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Genetic characterization of canine parvovirus type 2c from domestic dogs in Korea

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Abstract

Canine parvovirus type 2 (CPV-2) is an aetiological agent that causes acute haemorrhagic enteritis and fatal myocarditis in dogs. Since CPV-2 first emerged in the late 1970s, its rapid evolution has resulted in three antigenic variants: CPV-2a, CPV-2b and CPV-2c. Here, we report, for the first time in Korea, two cases of CPV-2c infection in two dogs with severe diarrhoea. The complete open reading frame (4,269nt) of CPV-2, encoding both non-structural (NS) and structural (VP) proteins, was sequenced. Based on the amino acid Gln present at residue 426 of the VP2 gene, these strains were typed as CPV-2c, and were named Korea CPV-2c_1 and Korea CPV-2c_2. These strains shared 99.48% reciprocal nucleotide sequence identity and had the highest nucleotide identity (99.77%–99.34%) with Asian CPV strains isolated in China, Italy (found in a dog imported from Thailand), and Vietnam from 2013 to 2017. Phylogenetic analysis based on the non-structural (NS1) and capsid (VP2) genes revealed that Korean CPV-2c strains clustered closely to Asian CPV strains, and separately from strains isolated in Europe, South America and North America. Amino acid changes never reported before were observed in NS1 (Thr70Pro, Cys287Tyr), VP1 (Lys17Arg, Phe33Leu) and VP2 (Gln365His, Ala516Val). Additional observed mutations, including Phe267Tyr, Tyr324Ile and Gln370Arg, have been previously reported in the recent CPV-2c strains with Asian origins. These results suggest that the Korean CPV-2c strains were potentially introduced via neighbouring Asian countries.

KEYWORDS

canine parvovirus 2c, epidemiology, genetic characteristics, South Korea

1 | INTRODUCTION

Canine parvovirus type 2 (CPV-2) is a viral agent that causes acute haemorrhagic enteritis and, less frequently, fatal myocarditis in domestic and wild carnivores. Non-immunized puppies are particularly susceptible, and the virus causes severe enteritis accompanied with loss of appetite, depression, high fever, vomiting, rapid dehydration and bloody diarrhoea, as well as a wide range of cardiac clinical signs ranging from tachypnea or dyspnoea with exercise to respiratory distress, and resulting in acute heart failure (Appel, Cooper, Greisen, Scott, & Carmichael, 1979; Decaro & Buonavoglia, 2017; Hayes, Russel, Mueller, & Lewis, 1979).

CPV-2 is a non-enveloped single-stranded linear DNA virus of the *Parvoviridae* family, with a genome of approximately 5,200nt in length. The genome contains two open reading frames (ORFs), which encode for non-structural (NS) and structural (VP) proteins (Cotmore et al., 2019; Reed, Jones, & Miller, 1988). Interestingly, the virus has a rapid mutation rate, similar to those observed in RNA viruses (Decaro et al., 2009; Shackelton, Parrish, Truyen, & Holmes, 2005). CPV-2 first emerged in the late 1970s through a host-switching event of feline panleukopenia virus (FPV) (Appel et al., 1979).

Since first emerging, mutations of the VP2 gene have generated the antigenic variants CPV-2a, CPV-2b and CPV-2c, which soon replaced the original CPV-2 virus worldwide (Buonavoglia et al., 2001; Miranda & Thompson, 2016; Parrish et al., 1991; Parrish, O'Connell, Evermann, & Carmichael, 1985). These variants have different biological properties from the original CPV-2 virus and can infect cats and proliferate in feline cell lines (Truyen & Parrish, 1992).

CPV-2c carrying a Glu on residue 426 of VP2 was first identified in Italy in 2000 (Buonavoglia et al., 2001). A retrospective analysis described circulation of CPV-2c in Germany already in 1996 (Decaro et al., 2007). Currently, the CPV-2c variants are distributed worldwide, including Asia, Africa, Australia, Europe, and North and South America (Miranda & Thompson, 2016; Woolford, Crocker, Bobrowski, Baker, & Hemmatzadeh, 2017). In the 2000s, this variant was found with high frequency in Europe and South America, but was relatively rare in Asia, where CPV-2a was predominant (Decaro & Buonavoglia, 2012). However, the distribution of CPV-2c has since expanded, and it has recently been found in Asia including China (Geng et al., 2015; Wang et al., 2016; Zhao et al., 2017), Taiwan (Chiang, Wu, Chiou, Chang, & Lin, 2016; Lin et al., 2017), Vietnam (Hoang et al., 2019), Laos (Vannamahaxay et al., 2017), Mongolia (Temuujin et al., 2019) and Thailand (Charoenkul et al., 2019).

