ORIGINAL ARTICLE

CrossMark

Prevalence and genomic characteristics of canine kobuvirus in southwest China

Mingxiang Li[1] · Nan Yan[1] · Min Wang[1] · Bin Zhang[1] · Hua Yue[1] · Cheng Tang[1]

Abstract

To investigate canine kobuvirus (CaKoV) infection in southwest China, 107 fecal samples were collected from dogs with obvious diarrhea in Sichuan and Chongqing regions, China. CaKoV infection was detected in 54 diarrheic samples (50.46%) by RT-PCR targeting a partial fragment (504 bp) of the *3D* gene. Comparison of these partial *3D* gene sequences from 14 of these CaKoV-positive samples show 95.4%–99.0% nucleotide (nt) identity within this group, and nt identities ranging from 93.1% to 98.2% with previously reported CaKoV *3D* gene sequences. Additionally, we amplified five *VP1* gene sequences and analyzed the inferred phylogeny. Amino acid (aa) identities of the five *VP1* gene sequences were 81.5%–89.4% with those previously reported. Furthermore, one complete CaKoV genome was successfully obtained from a positive sample and designated SMCD-59/CHN/2015. This genome consisted of 8,184 nt, and shared 92.9%–96.6% nt identity (97.6%–98.1% aa identity) with other reported CaKoV genomes. This study provides proof that CaKoV circulates in diarrheic dogs in southwest China, and that these viruses exhibit unique genetic characteristics.

Introduction

Canine kobuvirus (CaKoV) is classified as a novel member of the *Aichivirus A* species, which taxonomically belongs to the *Kobuvirus* genus in the family *Picornaviridae* [1]. According to previous studies, the CaKoV genome is 8.1–8.2 kb long, with a single open reading frame (ORF) containing 7,332–7,341 nucleotides (nt), encoding 2,442–2,475 amino acids (aa) [2, 3]. All CaKoV isolates have the same genomic organization: a 5′-untranslated region (UTR); a single ORF encoding a polyprotein precursor that is processed to generate a leader (L) protein, three structural proteins (VP0, VP3, and VP1), and seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D); a 3′-UTR; and a poly-(A) tail [1]. CaKoV structural proteins form the virion capsid, which is associated with the adsorption and invasion of virus particles. CaKoV nonstructural proteins and intermediates are involved in RNA replication, polyprotein cleavage, and assembly of virions [1].

CaKoV was first detected in 2011 in the feces of dogs with acute gastroenteritis in the USA [2, 3]. CaKoV has subsequently been detected in diarrheic as well as healthy dogs in Britain [4], Italy [5], South Korea [6, 7], Tanzania and Kenya [8], Japan [9], and China [10, 11], presenting a global distribution. The emerging virus has been detected in wild carnivores, including jackal, fox, and *Hyaenidae* [8, 12], in addition to domestic dogs. Previous studies have shown no significant difference in CaKoV prevalence between healthy versus diarrheic dog samples [6]. Therefore, it is unclear whether CaKoV alone, or together with other canine enteric pathogens causes gastroenteritis in dogs. Recently, the first report of CaKoV being detected in extra-intestinal tissue was published; the virus was found within the cerebellum, lung, tonsil, and liver of a 5-month-old puppy [13], suggesting that CaKoV may lead to systemic infection. In this study we report the prevalence of CaKoV in diarrheic dog feces from southwest China, and we characterize the genome of SMCD-59/CHN/2015, a Chinese CaKoV strain.

Handling Editor: Diego G. Diel.

✉ Cheng Tang
tangcheng101@163.com

1 College of Life Science and Technology, Southwest University for Nationalities, No. 16, South 4th Section 1st Ring Road, Chengdu 610041, China

Materials and methods

Sample collection

Fecal samples were collected from single-household dogs with diarrhea symptoms at six animal hospitals in Sichuan and Chongqing regions, China, during 2015 and 2016. The dogs were 2–6 months old, and were kept indoors, except for outdoor walks or runs of 1–2 hours daily. Labrador retriever, golden malinois, Tibetan mastiff, chihuahua, pomerania, schnauzer, and poodle breeds were all present. The samples were suspended in 5.0-mL sterile phosphate buffer saline (PBS) in sampling tubes, and then stored at −80°C for further testing.

