# Molecular Epidemiological Survey of Canine Parvovirus Circulating in China from 2014 to 2019

Bixia Chen[1], Xiaohui Zhang[2], and Endong Bao[1]

[1]Nanjing Agricultural University
[2]Nanjing Agricultural University

March 30, 2022

## Abstract

The global distribution of canine parvovirus (CPV-2), derived from a closely related carnivore parvovirus, has caused a considerable threat to the dog population. The virus continuously underwent genetic evolution, giving rise to several variants. To investigate the prevalence of Chinese CPV-2 strains in recent years, a total of 25 CPV-2 strains were isolated from 33 canine samples collected from 2018 to 2020, and then sequenced and analyzed. Two variants, New CPV-2a and CPV-2c were identified. Contrary to previous reports, the CPV-2c variant has gained an epidemiological advantage over the New CPV-2a variant in China. To make up for the relatively limited sample, 683 Chinese CPV-2 records identified between 2014 to 2019 were retrieved from Genbank and associated publications, whose result further supported our finding. That should be caught concern since the CPV-2c variant has been frequently related to vaccine failure in adult dogs. VP2 protein sequences analysis revealed several amino acid substitutions, including Ala5Gly, Pro13Ser, Phe267Tyr, Tyr324Ile, Gln370Arg, Thr440Ala, and Lys570Arg. Phylogenetic analysis indicated a close relationship between Chinese strains with Asian strains, suggesting the mutual transmission between Asian countries. Furthermore, the intercontinental transmission should be a cause for concern. Surprisingly, two feline panleukopenia (FPLV) strains with Ile101Thr mutation in VP2 protein were also successfully isolated from canine fecal samples, which was considered incapable of infecting dogs. This study clarified the epidemic characteristics of Chinese CPV-2 strains between 2014 and 2019, offering a reference for epidemic control. Besides, the detection of FPLV in canine samples may provide information for future studies on the evolution of carnivore parvovirus.

## Introduction

Canine parvovirus (CPV-2), a causative agent of hemorrhagic gastroenteritis and myocarditis in canids, belongs to the family Parvoviridae, subfamily Parvovirinae, and genus Protoparvovirus(Maclachlan et al., 2011). The clinical symptoms include fever, leukopenia, diarrhea, dehydration, anorexia with 100% morbidity, and mortality of 10% in adult dogs and 91% in pups(Nandi et al., 2019).

CPV-2 is a naked, icosahedral, linearized, and single-stranded DNA virus(Cavalli et al., 2018). The capsid consists of 60 subunits. Each subunit has the same eight-stranded antiparallel β-barrel motif with large insertions between strands of the β-barrel(Parrish, 2010). The features of capsid include spikes in the three-fold axes, a canyon-like depression circulating around each of the five-fold axes, and a dimple-like depression at the two-fold axes(Agbandje and Rossmann, 1995). The full genome is 5323bp, containing two open reading frames (ORFs). One is located in 3', encoding nonstructural proteins called NS1 and NS2. NS1 is necessary for duplicating(Maclachlan et al., 2011) while the function of NS2 is still unclear, which seems not required for efficient replication and assembly(Wang et al., 1998). Another ORF is located in 5', which encodes structural protein including VP1 and VP2 through alternative splicing of the same mRNAs. VP1 protein contains the complete sequence of VP2 protein and a 143 residues unique N-terminal sequence required for successful infection(Vihinen-Ranta et al., 2002). The VP2 protein is a favored location for mutation and a key molecule determining the host range, antigenic properties, and receptor binding. The antigens or

subtypes of CPV-2 can be identified by certain residues positioned within the VP2 protein(Vannamahaxy and Chuammitri, 2017). VP3 was derived from VP2 protein by host proteolytic cleavage presenting only on complete (DNA-containing) virions(Nandi and Kumar, 2010).

CPV-2 was regarded as a host-range variant derived from an FPLV-like virus via wild carnivores that gained the ability to bind the canine transferrin receptors (TfR), thus allowing the infection of dogs while failed to replicate in the feline host (Hueffer et al., 2003; Nandi et al., 2019; Stucker et al., 2012). CPV-2 was first reported in 1978 in the United States whereas the serological test indicated that dogs in Europe or Eurasia were widely infected between 1974 and 1976(Hoelzer and Parrish, 2010). At least six amino acid differences between CPV-2 and FPLV were found. VP2 amino acid residues 93 and 323 determined the canine host range(Chang et al., 1993). Within a few years, its variants arose and replaced the original CPV-2. The first CPV-2 variant, termed CPV-2a, emerged in 1979. The single mutation of VP2 residue 300, from Asp to Gly, is a key determinant of infecting cats(Organtini et al., 2015). In 1984 and 2001, another two variants, CPV-2b and CPV-2c were detected in the United States and Italy respectively. Antigenic differences among these three variants were based on the amino acid presented at residue 426 of VP2 protein (Asn in CPV-2a, Asp in CPV-2b and Glu in CPV-2c)(Miranda and Thompson, 2016).

Additional variants, New CPV-2a and New CPV-2b, were discovered in 1990. They differ from CPV-2a/b only at 297 residue (Ser to Ala) of VP2 protein without changing their antigenic properties even its proximity to epitope B(Ohshima et al., 2008), which is currently detected in most recent CPV-2 strains(Martella et al., 2006). Another two variants, CPV-2c(a) and CPV-2c(b), were isolated from Vietnamese leopard cats in 1997 with substitution at residue 300 (Gly to Asp) and lost canine host range(Ikeda et al., 2000; Ohshima et al., 2008). The few amino acid differences in VP2 protein between FPLV, CPV-2 and, CPV-2 variants appear to have modified important biological properties, such as antigenic properties, host ranges, interactions with TfR, and virulence(Cavalli et al., 2008).

In China, the first CPV-2 case was detected in 1982(Shizhe et al., 1982). CPV-2 prevailed during the early 1980s, then it was gradually replaced by CPV-2a after 1986(Zhijing et al., 2004). In the 1990s, most CPV-2 variants detected were New CPV-2a/2b, which seemed to completely replace CPV2a/2b. The New CPV-2a has been the dominant genotype since the 1990s. After 2000, the detection rate of New CPV-2b increased, which was identified as the dominant strains in some cities(Jianqing, 2003; Kegong et al., 2004; Zhijing et al., 2004). The CPV-2c variant was observed initially in 2009 and has developed a continuous uptrend since 2010(Qi et al., 2020). In this study, we collected samples in the Tianjin area, a city that hasn't been investigated before, along with other cities between 2018 and 2020. To further study the prevalence of CPV-2 in China, we retrieved CPV-2 sequences date with clear background collected in China from Genbank and related papers from 2014 to 2019 then examined those data in detail.