Although the VP2 gene of CPV-2 has been relatively well researched, the study of genetic variations in the NS gene has been limited. The NS gene is associated with viral replication, DNA packaging, cytotoxicity and pathogenicity. A recent study has described specific amino acids in NS that are associated with the differentiation of FPV and CPV-2 and CPV-2 NS also harbours more sites that are under selection (Mira, Canuti, et al., 2019).

Recent studies revealed that the migration of CPV-2 might occur across geographical barriers, resulting in dynamic changes in the CPV-2 population at the same location. For example, a CPV-2c strain with the genetic characteristics of Asian-origin CPV-2c was found in dogs imported from Thailand to Italy, and is currently circulating in Italy (Mira, Purpari, et al., 2019; Mira et al., 2018). In addition, the genetic divergent properties of CPV-2 in South America resulted from the migration of CPV-2 originating from other continents during the last two decades (Grecco et al., 2018; Miranda & Thompson, 2016; Perez et al., 2014).

Between 2003 and 2006, two CPV-2 variants, CPV-2a and CPV-2b, were detected in Korea, with a higher prevalence of the CPV-2a variant (Jeoung, Ahn, & Kim, 2008; Kang et al., 2008; Yoon, Jeong, Kim, & An, 2009). However, CPV-2c was not detected. Here, we report the first identification of CPV-2c variants in Korea, and compare their genetic characteristics and epidemiological relatedness with global CPV-2 strains.

2 | MATERIALS AND METHODS

2.1 | Samples

Two cases of suspected CPV-2 infection were reported in March and November 2017 in South Korea. First case (id:17–81) was a 7-month-old French bulldog from the Gyeong-gi province in South Korea that presented with vomiting and haemorrhagic diarrhoea, seven days after the administration of a single dose of a DHPP vaccination, preventing canine distemper, infectious canine hepatitis, canine parainfluenza and canine parvoviral enteritis. Second case (id:17–270) was a 5-month-old Korean jindo from the Jeollanam-do province of South Korea, with clinical signs including salivation, vomiting and watery diarrhoea. The vaccination status of the dog was unknown. A faecal specimen from 17–81 and the carcass of 17–270 were submitted to the Animal and Plant Quarantine Agency for diagnostic purposes. A necropsy was conducted for 17–270, and tissues obtained from the lymphatic gland, lung, spleen, heart, kidney, intestine and brain were used for histopathological examination and molecular assays.

3 | HISTOPATHOLOGICAL EXAMINATION

The parenchymal organs were removed from 17–270, fixed in 10% neutral buffered formalin and embedded in paraffin wax. The embedded tissues were sectioned and subsequently stained with haematoxylin and eosin.

4 | PCR AND SEQUENCING ASSAYS

PCR detection of canine viral agents was performed on the faeces of 17–81 and the necropsy tissues of 17–270. Viral DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN), according to the manufacturer's instructions, and stored at −20°C. PCR assay kits and specific primers were used to identify canine viral pathogens. CPV, canine distemper virus (CDV), canine parainfluenza virus (CPIV) and canine coronavirus (CCoV) were screened using Lilif™ CPV PCR kit, Lilif™ CDV Nested-PCR kit, Lilif™ CPIV RT-PCR kit, and Lilif™ CCoV RT-PCR kit (iNtRON Biotechnology), respectively, according to the manufacture's instructions. Canine herpesvirus (CHV) and canine adenovirus type 1 and 2 (CAdV-1 and CAdV-2) were detected using previously described PCR assays (Burr, Campell, Nicolson, & Onion, 1996; Hu et al., 2001). The 25 μL PCR mixtures contained 100 ng DNA template, primers, 10× HotStarTaq™ Master Mix kit (Qiagen) and distilled water. The PCR products were analysed by electrophoresis on a 2% agarose gel with ethidium bromide.