RNA extraction and cDNA synthesis

Fecal samples were resuspended by vigorous vortexing in PBS at approximately 0.2 g/mL. The suspensions were centrifuged at 12,000 xg for 5 min to remove cellular debris. Viral RNA was extracted from 300 μL of the fecal suspensions using RNAios Plus® (TaKaRa BIO INC, JPN) according to the manufacturer's instructions, and then reverse transcribed into cDNA using the PrimeScript™ RT Reagent Kit according to the manufacturer's instructions (TaKaRa BIO INC, JPN). The resulting cDNA was used as a template for the PCR assay, and stored at −20 °C.

Detection of CaKoV

CaKoV RNA was detected by an RT-PCR assay targeting a 504 bp fragment of the *3D* gene, with forward, CaKV/F (5′-CCCTGGAACACCCAAGGCCGCT-3′), and reverse primer, CaKV/R (5′-TCTGGTTGCCATAGATGTGGTG-3′), sequences, as described by Di Martino *et al.* [5]. PCR products were purified and cloned into the pMD19-T (TaKaRa BIO INC, JPN) simple vector, prior to sequencing. Sequences have been submitted to GenBank under accession No. MF062159–MF062171.

VP1 gene sequence amplification

The CaKoV *VP1* gene was also analyzed. A primer pair was designed, according to the genome sequence (JQ11763) available in GenBank, to amplify the VP1 capsid-coding sequences from positive samples (VP1/F: 5′-GTGGACATC TTCAACCCTGAT-3′ and VP1/R: 5′-CCCACGGTTTGC CGAGTT-3′). PCR conditions were as follows: 94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 54 °C for 45 s and 72 °C for 70 s, with a final extension step at 72 °C for 10 min. PCR products were 1,188-bp long, and contained the full VP1 gene sequence. After the amplification, products

were purified and cloned into the pMD19-T simple vector, and then sequenced. VP1 sequences have been submitted to GenBank under accession No. MF062172–MF062175.

CaKoV genome amplification and phylogenetic analysis

Nine pairs of sequence-specific oligonucleotide primers were designed using the conserved regions of two published CaKoV sequences (JQ911763 and KF924623) to determine the CaKoV genome sequence. PCR reaction conditions are shown in Table 1. PCR products were purified and cloned into the pMD19-T simple vector, prior to sequencing. The sequences were assembled using SeqMan software (version 7.0; DNASTAR). The single ORF was identified using an online ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/). The CaKoV genome sequence generated in this study has been deposited in GenBank under accession No. MF062158.

Sequence, recombination, and phylogenetic analyses

All sequence identity analyses were performed with the nt and aa sequences aligned with the ClustalW method using the MegAlign 7.2 program in the Lasergene software (DNASTAR, Madison, WI, USA). Neighbor-joining phylogenetic trees were built with the p-distance model, 1,000 bootstrap replicates and, otherwise, the default parameters in MEGA 6. The Recombination Detection Program version 4.0 (RDP) was used to identify possible parental sequences and recombinant strains.

Results

Detection of CaKoV

Of the 107 samples evaluated, 54 (50.46%) were positive for CaKoV. CaKoV positive rates were 50.8% and 50.0% in Sichuan and Chongqing regions, respectively, which is higher than in northeast China [10].

Partial 3D gene sequence and phylogenetic analyses

In this study 14 CaKoV partial *3D* genes (504 nt) were sequenced, of the 54 positive samples identified. The 14 sequences shared 95.4%–99.4% nt identity with each other, and 94.1%–98.2% nt identity with other sequences previously reported. Phylogenetic analysis indicates that the 14 CaKoV sequences in this study are more closely related to other CaKoV sequences from Asia and Europe, than with sequences from Africa and the Americas (Fig. 1), which is

Table 1 Oligonucleotide sequences used for PCR amplification and genomic sequencing