## Materials and Method

### Sample collection

A total of 33 samples were collected from small animal clinics between 2018 and 2020. When the dogs were tested positive using Rapid CPV/CCV Ag Test Kit (Anigen, Korea) or showed some specific clinical symptoms, their fecal swabs will be mixed with normal saline and preserved in centrifuge tubes, which will be packed and mailed to our department and kept in a -80 °C refrigerator.

### Virus isolation

The cell line used is the cat kidney F81 cell line. Added 1 mL PBS to each centrifuge tube and oscillated using an oscillator, then drew 500 μL liquid and filtered through a 0.22μm filter (Millipore, USA). At the same time, the cell suspension was prepared, which was digested by 0.05% pancreatin and maintained in Minimal Essential Medium (MEM, Gibco, USA) supplemented with 10% calf serum. The cell suspension was added to the culture flask and inoculated with CPV-2 with a proportion of 5%. The culture flask was kept in a $CO_2$ incubator (MEMMERT, German). We harvested the virus when the cytopathic effect (CPE) was about 80%. The third passage of viruses was used for analysis.

### Direct Immunofluorescent Assay

F81 cell incubated with CPV-2 in a 96-well plate (Corning, USA) was prepared. When the CPE was about 80%, the MEM was discarded. Cells were gently rinsed with PBS three times, then 80% acetone solution was used to fix cells for 10 min. After washing with PBS three times, CPV-2 fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (VMRD, USA) was placed on the wells, which were then incubated in a humid chamber at 30 °C for 30 min. The stained cells were observed with the fluorescence microscope (Nikon, Japan).

### Detection of CPV-2

A pair of primers were designed to detect CPV-2. The forward primer is CTGTGGGTAATGTTGGTTGTT (5'to 3'), and the reverse primer is TGGTCTTGATGTTGATGGATG (5' to 3'). The product's length is 1163bp. We used DNAiso Reagent (Takara, China) to extract the genome and ExTaq DNA Polymerase (Takara, China) to amplify the gene. The following amplification procedure was applied: pre-denaturation at 94 °C for 5 min, then 35 cycles of denaturation at 94 °C for 30 sec, annealing at 48 °C for 30 sec and extension at 72 °C for 1 min. The final extension at 72 °C for 7 min was performed after the cycles.

### Amplification and sequencing of VP2 gene

Another pair of primers were designed to amplify the whole VP2 gene. The forward primer is CACCAGATCATCCATCAACATC (5' to 3'), and the reverse primer is AACCACCCACACCATAAC (5' to 3'). The product is 2293bp in length encompassing the entire VP2 gene. The super-fidelity DNA Polymerase used was 2 × Phanta Max Master Mix (Vazyme, China). Amplification conditions were performed following the manufacturer's instructions: pre-denaturation at 95 °C for 30 sec, then 35 cycles of denaturation at 95 °C for 15 sec, annealing at 56 °C for 15 sec and extension at 72 °C for 1.5 min, and a final extension at 72 °C for 5 min.

The PCR product was purified by EasyPure Quick Gel Extraction Kit (TransGen, China) according to the manufacturer's instructions. Then it was cloned into pEASY-Blunt Cloning Vector (TransGen, China). The recombinant vector was transformed into Trans1-T1 Phage Resistant Chemically Competent Cell (TransGen, China). Positive clones were screened by blue-white selection and further verified by the PCR test, thus were sent to a third-party company (Huada Gene, Beijing) for sequencing.

### SequenceAnalysis and Phylogenetic Construction

Sequences were assembled using Seqman (DNASTAR, USA), then the nucleotide sequences and deduced amino acid sequences were aligned with the ClustalW method. To construct a nucleotide phylogenetic tree based on the full VP2 gene, MEGA-X was used to find the best DNA models with maximum likelihood (ML) method. The bootstrap values were 1000 to analyze the confidence level. All strains were analyzed with reference strains obtained from Genbank. Three CPV-2 vaccine strains (Genbank accession number: FJ197847, FJ011098, and FJ011097) and two FPLV vaccine strains (Genbank accession number: EU498681 and EU498680) were included.

### Temporal and Geographical Distribution Analysis

The reported Chinese CPV-2 strains between 2014 to 2019 with clear background were collected either from Genbank or related publications, along with CPV-2 strains in the study (the CPV-2 strains collected in 2020 were excluded since no other CPV-2 strains were isolated in 2020 currently). These data were subjected to temporal distribution analysis and geographical distribution analysis.

## Result

### Virus Isolation and PCR Result

### Hosted file

image1.emf available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

A total of 27 strains (positive rate=82%) were successfully isolated from fecal samples and showed positive in the PCR test. The specific CPE of CPV-2 in F81 cells was elongated and thinned cells with gaps between them(Figure 1). F81 cells incubated with CPV-2 emitted green fluorescence under the fluorescence microscope, indicating the reproduction and reproduction of CPV-2 in F81 cells.

Figure 1. CPV-2 isolation and direct immunofluorescent (DIF) assay results. (a) CPE of F81 cells incubated with CPV-2. (b) Normal F81 cell. (c) The DIF assay result of F81 cell incubated with CPV-2. (d) Negative control.

**Amplification of VP2 Gene and GenotypeAnalysis**

Full-length VP2 nucleotide sequences were amplified from 27 samples, which is 2293bp in length. After aligned with reference strains, extra sequences were cut off and simplified into 1755bp, namely the full VP2 gene. Table 1 provided the background information of CPV-2 strains isolated in this study.

**Hosted file**

image2.emf available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Table 1.** Information of CPV-2 strains in this study

The result revealed the co-circulation of New CPV-2a and CPV-2c strains in China. No CPV-2, CPV-2a, CPV-2b, New CPV-2b variants were detected. CPV-2c was the predominant variant detected in 19 samples (70.3%), while New CPV-2a variants were only detected in 6 samples (22%). Noteworthily, two FPLV were identified in dog samples and successfully isolated. It was reported that FPLV strains can replicate only in feline cells(Nakamura et al., 2001). However, this study provided two exceptions.