To analyse the complete sequence of the CPV coding regions, five primer pairs were designed based on the genome sequence of the CPV-N strain (NCBI RefSeq NC_001539) (Table 1). PCR was performed using Advantage® 2 Polymerase mix (Takara Bio) according to the manufacturer's instructions. The PCR protocol included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for l min, then a final extension at 72°C for 10 min. The five PCR amplicons were cloned into a pGEM®-T Easy vector system (Promega) in ECOS™ 101 competent cells [DH5α] (Yeastern Biotech). Selected individual clones were sequenced, and consensus sequences were

TABLE 1 Oligonucleotide primers used for CPV-2 sequencing in this study

Primer name	Sequence (5` to 3`)	Location*	Product (bp)
CPV 1 F	CTTCTTGTCTTTGACAGAGTGAA	224–246	1,198
CPV 1 R	TGCTATAGCGTGACAAACTTTA	1399–1420	
CPV 2 F	ATCTTGCAAATTCTAGAACATGTCA	1344–1368	843
CPV 2 R	TTGCACGTCTTTGTGAGTAAC	2165–2185	
CPV 3 F	ACGTAGTGGACCTTGCACTGGAA	2079–2101	776
CPV 3 R	GATCCTGTAGCTCTTTCATTTCT	2831–2853	
CPV 4 F	AATCTTGCACCAATGAGTGA	2774–2793	1,169
CPV 4 R	TGACCATGTTGTCTACCAAATGCAT	3918–3942	
CPV 5 F	TATGAGACCAGCTGAGGTTGGTTA	3775–3798	821
CPV 5 R	AATTTTTCTAGGTGCTAGTTGATATGTAAT	4566–4595	

*Nucleotide positions refer to the prototype CPV-*N* (RefSeq sequence: NC_001539).

assembled using the computational program SeqMan (Lasergene, DNASTAR). Sequence data of the CPV-2 strains obtained from cases id.17–81 and id.17–270 have been submitted to the GenBank database under accession numbers MK306289 and MK306290, respectively.

5 | SEQUENCE AND PHYLOGENETIC ANALYSES

The obtained novel CPV-2 nucleotide sequences were aligned together with 61 CPV-2 sequences randomly obtained from GenBank using the ClustalW algorithm in Bioedit software 7.2.0 (Ibis Therapeutics). The alignment was used to calculate nucleotide sequence identities using the pairwise distance algorithm.

Phylogenetic trees based on the nucleotide sequences of complete NS1 and VP2 gene were analysed with MEGA 6 software using the maximum-likelihood (ML) method according to Hasegawa-Kishino-Yano (HKY) and the Tamura 3-parameter (T92) models with Gamma distribution (five rate categories) (G) and 1,000 bootstrap replicates (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The best-fit model for nucleotide substitution was selected in MEGA 6 and used to determine HKY + G for the NS1 gene and T92 + G for the VP2 gene.

6 | RESULTS

6.1 | Identification of CPV-2

The necropsy organs of 17–270 showed typical lesions for CPV-2 infection. Grossly, the mucosa and serosa of the small intestine were congested and glistening, and the mucosa of the large intestine showed petechiae. Histopathological analysis revealed loss and dilation of crypts, and infiltration of inflammatory cells in the lamina propria in the intestine. PCR detection of canine viral pathogens was performed on samples from both dogs. All samples tested positive for CPV-2 and negative for CCoV, CDV, CPIV, CHV, and CAdV-1 and CAdV-2.

7 | CPV-2 SEQUENCE ANALYSIS

A consensus genome sequence containing 4,269nt with 37% GC content was obtained from the faeces of 17–81 and intestinal tissue of 17–270. The sequence includes two ORFs encoding the NS gene (2007nt), the VP gene (2256nt) and an untranslated region (6nt) between the two ORFs. The strains were typed as CPV-2c, based on the amino acid residue at position 426 (Gln) of the VP2 gene sequence, and were named as Korea CPV-2c_1 and Korea CPV-2c_2, respectively.

Table 2 shows the nucleotide sequence similarity of the two Korean CPV-2c strains compared with other CPV-2c strains in GenBank. The Korean CPV-2c strains had 99.48% reciprocal nucleotide identity. When compared with other CPV-2 strains, Korea CPV-2c_1 had 99.77%–98.94% identity, while Korea CPV-2c_2 had 99.74%–98.70% identity. Maximum similarity (99.77%–99.34%) to the Korean CPV-2c strain was observed with CPV-2c strains with Asian origins collected from China, Italy (isolated from a dog imported from Thailand) and Vietnam between 2013 and 2017. Conversely, the similarity with CPV-2c strains from Europe and South America was lower (99.13%–98.70%). Among these strains, the CPV-2c Argentina 2010 (Acc.nr. MF177249) strain shared the highest similarity of 98.95 and 98.70% with Korea CPV-2c_1 and Korea CPV-2c_2, respectively.