Primer name	Nucleotide sequence (5′→3′)	Amplicon (bp)	reaction condition
CaKoV-1-F CaKoV-1-R	1-TTTAAGTGTTGTGCCCAATCTCTTG 765-CAATAAGGACACGGGAAGTAG	765	94 °C for 2 min, followed by 38 cycles at 94 °C for 35 s, 52 °C for 35 s and 72 °C for 45 s and a final extension step at 72 °C for 8 min.
CaKoV-2-F CaKoV-2-R	399-TGTGCGTAGTTCTCATCCAG 1858-TGGGTACGCACGCACGAA	1459	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 54 °C for 45 s and 72 °C for 90 s and a final extension step at 72 °C for 10 min.
CaKoV-3-F CaKoV-3-R	1136-TACGGCAATGGCAACAAC 3330-GGACTCTGAGGGATTGTAGCA	2194	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 52 °C for 45 s and 72 °C for 140 s and a final extension step at 72 °C for 10 min.
CaKoV-4-F CaKoV-4-R	2786-ACCACAGGCTACCTCTCTGT 4116-GGTGGCTTGGGTGACAGATT	1330	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 90 s and a final extension step at 72 °C for 8 min.
CaKoV-5-F CaKoV-5-R	3849-TTGTCGCCTACGAACCAC 5219- GGAGGATGTAAGGAGCAGAA	1370	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 53 °C for 45 s and 72 °C for 90 s and a final extension step at 72 °C for 8 min.
CaKoV-6-F CaKoV-6-R	4440-GACTCCTCGTCATCATCTGTG 5892-TATGCCGCTTGCGTTTCA	1454	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 100 s and a final extension step at 72 °C for 10 min.
CaKoV-7-F CaKoV-7-R	5494-CTGGACTCCCTCCTTCTCCA 6918- TCATCAAAGAGGGATCGGCG	1424	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 100 s and a final extension step at 72 °C for 10 min.
CaKoV-8-F CaKoV-8-R	6575-CCATCAAGAAGGAACCGGC 8011-GTGGGTGGTCTTTATATCACCATG	1436	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 100 s and a final extension step at 72 °C for 10 min.
CaKoV-9-F CaKoV-9-R	7000-CTCCACCTTCCTGAAAGATGA 8184-AAGAACAGTTAGAAAAGTTCAAAGACAA	1184	94 °C for 2 min, followed by 40 cycles at 94 °C for 45 s, 52 °C for 45 s and 72 °C for 80 s and a final extension step at 72 °C for 8 min.

consistent with previous reports [5, 10]. Moreover, the 14 Chinese CaKoV sequences cluster into two major groups, albeit with little bootstrap support. Interestingly, one sequence (SMCQ-S6) clusters alone, with the remaining 13 sequences clustering into several different, loosely supported for the most part, groups, along with the other sequences previously reported. Six of the 13 sequences appear more closely related to the CaKoV strains from South Korea and England, whereas seven of the 13 sequences seem to be more closely related to the CaKoV strains from China and Japan.

VP1 genes sequence analysis

We obtained five CaKoV complete *VP1* gene sequences in this study, ranging in length between 835–838 bp, and encoding 278–279 aa. Nt and deduced aa identities among these five CaKoV *VP1* gene sequences range from 95.2% to 99.5% and 94.7% to 98.6%, respectively. Nt and aa identities are 83.0%–91.2% and 82.3%–89.7%, respectively, between these five CaKoV *VP1* gene sequences and other CaKoV *VP1* sequences previously reported. No reorganization of the gene was found using RDP software.

Phylogenetic analysis based on our and other reported CaKoV VP1 aa sequences divides the virus isolates into two major groups: one group of sequences from four sources,

the United States, England, Korea, and Africa; and another group of sequences from China (Fig. 2). However, one Chinese sequence, JQ11763/CH-1, is not strongly associated with either cluster. Our five Chinese VP1 aa sequences cluster into a single group, with 100% bootstrap support, but the relationship with strain JQ11763/CH-1 remains unclear based on VP1 sequences. These five VP1 sequences share nine identical aa substitution sites, compared with the other six aa sequences previously reported, all concentrated at positions 201–243 (Table 2).

SMCD-59/CHN/2015 genome analysis

We amplified, using nine pairs of primers, sequenced, and assembled the genome of the SMCD-59/CHN/2015 CaKoV Chinese strain (GenBank No. MF062158). This kobuvirus genome has 8,184 nt, excluding the poly-(A) tail and contains a 7,335 bp ORF encoding a putative polyprotein precursor of 2,444 aa, a 601 bp 5′-UTR, and a 248 bp 3′-UTR (Fig. 3). Base content, 19.94% A, 20.95% G, 21.89% T, and 37.21% C (GC content 58.16%), is characteristic of the family Picornaviridae [14]. Strain SMCD-59 is 92.9%–96.6% identical based on nts (97.6%–98.1% identical based on aas) to four other CaKoV strains with available complete genome sequences (JQ11763/CH-1, KC161964/UK003,