**Sequence Analysis**

22 nucleotide mutations result in nonsynonymous substitutions were identified (Table 2). All CPV-2 strains presented residue mutations at Phe267Tyr, and Tyr324Ile. All New CPV-2a strains carried a specific mutation Thr440Ala, and all CPV-2c strains exhibited unique mutation Ala5Gly and Gln370Arg while one New CPV-2a strain also harboured the mutation Ala5Gly. Mutation at residue Pro13Ser presented in two CPV-2c strains and one New CPV-2a strain. Another mutation found was Lys570Arg in one CPV-2c strain. Mutation Ile101Thr was observed in two FPLV strains and Pro238Gln in one FPLV strain.

**Table 2**Amino acid variations of CPV-2 strains in this study compared with reference strains

**Hosted file**

image3.emf available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Phylogenetic Analysis**

Based on the evaluation of MEGA-X, an ML phylogenetic tree based on the full VP2 gene was constructed with the Tamura 3-parameter model plus gamma distribution with five rate categories (T92+G+I).

According to the phylogenetic tree, the 27 strains fell into three clades(Figure 2). Clade CPV-2c I included all the CPV-2c variants in this study, and Asian CPV-2c strains, along with one Italian CPV-2c strain. This clade was separated from clade CPV-2c II, which contained CPV-2c strains circulating in European and American countries. CPV-2c strains belong to this clade were characterized by four mutations: Ala5Gly, Phe267Tyr, Tyr324Ile, Gln370Arg. Six New CPV-2a strains in this study were classified into another clade, New CPV-2a/b I, containing Asian New CPV-2a/b strains and two American New CPV-2a strains. All the strains located within this clade shared three mutations, Phe267Tyr, Tyr324Ile, and Thr440Ala, clustering

away from other clades containing New CPV-2a variants with different mutations. Two FPLV strains were located in clade FPLV which included all the FPLV strains.

**Hosted file**

`image4.emf` available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Figure 2**Maximum likelihood phylogenetic tree based on full VP2 gene. Bootstrap values less than 60% will not be shown on the branches. Strains analyzed in this study were marked with ·$Referencestrainswereexhibitedinthe\ following\ order$ : $Genbankaccessionnumber, isolatedcountry, isolatedtimeandgenotype.Themainresiduemutationsineverycladewerenotedwi$

**Temporal and Geographical Distribution Analysis.**

A total of 683 Chinese CPV-2 records were retrieved from Genbank and related publications. The details were presented in Supporting Information. Additionally, 23 strains (collected from 2018 to 2019) in this study were included.

The temporal distribution analysis was given in Figure 3. The detection rate of New CPV-2a has declined since 2016, and New CPV-2b showed the same tendency. In contrast, CPV-2c has been consistently growing since 2014, thereby replacing New CPV-2a as the predominant variant in China after 2018.

**Figure 3** Temporal Distribution of CPV-2 in China from 2014-2019.

Considering the dynamic change of the genotype of Chinese CPV-2 strains in recent years, the geographical distribution analysis was divided into two periods. The former was from 2014 to 2016, and the latter was from 2017 to 2019. Their respective data was given in Figure 4.

**Hosted file**

`image6.emf` available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Figure 4** Geographical Distribution of CPV-2 in China. (a) Geographical Distribution of CPV-2 in China from 2014 to 2016. (b) Geographical Distribution of CPV-2 in China from 2017 to 2019.

According to Figure 4(a), from 2014 to 2016, New CPV-2a mainly circulated in eastern and southwestern areas of China while New CPV-2b prevailed in Hubei. CPV-2c was the dominant subtype of central China. Figure 2(b) gave a clear message that CP-2c has become the dominant variant from 2017 to 2019, while New CPV-2a prevailed only in Beijing.

**Discussion**

According to the reports before 2015, CPV-2c was scarcely detected in Chinese strains(Han et al., 2011; Wang et al., 2016a; Zhang et al., 2010a; Zhang et al., 2010b; Zhao et al., 2016). After 2015, although its number increased, most reports indicated that New CPV-2a has the highest proportion in China(Geng et al., 2015; Wang et al., 2016b; Wu et al., 2018; Zhao et al., 2015; Zhuang et al., 2019). This study provided a different result compared to previous reports. By collecting available Chinese CPV-2 strains from Genbank and related publications, we further proved our finding, that CPV-2c has a dramatic increase in China and finally replaced the New CPV-2a variant as the predominant strain in most cities of China. However, since many cities were not investigated, the result was not conclusive. But anyway, this tendency should be caught concern since CPV-2c variants have been frequently associated with CPV-2 outbreak in vaccinated dogs(Cavalli et al., 2008; Decaro and Buonavoglia, 2012; Decaro et al., 2008a). So far, there is no consensus about the effectiveness of prototype CPV-2-based vaccines against heterologous CPV-2 variants(Decaro et al., 2020). However, the continuous and dynamic evolution of CPV-2c may pose a new challenge to the vaccines used currently.

In the past, CPV-2c has been found mainly in South American and European countries, and it was rarely detected in Asia, where more CPV-2a/2b variants prevailed(Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016). However, in recent years, the amount of CPV-2c increased in Asia(Moon et al., 2020). The result in this study was consistent with recent reports of Asian countries, such as Taiwan(Chiang et al., 2016; Lin et al., 2017), Laos(Vannamahaxay et al., 2017), Vietnam(Hoang et al., 2019; Nguyen Manh et al.), Thailand(Charoenkul et al., 2019; Inthong et al., 2020), and Korea (Moon et al., 2020). These reports either revealed the higher detection rate of CPV-2c or indicated that CPV-2c has become the predominant variant in recent years. It's noteworthy that these reports have pointed out that the strains isolated shared similar features between Asian strains, which seemed to demonstrate the mutual transmission of CPV-2 between neighboring Asian countries. We could see the synchronous changes of CPV-2c in some Asian countries since 2015, thus it's possible that CPV-2c variants have been continuing its evolution in Asia and gaining a stronger epidemiological advantage over other mutants.

In this study, some newly reported amino acid mutations in VP2 protein were found. Mutation Ala5Gly existed in all the CPV-2c strains, including one New CPV-2a strain. Mutation Ala5Gly first reported in Chinese strains in 2015, then detected in Asian countries like Taiwan(2017)(Lin et al., 2017), Thailand(2017)(Mira et al., 2018), and Vietnam(2018)(Hoang et al., 2019). In these studies, Ala5Gly was only observed in CPV-2c strains, while it also existed in one New CPV-2a strains. Formerly, this mutation was only observed in Asian strains. However, recently, it has also been found in CPV-2a/b variants in Australia(Kwan et al., 2020) and Italy(Mira et al., 2019), suggesting the possible introduction from Asian countries. Or they were not of unitary origin and evolved independently due to the increasingly selective pressure in this site since this mutation was not presented in the same variant. Currently, the potential biological function of this mutation is unclear.