Table 3 presents a total of 15 nucleotides substitutions that were detected in Korean CPV-2c strains, resulting in ten synonymous and five non-synonymous mutations. Korea CPV-2c_1 harboured four substitutions, including A50G (Lys17Arg), T97C (Phe33Leu), and G477A (Gln159) in VP1 and C1546T (Ala516Val) in VP2. Korea CPV-2c_2 harboured 11 substitutions, including G165A (Glu55), A208C (Thr70Pro), T405C (135His), G860A (Cys287Tyr), T1347C (Phe449) and T1401C (Ile467) in NS, C354A (Pro118) in VP1, and A1095T (Gln365His), A1383G (Pro461), A1686G (Val562) and T1704C (Gly568) in VP2.

TABLE 2 Nucleotide sequence similarity between Korea CPV-2c strains and other CPV-2 strains

	No.*	Details	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Korea CPV–2	1	CPV–2c_1 Korea 2017																
	2	CPV–2c_2 Korea 2017	99.48															
Proto CPV–2	3	CPV–2a Japan 1998	99.20	98.91														
	4	CPV–2b USA 1986	99.20	98.91	99.60													
	5	CPV–2c Italy 2001	99.22	98.98	99.60	99.67												
Asian-origin CPV–2	6	CPV–2c China 2017	99.77	99.72	99.20	99.20	99.27											
	7	CPV–2c Italy 2017	99.74	99.74	99.17	99.17	99.25	99.98										
	8	CPV–2c Vietnam 2013	99.46	99.46	98.94	98.98	99.06	99.69	99.72									
	9	CPV–2a China 2014	99.44	99.34	99.27	99.17	99.22	99.62	99.60	99.46								
CPV–2c strains with other origins	10	CPV–2c Italy 2010	99.13	98.89	99.51	99.58	99.91	99.17	99.15	98.96	99.17							
	11	CPV–2c France 2009	99.10	98.86	99.48	99.55	99.88	99.15	99.13	98.94	99.10	99.79						
	12	CPV–2c Uruguay 2007	99.08	98.84	99.46	99.53	99.86	99.13	99.10	98.91	99.08	99.77	99.74					
	13	CPV–2c Ecuador 2011	99.13	98.89	99.51	99.58	99.91	99.17	99.15	98.96	99.13	99.81	99.79	99.77				
	14	CPV–2c Argentina 2010	98.94	98.70	99.32	99.39	99.62	98.98	98.96	98.77	98.98	99.53	99.51	99.53	99.53			
	15	CPV–2c Brazil 2014	99.08	98.84	99.46	99.48	99.67	99.13	99.10	98.91	99.08	99.58	99.55	99.53	99.58	99.53		
	16	CPV–2c Australia 2016	99.01	98.77	99.39	99.46	99.79	99.06	99.03	98.84	99.01	99.69	99.67	99.69	99.69	99.46	99.46	

*GenBank accession numbers of CPV-2 strains; 1, MK306289; 2, MK306290; 3, D26079; 4, EU659120; 5, MF177239; 6, MG013488; 7, MF510157; 8, LC214969; 9, KR002800; 10, MF177240; 11, MF177227; 12, KM457109; 13, MF177263; 14, MF177249; 15, MF177255; 16, KU508691

