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

Bo-Youn Moon | Jiung Jang | Seong-Hee Kim | Yeon-Hee Kim | Hyun-kyoung Lee | ByungJae So | Choi-Kyu Park | Kyoung-Ki Lee

1Animal Disease Diagnosis Division, Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea
2College of Veterinary Medicine & Animal Disease Intervention Center, Kyungpook National University, Daegu, Republic of Korea

**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

**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, Chou, 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 DPPV vaccine, 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 Liif™ CPV PCR kit, Liif™ CDV Nested-PCR kit, Liif™ CPIV RT-PCR kit, and Liif™ CCoV RT-PCR kit (InTRON Biotechnology), respectively, according to the manufacturer’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 1 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] (Yeaster Biotech). Selected individual clones were sequenced, and consensus sequences were
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.

| Primer name | Sequence (5’ to 3’) | Location* | Product (bp) |
|-------------|----------------------|-----------|--------------|
| CPV 1 F     | CTCTTGTTCTTTGGACAGATGGA | 224–246   | 1,198        |
| CPV 1 R     | TGCATAGCCGTGACAAAATTTTA | 1399–1420 |             |
| CPV 2 F     | ATCTTGCAAAATCTGAACATGCA | 1344–1368 | 843         |
| CPV 2 R     | TTGCACGTCTTTGAGTAAC | 2165–2185 |             |
| CPV 3 F     | AGCTAGTGGACCTTGACACTGGA | 2079–2101 | 776         |
| CPV 3 R     | GATCCCTGAGCTTCTTTCCATTCT | 2831–2853 |             |
| CPV 4 F     | AATCTTGCAACAAATGTAAC | 2774–2793 | 1,169       |
| CPV 4 R     | TGCACATTGTTGCTCAAAATGCAT | 3918–3942 |             |
| CPV 5 F     | TATGGACCCAGCTGAAGTGTTGATTA | 3775–3798 | 821         |
| CPV 5 R     | AATTTTCTAGTTGCTAGTGATATGTAAT | 4566–4595 |             |

* Nucleotide positions refer to the prototype CPV-N (RefSeq sequence: NC_001539).
| No.* | Details                              | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|------|-------------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1    | CPV-2c_1 Korea 2017                 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2    | CPV-2c_2 Korea 2017                 | 99.48 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 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 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 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 |    |    |    |    |    |    |    |    |    |
| 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
| Gene | NS | VP | VP1 | VP2 |
|------|----|----|-----|-----|
| Nucleotides | 163–165 | 208–210 | 403–405 | 859–861 | 1345–1347 | 1399–1401 | 352–354 | 475–477 | 799–801 | 970–972 | 1093–1095 | 1108–1110 | 1276–1328 | 1318–1383 | 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
  - (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
  - (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
  - (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
  - (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
  - (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
  - (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
  - (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
  - (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 Ca2+ 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.

ACKNOWLEDGEMENTS

This work was supported by the grant from Animal and plant quarantine agency (B-1543069-2019-21-04).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

The authors confirm that the ethical policies 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.

ORCID

Bo-Youn Moon https://orcid.org/0000-0002-8474-5506
Choi-Kyu Park https://orcid.org/0000-0002-0784-9061

REFERENCES

Appel, M. J., Cooper, B. J., Greisen, H., Scott, F., & Carmichael, L. E. (1979). Canine viral enteritis. I. Status report on corona- and parvo-like viral enteritides. Cornell Veterinarian, 69(3), 123–133.
identification of canine parvovirus (CPV) type 2c variants during 2016–2018 genetic surveillance of dogs in Mongolia. *Infect Genet Evol*, 73, 269–275. https://doi.org/10.1016/j.meegid.2019.05.006

Truyen, U., & Parrish, C. R. (1992). Canine and feline host ranges of canine parvovirus and feline panleukopenia virus: Distinct host cell tropisms of each virus in vitro and in vivo. *Journal of Virology*, 66(9), 5399–5408. https://doi.org/10.1128/JVI.66.9.5399-5408.1992

Vannamahaxay, S., Vongkhamchanh, S., Intanon, M., Tangtrongsup, S., Tiwananthagorn, S., Pringproa, K., & Chuammitri, P. (2017). Molecular characterization of canine parvovirus in Vientiane. *Archives of Virology*, 162(5), 1355–1361. https://doi.org/10.1007/s00705-016-3212-1

Vihinen-Ranta, M., Wang, D., Weichert, W. S., & Parrish, C. R. (2002). The VP1 N-terminal sequence of canine parvovirus affects nuclear transport of capsids and efficient cell infection. *Journal of Virology*, 76(4), 1884–1891. https://doi.org/10.1128/JVI.76.4.1884-1891.2002

Wang, J., Lin, P., Zhao, H., Cheng, Y., Jiang, Z., Zhu, H., … Cheng, S. (2016). Continuing evolution of canine parvovirus in China: Isolation of novel variants with an Ala5Gly mutation in the VP2 protein. *Infection, Genetics and Evolution*, 38, 73–78. https://doi.org/10.1016/j.meegid.2015.12.009

Woolford, L., Crocker, P., Bobrowski, H., Baker, T., & Hemmatzadeh, F. (2017). Detection of the canine parvovirus 2c subtype in Australian dogs. *Viral Immunology*, 30(5), 371–376. https://doi.org/10.1089/vim.2017.0019

Yoon, S. H., Jeong, W., Kim, H. J., & An, D. J. (2009). Molecular insights into the phylogeny of canine parvovirus 2 (CPV-2) with emphasis on Korean isolates: A Bayesian approach. *Archives of Virology*, 154(8), 1353–1360. https://doi.org/10.1007/s00705-009-0444-3

Zhao, H., Wang, J., Jiang, Y., Cheng, Y., Lin, P., Zhu, H., … Cheng, S. (2017). Typing of canine parvovirus strains circulating in North-East China. *Transboundary and Emerging Diseases*, 64(2), 495–503. https://doi.org/10.1111/tbed.12390

How to cite this article: Moon B-Y, Jang J, Kim S-H, et al. Genetic characterization of canine parvovirus type 2c from domestic dogs in Korea. *Transbound Emerg Dis*. 2020;67:1645–1653. https://doi.org/10.1111/tbed.13501