Another specific amino acid mutation of CPV-2c was Gln370Arg, except for two CPV-2c strains. In 2012, this mutation was first observed in a New CPV-2a strain isolated from a giant panda(Guo et al., 2013). Then in 2015, it was reported again in Chinese CPV-2c strains from dogs(Geng et al., 2015; Zhao et al., 2015). After that, most Chinese CPV-2c strains harboured the same mutation(Wang et al., 2016a; Wang et al., 2016b; Wu et al., 2018; Zhuang et al., 2019). Interestingly, it was first observed in a New CPV-2a strain, but only presented in CPV-2c strains when it prevailed in China. It's possible that this mutation occurred during the further adaptation to giant pandas and has spread back to dogs(Wang et al., 2016b). Currently, this mutation is not only prevailing in China, but also occurring in neighboring countries like Taiwan, Thailand, Korea, Laos, and African countries like Nigeria(Ukwueze et al., 2020) and Zambia(Kapiya et al., 2019). Probably, this mutation was exported from China. It was reported that amino acid site 370 is adjacent to residue 375, which affects the pH dependence of hemagglutination(Chang et al., 1993). Also, residue 370 is located in a flexible surface loop consisting of residues 359 to 375 of the capsid protein, and residues within this loop were considered essential for virus infectivity(Simpson et al., 2000). Then, the mutation in residue 370 may also affect the loop's conformation then influence the host range.

In this study, mutation Thr440Ala was specific to New CPV-2a strains. According to previous reports, it also occurred in New CPV-2b strains(Geng et al., 2015; Zhang et al., 2010a; Zhuang et al., 2019). It was first detected in 1993 and became prevalent after 2005(Zhou et al., 2017). In China, Korea(Jeoung et al., 2008), Thailand(Inthong et al., 2020), India(Mittal et al., 2014), Colombia(Giraldo-Ramirez et al., 2020), Italy(Decaro et al., 2009), Brazil(de Oliveira et al., 2019), it presented in New CPV-2a/2b variants. However, in the United States(Kapil et al., 2008), Argentina(Calderon et al., 2011), and Mexico(Faz et al., 2019), it was unique to CPV-2c strains. Amino acid residue 440 is located in the GH loop, a region with the greatest variability due to its exposure on the capsid surface(Decaro et al., 2009). Also, it was found in the threefold axis, a site containing two major epitopes – epitope A and epitope B(Strassheim et al., 1994). Thus, mutations in this site could have antigenic significance and affect the host immune response. This mutation was estimated to have multiple evolutionary origins(Voorhees et al., 2019), which may explain why it presented in different CPV-2 variants in different areas.

Another substitution found was Pro13Ser, which presented both in New CPV-2a(n=1) and CPV-2c(n=2)

strains. This mutation was identified in Uruguayan, Vietnamese, and Japanese dogs, European cats(Maya et al., 2013), and Chinese raccoon dogs (Lu et al., 2020). In Italy, it was observed in CPV-2b strains but was Ala rather than Ser(Decaro et al., 2009). It was estimated that this mutation may not be antigenically significant since residue 13 was not exposed to the surface(Lu et al., 2020).

Mutation Phe267Tyr and Tyr324Ile were identified in all CPV-2 strains, and it's thought to be Asian strains characteristic(Mira et al., 2018). Phe267Tyr first appeared in 2002 and has become predominant since 2014, Tyr324Ile first appeared in 2006 and has consistently occurred, then becoming predominant in 2014(Zhou et al., 2017). As mentioned above, residue 267 and 324 are located in the GH loop, and they are subjected to positive selection(Giraldo-Ramirez et al., 2020; Zhao et al., 2015). Then they were thought to play an important role in the evolution of new variants (Nandi et al., 2019). Also, residue 324 is adjacent to 323, a key residue determining the host range. Then, it may also affect the host range. Recently, it was also reported in Australian(Kwan et al., 2020), Uruguayan(Maya et al., 2013), Colombian(Giraldo-Ramirez et al., 2020), and Italian(Mira et al., 2019) strains. The phylogenetic analysis compellingly indicated the intercontinental dissemination, possibly originated from Asian countries.

The last mutation was observed in residue 570, from Lys to Arg. However, in two Australian strains and one vaccine strain, it was Lys to Glu(Kwan et al., 2020). And it seemed to be the first case in China. Residue 570 is lying on the capsid surface and it closely approaches to the residue 300, a residue that has the greatest variability determining the cross-species transfer of viruses between different carnivores(Allison et al., 2016). Thus, its alteration may also affect the host TfR binding ability.

Based on the phylogenetic tree, all the CPV-2c strains in this study, along with other newly sequenced Chinese CPV-2c strains, were embraced in the same clade, indicating the intimate relationship between Chinese CPV-2c strains, which suggested the mutual transmission between different cities of China. In recent years, pets can be sold by online trading. Thus, the transportation of pets from cities to cities has increased, which could greatly contribute to the transmission of CPV-2. Other Asian strains were included in the same clade, revealing the possible introduction from neighboring Asian countries. Noteworthily, one Italian strain was also included. As the report concluded, this strain was of Asian origin(Mira et al., 2019). In recent years, several essays demonstrated the detection of CPV-2 strains originated from Asia in these Oceanian, African, American, and European countries(Giraldo-Ramirez et al., 2020; Kapiya et al., 2019; Kwan et al., 2020; Maya et al., 2013; Mira et al., 2019; Zaher et al., 2020). Therefore, stricter border controls may be needed as a preventive measure. All the strains located in this clade carried four important substitutions: Ala5Gly, Phe267Tyr, Tyr324Ile, and Gln370Arg. While clade CPV-2c II contained CPV-2c strains retaining initial amino acids in these sites and circulated in American and European countries, suggesting the geographical isolation of CPV-2c variants with Asian countries.