Fig. 1 A neighbor-joining phylogenetic tree based on a sequence alignment of 504 nucleotides of the *3D* gene from CaKoV strains; isolates from this study are marked with black boxes. Bootstrap values based on 1,000 replicates are shown at the nodes
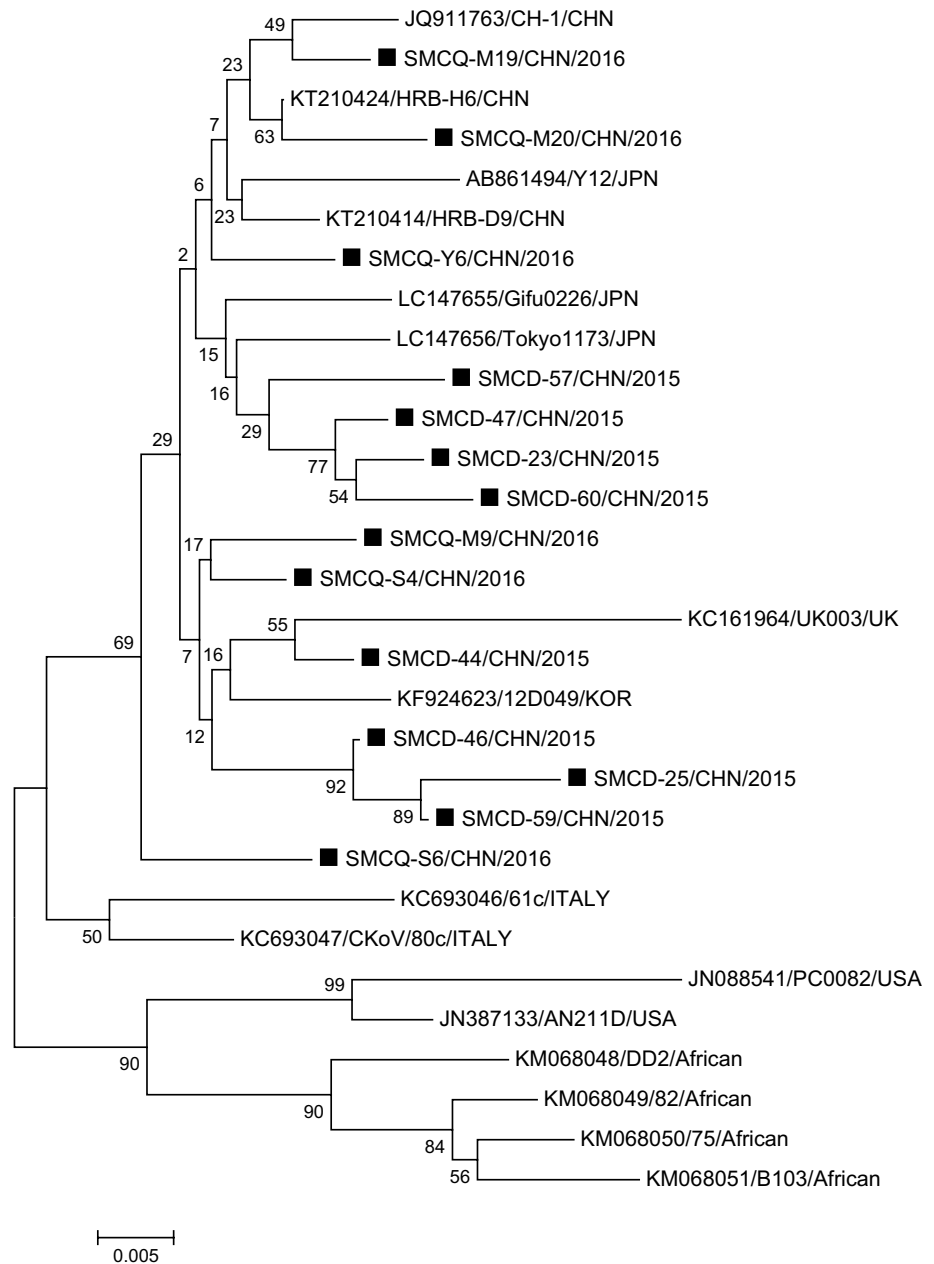

KF924623/12D049, and JN088541/PC0082). Aa identities of VP1, 3A, and 3B were 82.6%–87.9%, 81.9%–90.4%, and 76.9%–88.5%, respectively, with the other CaKoV strains. Results indicate that the VP1, 3A, and 3B genes have undergone greater evolutionary variation than previously reported (Table 3).