TABLE 3 Nucleotide and amino acid divergences in CPV-2c strains from Korea

Gene	NS						VP													
							VP1				VP2									
Nucleotides	163–165	208–210	403–405	859–861	1345–1347	1399–1401	49–51	97–99	352–354	475–477	799–801	970–972	1093–1095	1108–1110	1276–1278	1318–1320	1381–1383	1546–1548	1684–1686	1701–1704
Amino acid	55	70	135	287	449	467	17	33	118	159	267	324	365	370	426	440	461	516	562	568
Korea CPV–2c																				
CPV–2c_1 Korea 2017	GAG	ACC	CAT	TGT	TTT	ATT	A**GG**	**C**TT	CCC	CA**G**	TAT	ATT	CAA	CGA	GAA	ACA	CCA	G**T**T	GTA	GGT
(MK306289)	Glu	Thr	His	Cys	Phe	Ile	**Arg**	**Leu**	Pro	Gln	Tyr	Ile	Gln	Arg	Glu	Thr	Pro	**Val**	Val	Gly
CPV–2c_2 Korea 2017	GA**A**	**C**CC	CA**C**	**TAT**	TT**C**	AT**C**	AAG	TTT	CC**A**	CAA	TAT	ATT	CA**T**	CGA	GAA	ACA	CC**G**	GCT	GT**G**	GG**C**
(MK306290)	Glu	**Pro**	His	**Tyr**	Phe	Ile	Lys	Phe	Pro	Gln	Tyr	Ile	**His**	Arg	Glu	Thr	Pro	Ala	Val	Gly
Prototype CPV–2																				
CPV–2a Japan 1998	GAG	ACC	CAT	TGT	TTT	ATT	AGG	TTT	CCA	CAA	TTT	TAT	CAA	CAA	AAT	ACA	CCA	GCT	GTA	GGT
(D26079)	Glu	Thr	His	Cys	Phe	Ile	Lys	Phe	Pro	Gln	Phe	Tyr	Gln	Gln	Asn	Thr	Pro	Ala	Val	Gly
CPV–2b USA 1986	GAG	ACC	CAT	TGT	TTT	ATT	AGG	TTT	CCA	CAA	TTT	TAT	CAA	CAA	GAT	ACA	CCA	GCT	GTA	GGT
(EU659120)	Glu	Thr	His	Cys	Phe	Ile	Lys	Phe	Pro	Gln	Phe	Tyr	Gln	Gln	Asp	Thr	Pro	Ala	Val	Gly
CPV–2c Italy 2001	GAG	ACC	CAT	TGT	TTT	ATT	AGG	TTT	CCA	CAA	TTT	TAT	CAA	CAA	GAA	ACA	CCA	GCT	GTA	GGT
(MF177239)	Glu	Thr	His	Cys	Phe	Ile	Lys	Phe	Pro	Gln	Phe	Tyr	Gln	Gln	Glu	Thr	Pro	Ala	Val	Gly
Recent Asia CPV–2																				
CPV–2c China 2017	GAG	ACC	CAT	TGT	TTT	ATT	AGG	TTT	CCA	CAA	TAT	ATT	CAA	CGA	GAA	ACA	CCA	GCT	GTA	GGT
(MG013488)	Glu	Thr	His	Cys	Phe	Ile	Lys	Phe	Pro	Gln	Tyr	Ile	Gln	Arg	Glu	Thr	Pro	Ala	Val	Gly
CPV–2c Italy 2017	GAG	ACC	CAT	TGT	TTT	ATT	AGG	TTT	CCA	CAA	TAT	ATT	CAA	CGA	GAA	ACA	CCA	GCT	GTA	GGT
(MF510157)	Glu	Thr	His	Cys	Phe	Ile	Lys	Phe	Pro	Gln	Tyr	Ile	Gln	Arg	Glu	Thr	Pro	Ala	Val	Gly
CPV–2c Vietnam 2013	GAG	ACC	CAT	TGT	TTT	ATT	AGG	TTT	CCA	CAA	TAT	ATT	CAA	CGA	GAA	ACA	CCA	GCT	GTA	GGT
(LC214969)	Glu	Thr	His	Cys	Phe	Ile	Lys	Phe	Pro	Gln	Tyr	Ile	Gln	Arg	Glu	Thr	Pro	Ala	Val	Gly

Note: Bold character indicates the mutation of nucleotide and amino acid from Korean CPV-2c strains.