Compared to CPV-2c variants, the molecular evolution of New CPV-2a strains was more complicated due to its genetic diversity. New CPV-2a variants were marked by residue 297Ala. However, in Colombia, Brazil, Uruguay and Argentina(Giraldo-Ramirez et al., 2020), residue 297 can be substituted with Asn. Thus, such categorization can be confusing. All the New CPV-2a strains isolated in this study were located in the same clade, characterized by mutations Phe267Tyr, Tyr324Ile and Thr440Ala. In contrast, clade New CPV-2a II contained New CPV-2a strains with only one mutation Thr440Ala, and clade New CPV-2a III included New CPV-2a variants with original residues in the sites mentioned above. The Phe267Tyr and Tyr324Ile changes together resulted in a distant phylogenetic relationship between clade New CPV-2a I and clade New CPV-2a II/III, which highlighted the importance of Phe267Tyr and Tyr324Ile. All the FPLV strains were located in the FPLV clade. Compared with CPV-2, FPLV strains were highly conserved and exhibited a closer relationship with Asian strains.

Remarkably, two FPLV strains were detected in dog samples and both harboured a mutation Ile101Thr, which is CPV-2 mutants specific. Most FPLV detected recently owned the same mutation compared to original FPLV strains(Decaro et al., 2008b; Li et al., 2018). It is strange since FPLV was unable to replicate in the small intestine or mesenteric lymph nodes of dogs without being shed in the feces(Truyen and Parrish, 1992). Similar findings have been reported in Pakistani and Thai strains. This phenomenon may

suggest the dynamic evolution of carnivore parvovirus, or the existence of the recombinant virus of CPV and FPLV(Ahmed et al., 2018; Inthong et al., 2020). Meanwhile, it may be caused due to the mistake made by a veterinary assistant, mislabeling cat samples with dog samples. However, similar reports in recent years may disprove this postulation. Also, as Nisar Ahmed suggested, it was possibly due to the coprophagous behavior of dogs(Ahmed et al., 2018). Anyway, further investigations are needed to explain this phenomenon.

In conclusion, 6 New CPV-2a, 19 CPV-2c, and 2 FPLV strains were isolated from 33 canine samples, and CPV-2c emerged as the dominant genotype, which was further proved by the analysis of 683 reported Chinese strains collected from 2014 to 2019. Currently, no ascertainable facts prove that CPV-2c could result in immunization failure. However, the obvious epidemic superiority of CPV-2c strains with mutation Ala5Gly and Gln370Arg mutation in Asian areas may pose a new challenge to the vaccines used at present. Further research should be undertaken to investigate the relationship between immunization failure with current CPV-2c strains. Phylogenetic tree revealed the national and international spread of CPV-2 variants, thus boundary administration should be stricter. One unanticipated finding was the identification of two FPLV strains in canine samples, which is an important issue for future research.

**Acknowledgements**

We would like to thank the veterinary clinics involved in the sampling, and we appreciated the staff who taught us how to do the experiments.

**Conflict of Interest Statement**

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

**Ethics Statement**

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the samples used in this study were a part of standard clinical examination.

**Supporting Information**

The data of 683 Chinese CPV-2 strains were given in the Excel file.

**Data Availability Statement**

The data used to support the findings of this study are available from the corresponding author upon request.

**Reference**

Agbandje, M., Rossmann, M., 1995. The Structure of Parvoviruses. Seminars in Virology 6, 299-309. https://doi.org/10.1006/smvy.1995.0036

Ahmed, N., Riaz, A., Zubair, Z., Saqib, M., Ijaz, S., Nawaz-Ul-Rehman, M.S., Al-Qahtani, A., Mubin, M., 2018. Molecular analysis of partial VP-2 gene amplified from rectal swab samples of diarrheic dogs in Pakistan confirms the circulation of canine parvovirus genetic variant CPV-2a and detects sequences of feline panleukopenia virus (FPV). Virology Journal 15, 45. https://doi.org/10.1186/s12985-018-0958-y

Allison, A.B., Organtini, L.J., Zhang, S., Hafenstein, S.L., Holmes, E.C., Parrish, C.R., 2016. Single Mutations in the VP2 300 Loop Region of the Three-Fold Spike of the Carnivore Parvovirus Capsid Can Determine Host Range. Journal of Virology 90, 753-767. https://doi.org/10.1128/jvi.02636-15

Calderon, M.G., Romanutti, C., Antuono, A.D., Keller, L., Mattion, N., La Torre, J., 2011. Evolution of Canine Parvovirus in Argentina between years 2003 and 2010: CPV2c has become the predominant variant affecting the domestic dog population. Virus Research 157, 106-110. https://doi.org/10.1016/j.virusres.2011.02.015

Cavalli, A., Marinaro, M., Desario, C., Corrente, M., Camero, M., Buonavoglia, C., 2018. In vitro virucidal activity of sodium hypochlorite against canine parvovirus type 2. Epidemiology and Infection 146, 2010-2013.

Cavalli, A., Martella, V., Desario, C., Camero, M., Bellacicco, A.L., De Palo, P., Decaro, N., Elia, G., Buonavoglia, C., 2008. Evaluation of the antigenic relationships among canine parvovirus type 2 variants. Clin Vaccine Immunol 15, 534-539. https://doi.org/10.1017/s0950268818002431

Chang, S.-F., Sgro, J.Y., Parrish, C., 1993. Multiple amino acids in the capsid structure of Canine parvovirus coordinately determine the canine host range and specific antigenic and hemagglutination properties. Journal of virology 66, 6858-6867. https://doi.org/10.1128/jvi.66.12.6858-6867.1992

Charoenkul, K., Tangwangvivat, R., Janetanakit, T., Boonyapisitsopa, S., Bunpapong, N., Chaiyawong, S., Amonsin, A., 2019. Emergence of canine parvovirus type 2c in domestic dogs and cats from Thailand. Transboundary and Emerging Diseases 66, 1518-1528. https://doi.org/10.1111/tbed.13177

Chiang, S.-Y., Wu, H.-Y., Chiou, M.-T., Chang, M.-C., Lin, C.-N., 2016. Identification of a novel canine parvovirus type 2c in Taiwan. Virology Journal 13, 160. https://doi.org/10.1186/s12985-016-0620-5

de Oliveira, P.S.B., Cargnelutti, J.F., Masuda, E.K., Weiblen, R., Flores, E.F., 2019. New variants of canine parvovirus in dogs in southern Brazil. Arch Virol 164, 1361-1369. https://doi.org/10.1007/s00705-019-04198-w

Decaro, N., Buonavoglia, C., 2012. Canine parvovirus–a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol 155, 1-12. https://doi.org/10.1016/j.vetmic.2011.09.007