Phylogenetic analysis of our genome sequence with other published CaKoV genomes shows strain SMCD-59 to be more closely related to a Chinese (CH-1) and Korean (12D049) canine strain, than to other strains (Fig. 4A). The *3A* gene phylogeny (Fig. 4B) places four canine strains (MF062158/SMCD-59, JQ11763/CH-1, KC161964/UK003, and KF924623/12D049) together, indicating a closer

relationship among these strains from Asia and Europe, than to other, more distantly related strains from Africa and North America. Interestingly, phylogenetic analysis of the *3B* gene shows all strains from Asia clustering together, separate from areas outside of Asia (Fig. 4C).

Discussion

CaKoV infection was identified in 50.46% of all the diarrheic fecal samples that we collected in southwest China, which is higher than other Chinese regions previously reported [10, 11]. This suggests that CaKoV has widely
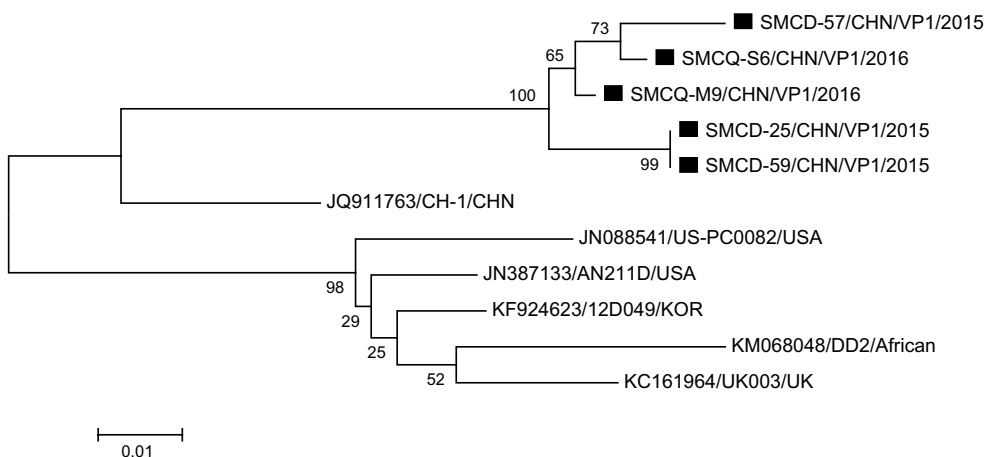
Fig. 2 A neighbor-joining phylogenetic tree based on a sequence alignment of 279 amino acids inferred from the *VP1* gene of CaKoV strains; isolates from this study are marked with black boxes. Bootstrap values based on 1,000 replicates are shown at the nodes

Table 2 Amino acid substitutions in VP1, when compared with GenBank strains

Strains	Amino acid at position								
	201	204	205	206	210	213	218	241	243
JN387133/AN211D/USA	T	V	E	D	S	S	I	A	T
JN088541/PC0082/USA	T	V	E	D	S	S	I	A	A
KC161964/UK003/UK	T	V	E	D	S	S	I	T	T
KF924623/12D049/KOR	T	V	E	D	S	S	I	A	A
JQ911763/CH-1/CHN	T	V	E	D	S	S	I	A	A
SMCD-59/CHN/VP1/2015[*]	S	Q	Q	I	Q	T	V	K	I
SMCD-25/CHN/VP1/2015[*]	S	Q	Q	I	Q	T	V	E	N
SMCD-57/CHN/VP1/2015[*]	S	Q	Q	T	Q	T	V	E	N
SMCQ-S6/CHN/VP1/2016[*]	S	Q	Q	T	Q	T	V	E	N
SMCQ-M9/CHN/VP1/2016[*]	S	Q	Q	T	Q	T	V	E	N

