-intestinal detection of canine kobuvirus in a puppy from Southern Brazil. *Arch Virol* 162:867–872
 19. Soma T, Matsubayashi M, Sasai K (2016) Detection of kobuvirus RNA in Japanese domestic dogs. *J Vet Med Sci* 78:1731–1735
 20. Yamashita T, Sakae K, Tsuzuki H, Suzuki Y, Ishikawa N, Takeda N, Miyamura T, Yamazaki S (1998) Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. *J Virol* 72:8408–8412
 21. Yamashita T, Ito M, Kabashima Y, Tsuzuki H, Fujiura A, Sakae K (2003) Isolation and characterization of a new species of kobuvirus associated with cattle. *J Gen Virol* 84:3069–3077

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.