Decaro, N., Buonavoglia, C., Barrs, V.R., 2020. Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? Veterinary Microbiology 247, 108760. https://doi.org/10.1016/j.vetmic.2020.108760

Decaro, N., Desario, C., Elia, G., Martella, V., Mari, V., Lavazza, A., Nardi, M., Buonavoglia, C., 2008a. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. The new microbiologica 31, 125-130. DOI:10.1080/14636770802485467

Decaro, N., Desario, C., Miccolupo, A., Campolo, M., Parisi, A., Martella, V., Amorisco, F., Lucente, M.S., Lavazza, A., Buonavoglia, C., 2008b. Genetic analysis of feline panleukopenia viruses from cats with gastroenteritis. J Gen Virol 89, 2290-2298. https://doi.org/10.1099/vir.0.2008/001503-0

Decaro, N., Desario, C., Parisi, A., Martella, V., Lorusso, A., Angela, M., Mari, V., Colaianni, M., Cavalli, A., Trani, L., Buonavoglia, C., 2009. Genetic analysis of canine parvovirus type 2c. Virology 385, 5-10. https://doi.org/10.1016/j.virol.2008.12.016

Faz, M., Martinez, J.S., Gomez, L.B., Quijano-Hernandez, I., Fajardo, R., Del Angel-Caraza, J., 2019. Origin and genetic diversity of canine parvovirus 2c circulating in Mexico. Arch Virol 164, 371-379. https://doi.org/10.1007/s00705-018-4072-7

Geng, Y., Guo, D., Li, C., Wang, E., Wei, S., Wang, Z., Yao, S., Zhao, X., Su, M., Wang, X., Wang, J., Wu, R., Feng, L., Sun, D., 2015. Co-Circulation of the Rare CPV-2c with Unique Gln370Arg Substitution, New CPV-2b with Unique Thr440Ala Substitution, and New CPV-2a with High Prevalence and Variation in Heilongjiang Province, Northeast China. PLOS ONE 10, e0137288. https://doi.org/10.1371/journal.pone.0137288

Giraldo-Ramirez, S., Rendon-Marin, S., Ruiz-Saenz, J., 2020. Phylogenetic, Evolutionary and Structural Analysis of Canine Parvovirus (CPV-2) Antigenic Variants Circulating in Colombia. Viruses 12, 500. https://doi.org/10.3390/v12050500

Guo, L., Yang, S.-l., Chen, S.-j., Zhang, Z., Wang, C., Hou, R., Ren, Y., wen, X., Cao, S., Guo, W., Hao, Z., Quan, Z., Zhang, M., Yan, Q.-g., 2013. Identification of canine parvovirus with the Q370R

point mutation in the VP2 gene from a giant panda (Ailuropoda melanoleuca). Virology Journal 10, 163. https://doi.org/10.1186/1743-422x-10-163

Han, S., qi, B., Zhang, X., 2011. A Retrospective Analysis on Phylogeny and Evolution of CPV Isolates in China. Asian Journal of Animal and Veterinary Advances 6, 1204-1213. https://doi.org/10.3923/ajava.2011.1204.1213

Hoang, M., Lin, W.-H., Le, V.P., Nga, B.T.T., Chiou, M.-T., Lin, C.-N., 2019. Molecular epidemiology of canine parvovirus type 2 in Vietnam from November 2016 to February 2018. Virology Journal 16, 52. https://doi.org/10.1186/s12985-019-1159-z

Hoelzer, K., Parrish, C.R., 2010. The emergence of parvoviruses of carnivores. Vet. Res. 41, 39. https://doi.org/10.1051/vetres/2010011

Hueffer, K., Parker, J.S., Weichert, W.S., Geisel, R.E., Sgro, J.Y., Parrish, C.R., 2003. The natural host range shift and subsequent evolution of canine parvovirus resulted from virus-specific binding to the canine transferrin receptor. J Virol 77, 1718-1726. https://doi.org/10.1128/jvi.77.3.1718-1726.2003

Ikeda, Y., Mochizuki, M., Naito, R., Nakamura, K., Miyazawa, T., Mikami, T., Takahashi, E., 2000. Predominance of canine parvovirus (CPV) in unvaccinated cat populations and emergence of new antigenic types of CPVs in cats. Virology 278, 13-19. https://doi.org/10.1006/viro.2000.0653

Inthong, N., Kaewmongkol, S., Meekhanon, N., Sirinarumitr, K., Sirinarumitr, T., 2020. Dynamic evolution of canine parvovirus in Thailand. Vet World 13, 245-255. https://doi.org/10.14202/vetworld.2020.245-255

Jeoung, S.Y., Ahn, S.J., Kim, D., 2008. Genetic analysis of VP2 gene of canine parvovirus isolates in Korea. J Vet Med Sci 70, 719-722. https://doi.org/10.1292/jvms.70.719

Jianqing, S., 2003. Studies on the Genetic Variation of Canine Parvovirus Strains Isolated in Beijing Area of China. China Agricultural University. https://doi.org/10.1542/peds.2007-2022ss

Kapil, S., Cooper, E., Lamm, C., Murray, B., Rezabek, G., Johnston, L., Campbell, G., Johnson, B., 2008. Canine Parvovirus Types 2c and 2b Circulating in North American Dogs in 2006 and 2007. Journal of clinical microbiology 45, 4044-4047. https://doi.org/10.1128/jcm.01300-07

Kapiya, J., Nalubamba, K.S., Kaimoyo, E., Changula, K., Chidumayo, N., Saasa, N., Simuunza, M.C., Takada, A., Mweene, A.S., Chitanga, S., Simulundu, E., 2019. First genetic detection and characterization of canine parvovirus from diarrheic dogs in Zambia. Arch Virol 164, 303-307. https://doi.org/10.1007/s00705-018-4068-3

Kegong, T., Tiezhu, Z., Jianqing, S., Chunling, L., Ming, S., 2004. Study on the Type and Identification of Canine Parvovirus Isolated from Different Years in Beijing by PCR, 2004 Academic Annual Meeting of Chinese Society of Animal Husbandry and Veterinary Medicine, Beijing, p. 4.