[*] represents the CaKoV strains in this study
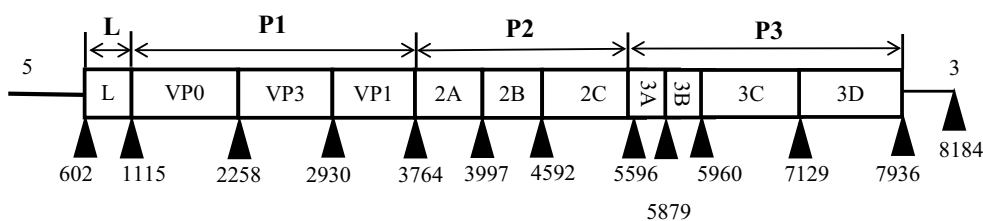
Fig. 3 Graphical depiction of the complete SMCD-59/CHN/2015 CaKoV genome; predicted nucleotide cleavage site positions are shown

circulated among dogs in China. However, the pathogenic potential of CaKoV remains unclear. Moreover, previous studies have shown no significant difference in CaKoV prevalence between healthy and diarrheic dog samples [6]. Recently, it has been reported that CaKoV can cause systemic infections [13]. The pathogenicity of CaKoV needs to be studied in detail within the context of virus load in diarrheic versus healthy dogs, and in the role of co-infection with other enteroviruses.

Phylogenetic analysis of the 504 bp partial *3D* gene sequence shows the 14 sequences in the study cluster into several different, loosely supported groups (Fig. 1). The CaKoV 3D sequences demonstrate considerable genetic diversity amongst themselves, as represented by horizontal branch lengths in the figure. This result is similar to a previous report in northeast China [10]. Interestingly, strain SMCQ-S6 clusters separately from the other strains, although bootstrap support for this distinction is not that

Table 3 Nucleotide and putative amino acid identities between SMCD-59 and other CaKoV strains

Gene region		Nucleotide identity (%)/amino acid identity (%)			
		JQ911763/CH-1/CHN	JN088541/PC0082/USA	KC161964/UK003/UK	KF924623/12D049/KOR
L		96.0/98.3	93.4/95.3	92.8/96.5	92.8/97.2
P1	VP0	96.7/99.2	91.5/98.2	92.0/98.4	92.7/98.2
	VP3	97.6/99.6	92.3/99.6	93.9/99.6	92.3/99.6
	VP1	90.8/87.9	84.2/82.6	85.1/85.8	85.6/86.8
P2	2A	98.2/100	96.1/100	95.2/99.1	97.3/100
	2B	97.0/100	94.4/100	92.3/99.4	97.2/100
	2C	97.5/99.7	95.4/100	95.5/99.7	96.2/99.1
P3	3A	96.8/90.4	94.4/84.0	93.7/81.9	95.4/86.2
	3B	95.0/88.5	90.0/76.9	90.0/76.9	91.2/76.9
	3C	97.9/99.2	95.3/98.7	95.6/98.7	96.8/98.2
	3D	97.6/100	95.4/99.6	96.4/100	95.9/98.5
Complete		96.6/98.0	92.9/97.6	93.2/98.1	94.1/97.8

P1 indicates the structural protein, P2 and P3 indicates non-structural proteins

strong (69%). Strain SMCQ-S6 has three nt replacements compared with the other 3D sequences in the study (positions 69, 111, and 189 in the alignment). Further targeted research is needed to determine the effect of these changes.

The VP1 protein of the Aichivirus is the most exposed and immunodominant portion of the kobuvirus capsid, and is the most variable structural protein in kobuviruses [14–16]. Furthermore, a proline-rich fragment of the Aichivirus VP1 protein (P228–P240) has been identified as a recognition motif for enteric receptor binding [17]. Regardless, detailed information regarding the CaKoV VP1 protein is quite limited.

The CaKoV VP1 aa sequences from our study have a similar proline-rich fragment at aa site P234–P244. Two aa substitutions occur in this region in these sequences, compared with other published sequences; whether this change affects function remains to be clarified. The five VP1 sequences from China that we generated and cluster together significantly (100% bootstrap), loosely associated with another Chinese sequence, and strongly separated from the other published VP1 sequences (98% bootstrap) in our phylogenetic analysis (Fig. 2). This suggests a unique evolutionary trend in the kobuviruses circulating among Chinese dogs, when compared with other countries.

