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Canine parvovirus type 2c is the dominant variant circulating in Jilin Province, Northeast China

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Abstract

To understand the current situation of canine parvovirus (CPV) epidemic strains in Jilin Province and to analyze the molecular biological characteristics and genetic evolution of the epidemic strains, 44 fecal or intestinal tissue samples detected by canine parvovirus test strips in Changchun and Liaoyuan City, Jilin Province, from February 2018 to November 2019 were collected for cloning, sequencing and genetic evolution analysis of the gene encoding the virus structural protein VP2. The results showed that 44 of the 44 samples were CPV-2 positive by PCR, among which CPV-2c was the dominant variant (70.4%). In addition, new-CPV-2a (18.2%), new-CPV-2b (9.1%) and CPV-2 (2.3%) also existed. This is the highest frequency of CPV-2c subtypes observed in canine populations in Jilin Province so far. In addition to the substitutions of Ala5Gly, Phe267Tyr, Tyr324Ile, and Gln370Arg in the VP2 protein, a novel variant with an Arg481Lys mutation was observed in a CPV-2c strain. Hence, there is a subsequent need for further and extensive epidemiological investigation and eventual adaptation of current vaccines.

Canine parvovirus type 2c is the dominant variant circulating in Jilin Province, Northeast China

Short title: Canine parvovirus type 2c circulating in China

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SUMMARY

To understand the current situation of canine parvovirus (CPV) epidemic strains in Jilin Province and to analyze the molecular biological characteristics and genetic evolution of the epidemic strains, 44 fecal or intestinal tissue samples detected by canine parvovirus test strips in Changchun and Liaoyuan City, Jilin Province, from February 2018 to November 2019 were collected for cloning, sequencing and genetic evolution analysis of the gene encoding the virus structural protein VP2. The results showed that 44 of the 44 samples

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were CPV-2 positive by PCR, among which CPV-2c was the dominant variant (70.4%). In addition, new-CPV-2a (18.2%), new-CPV-2b (9.1%) and CPV-2 (2.3%) also existed. This is the highest frequency of CPV-2c subtypes observed in canine populations in Jilin Province so far. In addition to the substitutions of Ala5Gly, Phe267Tyr, Tyr324Ile, and Gln370Arg in the VP2 protein, a novel variant with an Arg481Lys mutation was observed in a CPV-2c strain. Hence, there is a subsequent need for further and extensive epidemiological investigation and eventual adaptation of current vaccines.

Keywords: CPV, CPV-2c, Genetic variation

1 | INTRODUCTION

Canine parvovirus causes severe and fatal epidemic diseases of hemorrhagic gastroenteritis and subacute myocarditis in dogs, cats, and several wild carnivore species around the world (Miranda & Thompson, 2016). As a member of the Genus Protoparvovirus Parvovirinae subfamily in the Parvoviridae family, parvovirus is a small (diameter of 25 nm), nonenveloped virus (Cotmore et al., 2019). It has a linear, single-stranded, negative-sense DNA genome, which consists of approximately 5200 nucleotides (nt), including two large gene cassettes. One of them encodes two structural (VP1 and VP2) proteins and the other encodes two nonstructural (NS1 and NS2) proteins by alternative splicing of the same mRNAs (Reed, Jones, & Miller, 1988). VP1 contains the full-length VP2 sequence. However, the most abundant structural protein, VP2, accounts for 90% of the viral capsid, representing the major determinant of host range and virus-host interactions, and it is cleaved to VP3 by host proteases (Decaro & Buonavoglia, 2012).

It has been estimated that this virus has a significantly higher nucleotide substitution rate with values of approximately 10^{-4} substitutions/site/year, considering the genes of VP2, NS1 and the full-length genome (Hoelzer, Shackelton, Parrish, & Holmes, 2008). The frequent variation and evolution of CPV changes its host range and forms virus strains with different geographical characteristics. Amino acid substitutions in the VP2 gene have been responsible for changes in its genetic and antigenic properties.