Kwan, E., Carrai, M., Lanave, G., Hill, J., Parry, K., Kelman, M., Meers, J., Decaro, N., Beatty, J., Martella, V., Barrs, V., 2020. Analysis of canine parvoviruses circulating in Australia reveals predominance of variant 2b and identifies feline parvovirus-like mutations in the capsid proteins. Transboundary and Emerging Diseases. https://doi.org/10.22541/au.158920818.85252579

Li, X., Wu, H., Wang, L., Spibey, N., Liu, C., Ding, H., Liu, W., Yuxiu, L., Tian, K., 2018. Genetic characterization of parvoviruses in domestic cats in Henan province, China. Transboundary and Emerging Diseases 65. https://doi.org/10.1111/tbed.13014

Lin, Y.-C., Chiang, S.-Y., Wu, H.-Y., Lin, J.-H., Chiou, M.-T., Liu, H.-F., Lin, C.-N., 2017. Phylodynamic and Genetic Diversity of Canine Parvovirus Type 2c in Taiwan. Int J Mol Sci 18, 2703. https://doi.org/10.3390/ijms18122703

Lu, R., Yu, Y., Zhu, X., Shi, Q., Wang, Y., Wang, J., Lv, S., Shi, N., Liu, H., Deng, X., Lian, S., Yan, M., Zhao, H., Hu, B., Liu, W., Yan, X., 2020. Molecular characteristics of the capsid protein VP2

gene of canine parvovirus type 2 amplified from raccoon dogs in Hebei province, China. Arch Virol 165. https://doi.org/10.1007/s00705-020-04714-3

Maclachlan, N.J., Dubovi, E.J., Fenner, F., 2011. Fenner's veterinary virology, 4th ed. Elsevier/AP, Amsterdam ; Boston.

Martella, V., Decaro, N., Buonavoglia, C., 2006. Evolution of CPV-2 and Implicance for Antigenic/Genetic Characterization. Virus Genes 33, 11-13. https://doi.org/10.1007/s11262-005-0034-8

Maya, L., Calleros, L., Francia, L., Hernandez, M., Iraola, G., Panzera, Y., Sosa, K., Perez, R., 2013. Phylodynamics analysis of canine parvovirus in Uruguay: evidence of two successive invasions by different variants. Arch Virol 158, 1133-1141. https://doi.org/10.1007/s00705-012-1591-5

Mira, F., Purpari, G., Di Bella, S., Colaianni, M.L., Schiro, G., Chiaramonte, G., Gucciardi, F., Pisano, P., Lastra, A., Decaro, N., Guercio, A., 2019. Spreading of canine parvovirus type 2c mutants of Asian origin in southern Italy. Transboundary and Emerging Diseases 66, 2297-2304. https://doi.org/10.1111/tbed.13283

Mira, F., Purpari, G., Lorusso, E., Di Bella, S., Gucciardi, F., Desario, C., Macaluso, G., Decaro, N., Guercio, A., 2018. Introduction of Asian canine parvovirus in Europe through dog importation. Transboundary and Emerging Diseases 65, 16-21. https://doi.org/10.1111/tbed.12747

Miranda, C., Thompson, G., 2016. Canine parvovirus: the worldwide occurrence of antigenic variants. Journal of General Virology 97, 2043-2057. https://doi.org/10.1099/jgv.0.000540

Mittal, M., Chakravarti, S., Mohapatra, J.K., Chug, P.K., Dubey, R., Upmanuyu, V., Narwal, P.S., Kumar, A., Churamani, C.P., Kanwar, N.S., 2014. Molecular typing of canine parvovirus strains circulating from 2008 to 2012 in an organized kennel in India reveals the possibility of vaccination failure. Infection, Genetics and Evolution 23, 1-6. https://doi.org/10.1016/j.meegid.2014.01.015

Moon, B.-Y., Jang, J., Kim, S.-H., Kim, Y.-H., Lee, H.-k., So, B., Park, C.-K., Lee, K.-K., 2020. Genetic characterization of canine parvovirus type 2c from domestic dogs in Korea. Transboundary and Emerging Diseases 67, 1645-1653. https://doi.org/10.1111/tbed.13501

Nakamura, K., Sakamoto, M., Ikeda, Y., Sato, E., Kawakami, K., Miyazawa, T., Tohya, Y., Takahashi, E., Mikami, T., Mochizuki, M., 2001. Pathogenic Potential of Canine Parvovirus Types 2a and 2c in Domestic Cats. Clinical and Diagnostic Laboratory Immunology 8, 663. https://doi.org/10.1128/cdli.8.3.663-668.2001

Nandi, S., Kumar, M., 2010. Canine Parvovirus: Current Perspective. Indian Journal of Virology 21, 31-44. https://doi.org/10.1007/s13337-010-0007-y

Nandi, S., Sharma, G., Gupta, V., Deol, P., Chander, V., 2019. Global Scenario of Canine Parvovirus Mutants: Epidemiology, Diagnostics and Immunoprophylactic Agents. JSM Vet Med Res 2, 12.

Nguyen Manh, T., Piewbang, C., Rungsipipat, A., Techangamsuwan, S., Molecular and phylogenetic analysis of Vietnamese canine parvovirus 2C originated from dogs reveals a new Asia-IV clade. Transboundary and Emerging Diseases n/a. https://doi.org/10.1111/tbed.13811

Ohshima, T., Hisaka, M., Kawakami, K., Kishi, M., Tohya, Y., Mochizuki, M., 2008. Chronological analysis of canine parvovirus type 2 isolates in Japan. J Vet Med Sci 70, 769-775. https://doi.org/10.1292/jvms.70.769

Organtini, L.J., Allison, A.B., Lukk, T., Parrish, C.R., Hafenstein, S., 2015. Global displacement of canine parvovirus by a host-adapted variant: structural comparison between pandemic viruses with distinct host ranges. J Virol 89, 1909-1912. https://doi.org/10.1128/jvi.02611-14

Parrish, C.R., 2010. Structures and functions of parvovirus capsids and the process of cell infection. Curr Top Microbiol Immunol 343, 149-176. https://doi.org/10.1007/82_2010_33

Qi, S., Zhao, J., Guo, D., Sun, D., 2020. A Mini-Review on the Epidemiology of Canine Parvovirus in China. Front Vet Sci 7, 5. https://doi.org/10.3389/fvets.2020.00005

Shizhe, L., Xiren, W., Wenguang, F.,1982. Study on Infectious Enteritis in Dogs: Parvovirus Like Particles Detected in Gog Feces with Diarrhea. Shanghai Journal of Animal Husbandry and Veterinary Medicine, 172-175.