We successfully obtained a complete CaKoV genome in this study. Sequence identity shows strain SMCD-59 to be more similar to both Asian and European strains than to North American strains. Phylogenetic analysis based on genome sequences shows CaKoV strains from Asia to be more closely related to each other than to European and American strains. Phylogenetic analyses of partial *3D* and complete *3A* genes are consistent with this result. Further studies will be required to ascertain whether the evolutionary relationships of any particular CaKoV gene can be used as

an estimate of the overall evolutionary history of the various CaKoV strains.

Aa identities between the VP1, 3A, and 3B sequences of strain SMCD-59 and other strains ranges from 82.6%–87.9%, 81.9%–90.4%, and 76.9%–88.5%, respectively (Table 3). Until recently, the precise function of the 3A and 3B proteins were unclear for CaKoV. However, Klima *et al.* report that the 3A protein of the kobuvirus genus (*i.e.* Aichivirus A and Aichivirus B) act as a molecular harness to hijack host acyl-CoA-binding domain-containing protein-3 (ACBD3), which creates kobuvirus replication organelles at specific replication sites within the host cell [18]. Furthermore, the 3A protein of many picornaviruses plays an important role in viral replication [19]. Thus, we speculate that the 3A protein in CaKoV also participates in the replication of the virus. Therefore, further study should provide interesting results as to whether the 3A aa mutations we noted affect viral replication in CaKoV.

Conclusions

In conclusion, this study provides proof that CaKoV widely circulates in diarrheic dogs in southwest China, and shows considerable genetic diversity based on complete genomes, and on partial and entire gene sequences. Interestingly, the strains circulating in southwest China show unique evolutionary trends based on *VP1* gene sequences. This may relate to the prominent role VP1 plays in host immune response. Furthermore, we successfully sequenced a complete genome from one of our samples. These findings contribute to our further understanding of epidemics and genetics, and expedite further study of CaKoV in China.

Fig. 4 A neighbor-joining phylogenetic trees are shown based on the nucleotide sequences of strain SMCD-59/CHN/2015 CaKoV, together with those of other published CaKoV reference strains: specifically, the entire genome sequence (A), the *3A* gene (B), and the *3B* gene (C). The numbers at the nodes represent the bootstrap percentages supporting a particular branch occurring after 1,000 replicates. Isolates from this study are marked with black boxes
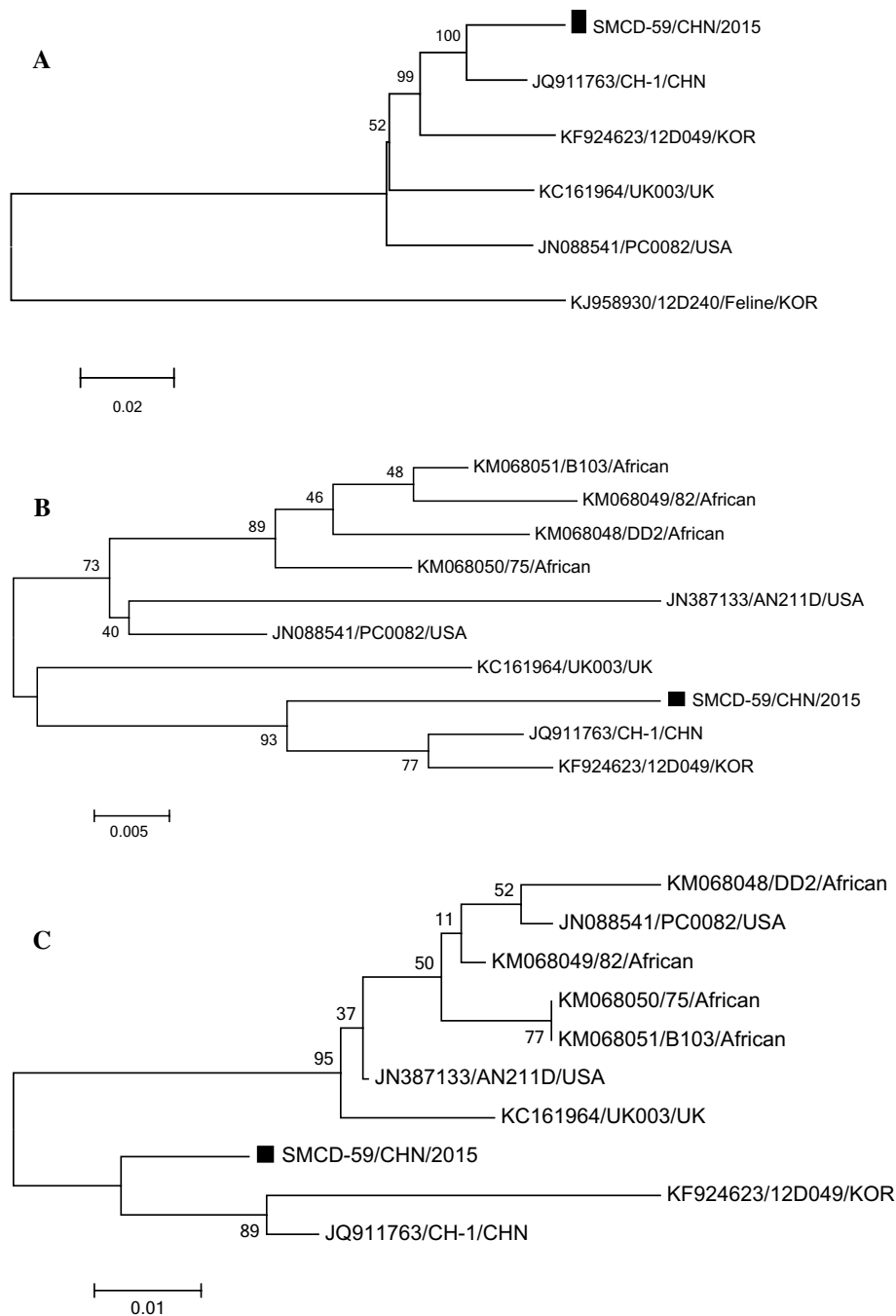
Compliance with ethical standards