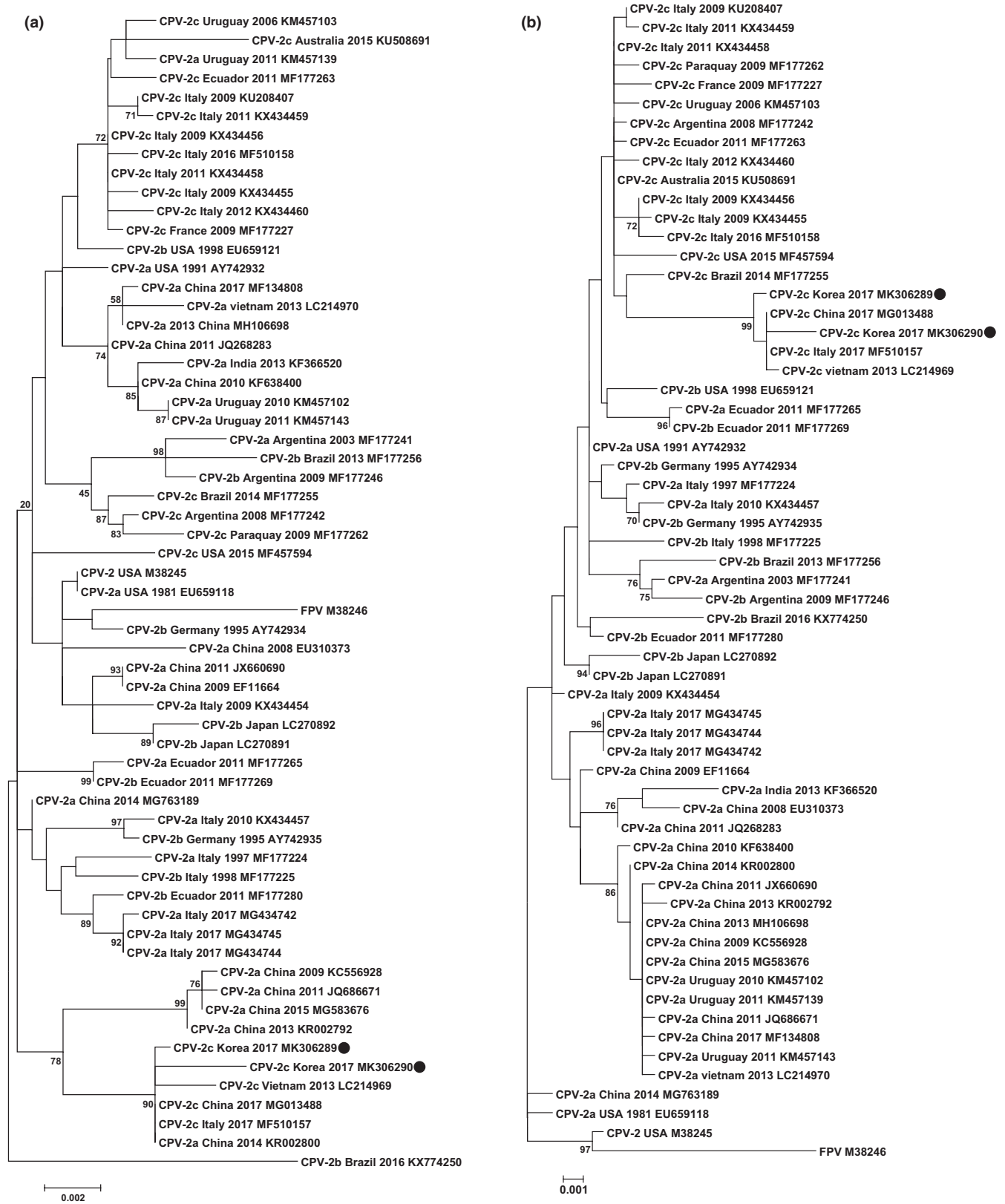

FIGURE 1 Maximum-likelihood trees based on the complete NS1 (a) and VP2 (b) nucleotide sequences of canine parvovirus type 2 (CPV-2). Scale bars indicate nucleotide substitutions per site. Bootstrap values at nodes are based on 1,000 replicates and bootstrap values greater than 70 are shown. CPV-2 strain was shown as 'CPV variants', ' country' 'year of isolation' and 'accession number' and Korean CPV-2c strain obtained from this study was indicated by black dot marking (●)

Additional mutations detected at Phe267Tyr, Tyr324Ile and Gln370Arg were identical to those reported in recent Asian CPV-2c strains (Chiang et al., 2016; Geng et al., 2015; Hoang et al., 2019).