Simpson, A.A., Chandrasekar, V., Hebert, B.t., Sullivan, G.M., Rossmann, M.G., Parrish, C.R., 2000. Host range and variability of calcium binding by surface loops in the capsids of canine and feline parvoviruses 11Edited by I. A. Wilson. Journal of Molecular Biology 300, 597-610. https://doi.org/10.1006/jmbi.2000.3868

Strassheim, M.L., Gruenberg, A., Veijalainen, P., Sgro, J.Y., Parrish, C.R., 1994. Two dominant neutralizing antigenic determinants of canine parvovirus are found on the threefold spike of the virus capsid. Virology 198, 175-184. https://doi.org/10.1006/viro.1994.1020

Stucker, K.M., Pagan, I., Cifuente, J.O., Kaelber, J.T., Lillie, T.D., Hafenstein, S., Holmes, E.C., Parrish, C.R., 2012. The role of evolutionary intermediates in the host adaptation of canine parvovirus. J Virol 86, 1514-1521. https://doi.org/10.1128/jvi.06222-11

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, 5399. https://doi.org/10.1128/jvi.66.9.5399-5408.1992

Ukwueze, C.S., Nwosuh, C.I., Obishakin, E.F., Anene, B.M., Ezeokonkwo, R.C., Owoludun, O.A., Chima, N.C., Luka, P.D., 2020. Genetic analysis and emergence of canine parvovirus type 2c in South Eastern Nigeria. Iran J Vet Res 21, 141-145.

Vannamahaxay, S., Vongkhamchanh, S., Intanon, M., Tangtrongsup, S., Tiwananthagorn, S., Pringproa, K., Chuammitri, P., 2017. Molecular characterization of canine parvovirus in Vientiane, Laos. Arch Virol 162, 1355-1361. https://doi.org/10.1007/s00705-016-3212-1

Vannamahaxy, S., Chuammitri, P., 2017. Update on Canine Parvovirus: Molecular and Genomic Aspects, with Emphasis on Genetic Variants Affecting the Canine Host. Kafkas University. https://doi.org/10.9775/kvfd.2017.17673

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, 1884. https://doi.org/10.1128/jvi.76.4.1884-1891.2002

Voorhees, I.E.H., Lee, H., Allison, A.B., Lopez-Astacio, R., Goodman, L.B., Oyesola, O.O., Omobowale, O., Fagbohun, O., Dubovi, E.J., Hafenstein, S.L., Holmes, E.C., Parrish, C.R., 2019. Limited Intrahost Diversity and Background Evolution Accompany 40 Years of Canine Parvovirus Host Adaptation and Spread. J Virol 94. https://doi.org/10.1101/714683

Wang, D., Yuan, W., Davis, I., Parrish, C.R., 1998. Nonstructural Protein-2 and the Replication of Canine Parvovirus. Virology 240, 273-281. https://doi.org/10.1006/viro.1997.8946

Wang, H., Jin, H., Li, Q., Zhao, G., Cheng, N., Feng, N., Zheng, X., Wang, J., Zhao, Y., Li, L., Cao, Z., Yan, F., Wang, L., Wang, T., Gao, Y., Yang, S., Xia, X., 2016a. Isolation and sequence analysis of the complete NS1 and VP2 genes of canine parvovirus from domestic dogs in 2013 and 2014 in China. Arch Virol 161, 385-393. https://doi.org/10.1007/s00705-015-2620-y

Wang, J., Lin, P., Zhao, H., Cheng, Y., Jiang, Z., Zhu, H., Wu, H., Cheng, S., 2016b. 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

Wu, H., Li, X., Wang, L., Liu, Y., Tian, K., 2018. Molecular epidemiological survey of canine parvovirus in domestic dogs in four provinces, China. VirusDisease 29, 113-117. https://doi.org/10.1007/s13337-018-0427-7

Zaher, K.S., El-Dabae, W.H., El-Sebelgy, M.M., Aly, N.I., Salama, Z.T., 2020. Genotyping and phylogenetic analysis of canine parvovirus circulating in Egypt. Vet World 13, 326-333. https://doi.org/10.14202/vetworld.2020.326-333

Zhang, Q., Xu, X., Zhai, G., Wang, Z., Hou, S., Zhu, H., 2010a. Molecular characterisation of canine parvovirus strains circulating in China. African Journal of Biotechnology.

Zhang, R., Yang, S., Zhang, W., Zhang, T., Xie, Z., Feng, H., Wang, S., Xia, X., 2010b. Phylogenetic analysis of the VP2 gene of canine parvoviruses circulating in China. Virus Genes 40, 397-402. https://doi.org/10.1007/s11262-010-0466-7

Zhao, H., Wang, J., Jiang, Y., Cheng, Y., Lin, P., Zhu, H., Han, G., Yi, L., Zhang, S., Guo, L., Cheng, S., 2015. Typing of Canine Parvovirus Strains Circulating in North-East China. Transboundary and emerging diseases 64. https://doi.org/10.1111/tbed.12390

Zhao, Z., Liu, H., Ding, K., Peng, C., Xue, Q., Yu, Z., Xue, Y., 2016. Occurrence of canine parvovirus in dogs from Henan province of China in 2009–2014. BMC Veterinary Research 12, 138. https://doi.org/10.1186/s12917-016-0753-1

Zhijing, X., Xianzhu, X., Rongliang, H., Zhongpeng, Z., Yuwei, G., Huanggeng, 2004. Investigation of CanineParvovirus Genotypes. Chinese Journal of Veterinary Science, 421-424.

Zhou, P., Zeng, W., Zhang, X., Li, S., 2017. The genetic evolution of canine parvovirus – A new perspective. PLoS ONE 12. https://doi.org/10.1371/journal.pone.0175035

Zhuang, Q.-y., Qiu, Y., Pan, Z.-h., Wang, S.-c., Wang, B., Wu, W.-k., Yu, J.-m., Yi, Y., Sun, F.-L., Wang, K.-C., 2019. Genome sequence characterization of canine parvoviruses prevalent in the Sichuan province of China. Transboundary and Emerging Diseases 66, 897-907. https://doi.org/10.1111/tbed.13100

**Hosted file**

`Figure 1.emf` available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Hosted file**

`Table 1.xlsx` available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Hosted file**

`Table 2.xlsx` available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Hosted file**

`Figure 2.emf` available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Hosted file**

`Figure 3.emf` available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019

**Hosted file**

Figure 4.emf available at https://authorea.com/users/469211/articles/562231-molecular-epidemiological-survey-of-canine-parvovirus-circulating-in-china-from-2014-to-2019