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

References

1. Khamrin P, Maneekarn N, Okitsu S, Ushijima H (2014) Epidemiology of human and animal kobuviruses. Virusease 25(2):195–200
2. Kapoor A, Simmonds P, Dubovi EJ, Qaisar N, Henriquez JA, Medina J, Shields S, Lipkin WI (2011) Characterization of a canine homolog of human aichivirus. J Virol 85(21):11520–11525
3. 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

4. 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(3–4):246–252

5. Di Martino B, Di Felice E, Ceci C, Di Profio F, Marsilio F (2013) Canine kobuviruses in diarrhoeic dogs in Italy. Vet Microbiol 166(1–2):246–249

6. Oem JK, Choi JW, Lee MH, Lee KK, Choi KS (2014) Canine kobuvirus infections in Korean dogs. Arch Virol 159(10):2751–2755

7. 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(3):1141–1145

8. 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

9. Soma T, Matsubayashi M, Sasai K (2016) Detection of kobuvirus RNA in Japanese domestic dogs. J Vet Med Sci 78(11):1731–1735

10. Li C, Wei S, Guo D, Wang Z, Geng Y, Wang E, Zhao X, Su M, Wang X, Sun D (2015) Prevalence and phylogenetic analysis of canine kobuviruses in diarrhoetic dogs in northeast China. J Vet Med Sci 78(1):7–11

11. Kong N, Zuo Y, Wang Z, Yu H, Zhou EM, Shan T, Tong G (2016) Molecular characterization of new described kobuvirus in dogs with diarrhea in China. Springerplus 5(1):2047

12. 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(7):1803–1806

13. 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(3):867–872

14. Reuter G, Boros Ákos, Pankovics P (2011) Kobuviruses—a comprehensive review. Rev Med Virol 21(1):32–41

15. 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

16. 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

17. Zhu L, Wang X, Ren J, Kotecha A, Walter TS, Yuan S, Yamashita T, Tuthill TJ, Fry EE, Rao Z, Stuart DI (2016) Structure of human Aichi virus and implications for receptor binding. Nat Microbiol 1(11):16150

18. Klima M, Chalupska D, Różycki B, Humpolickova J, Rezabkova L, Silhan J, Baumlova A, Dubankova A, Boura E (2017) Kobuviral non-structural 3A proteins act as molecular harnesses to hijack the host ACBD3 protein. Structure 25(2):219–230

19. Fujita K, Krishnakumar SS, Franco D, Paul AV, London E, Wimmer E (2007) Membrane topography of the hydrophobic anchor sequence of poliovirus 3a and 3ab proteins and the functional effect of 3a/3ab membrane association upon RNA replication. Biochemistry 46(17):5185–5199