Phylogenetic analysis of the NS1 and VP2 genes of Korean CPV-2c strains was performed including a data set of 61 CPV-2 genome sequences available in GenBank. This data set includes sequences of CPV-2 strains isolated in Asia (China, Japan and Vietnam), Australia, Europe (Italy, France and Germany), South America (Argentina, Brazil, Ecuador and Uruguay) and the USA. The NS and VP genes of Korean CPV-2c strains closely clustered with those of Asian CPV-2 strains but were separated from those of strains originating in Europe, South America and the USA (Figure 1).

8 | DISCUSSION

The present study reports the first evidence of CPV-2c infection resulting in severe enteritis in dogs in Korea, where CPV-2a had previously been predominant. Unique non-synonymous substitutions were found in the NS and VP genes of the Korean CPV-2c strains, and phylogenetic analysis indicated their close relationship with Asian CPV-2c variants. These findings suggest the potential introduction of CPV-2c into South Korea from neighbouring countries.

CPV-2c variants have been distributed throughout the world in the last two decades (Miranda & Thompson, 2016). Interestingly, CPV-2c was observed in Asia with low frequency in India (Nandi, Chidri, Kumar, & Chauhan, 2010) after the first identification of CPV-2c in Vietnam in 2002 (Nakamura et al., 2004). Recently, CPV-2c has been also found more frequently in Asia. Phe267Tyr, Tyr324Ile and Gln370Arg are among the most frequently observed mutations in the VP2 gene of Asian-origin CPV-2c variants (China, Laos, Taiwan, Thailand and Vietnam), and were also identified in the present study. Phe267Tyr, Tyr324Ile and Thr440Ala are also frequently detected in CPV-2a and CPV-2b worldwide, including Asia, and may contribute to antigenic drift that results in immune escape and vaccination failure (Geng et al., 2015). Gln370Arg was first observed in CPV-2c recovered from a giant panda in China in 2014 (Guo et al., 2013). The residue is located between residues 359 and 375, which are part of a flexible loop dependent on pH and Ca^{2+} levels. Mutations in this loop may result in a change in host range (canine or feline), and the haemagglutination of host erythrocytes (Simpson et al., 2000). Taken together, although the role of these residues needs to be further elucidated, these mutated residues may provide a survival advantage, allowing CPV-2c to adapt to a new host or environment.

In the present study, unique mutations were found scattered throughout the Korea CPV-2c strain genomes. Analysis of NS1 mutations among global CPV-2 variants has so far been limited. Korea CPV-2c_2 harboured two unique mutations (Thr70Pro and Cys287Tyr) in NS1, which is essential for viral replication and host apoptosis. Apoptosis is typically a host defence mechanism against harmful agents; however, some studies have demonstrated that cell death and cell cycle arrest are beneficial to the virus in parvovirus-infected cells, aiding in viral DNA replication and nuclear egress (Chen & Qiu, 2010). Two mutations in the VP1 region were observed in Korea CPV-2c_1 (Lys17Arg and Leu33Phe). These mutations are located in unique region of VP1, which is responsible for nuclear transport and phospholipase A2 activity during cell infection (Vihinen-Ranta, Wang, Weichert, & Parrish, 2002). Unique mutations in VP2, Gln365His in Korea CPV-2c_2 and Ala516Val in Korea CPV-2c_1, were reported in this study for the first time. Residue 365 is also located with residues 370 and 375 in the flexible loop that determines host range. Therefore, these mutations may contribute to host range extension (Simpson et al., 2000). The substitution at residue 516, Ala516Thr, has been reported as a specific mutation in Hungarian CPV-2 (Csagola, Varga, Lorincz, & Tuboly, 2014). Continuous monitoring of the genetic characteristics of Korean CPV-2 is therefore necessary for effective disease control.

In conclusion, the present study documents the genetic characteristics of the first reported cases of CPV-2c infection in Korea. The Korean CPV-2c strains possess mutations distinct from those observed in CPV-2c strains of other countries. Based on their phylogenetic divergence, the Korean CPV-2c strains clustered with CPV strains of Asian origin. These results suggest that CPV-2c was potentially introduced into South Korea by the transport of dogs and/or fomites contaminated with CPV-2c from neighbouring Asia countries.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

The authors confirm that the ethical polices of the journal, as noted on the journal's author guidelines page, have been adhered to. All samples (the gathered faeces and carcasses of dogs) used in this study were those that were submitted Animal and Plant Quarantine Agency for diagnosis of disease. Ethical approval was not required as per institutional guidelines and recommendations.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in 41 references.

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