CPV was first reported in the late 1970s as the original type 2 (CPV-2) and rapidly spread worldwide (Appel, Scott, & Carmichael, 1979). Within a few years of its emergence in dogs, five amino acid mutation in VP2 (Met87Leu, Ile101Thr, Ala300Gly, Asp305Tyr, and Val555Ile) occurred, resulting in a distinct antigenic type CPV-2a, and it completely replaced CPV-2 as the main epidemic strain in the world in 1980 (Shackelton, Parrish, Truyen, & Holmes, 2005; Stucker et al., 2012). Another early variant CPV-2b appeared in 1984, which has the Asn426Asp substitution in VP2 compared with CPV-2a (Parrish et al., 1991; Pratelli et al., 2001). In 1990, the Ser297Ala mutation appeared in VP2 of CPV-2a and CPV-2b, which were designated as new CPV-2a and new CPV-2b accordingly (Ohshima et al., 2008). In 2000, another antigenic variant having an amino acid substitution (Asp426Glu), named CPV-2c, was first reported in Italy (Buonavoglia et al., 2001), and then distributed to many parts of the world rapidly, even becoming the predominant variant in some European and American countries (Calderón et al., 2011; H. Wang et al., 2016). Currently, new CPV-2a and new CPV-2b appear to have replaced the prototype CPV-2a and CPV-2b in many countries (Zhuang et al., 2019).

In China, recent epidemiological surveys showed that the new CPV-2a and new CPV-2b strains have been in cocirculation. Although the CPV-2c strain has also been increasingly found since it was first discovered in 2010, the new CPV-2a and new CPV-2b strains are still the prominent CPV genotypes in many parts of China (H. Wang et al., 2016; Wu, Li, Wang, Liu, & Tian, 2018; Zhao et al., 2017). To further understand the prevalence and genetic evolution of CPV-2 in Jilin province, 44 positive samples were collected from animal hospitals in Changchun and Liaoyuan. The VP2 genes were amplified and analyzed to determine whether the epidemic CPV genotype has changed to provide a scientific basis for epidemic surveillance, control and vaccine research of CPV-2.

2 | MATERIALS AND METHODS

2.1 | Sample collection

A total of 44 fecal swabs or intestinal tissues from domestic dogs suspected of having CPV-2 infection

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were collected from animal hospitals in Changchun and Liaoyuan in Jilin Province from February 2018 to November 2019. These samples were initially certified to be infected with CPV-2 using colloidal gold test strips.

2.2 | Extraction of CPV genome

Fecal swabs were rinsed with 1ml sterile phosphate-buffered saline (PBS), and centrifuged at 12,000 × g and 4°C for 10 min, and the supernatants were stored at -80. The small intestinal tissue homogenates were clarified by centrifuging at 12,000xg and 4degC for 10 min to separate the supernatant. Then, 200 μL of supernatant from each sample was filtered through a 0.22 μm filter (Millipore, USA). The viral DNA was extracted from the supernatant of the treated samples by an AxyPrep^{TM}viral DNA miniprep kit (Axygen Scientific, Tewksbury, MA, USA) according to the manufacturer's instructions.

2.3 | Amplification and sequencing of the VP2 gene

The complete VP2 nucleotide sequence was amplified using the following primers: VP2-F complete: 5'-GGACAAGTAAAAAGAGAC-3' and VP2-R complete: 5'-TACAAGTACAATATTTCTATGCTG-3'. The length of the amplified fragment was 1925 bp, which covered the entire 1755 bp open reading frame (ORF) of VP2. A CPV DNA preserved in the laboratory and sterile water were used as positive and negative controls, respectively.

The amplified products were purified by a Gel Extraction Kit (Cwbio, China) and cloned into the pEASY blunt vector (TransGen Biotech Co., Ltd, Beijing, China). Then, the positive plasmids were sequenced at least 3 times by Sangon Biotech (Shanghai) Co., Ltd. The 44 sequences of the VP2 gene were submitted to GenBank with accession numbers MN810876-MN810919.

2.4. Phylogenetic analysis

The nucleotide sequences of VP2 and the deduced amino acid sequences were aligned with 18 reference CPV sequences from GenBank using the MegAlign program of DNASTAR (DNASTAR, USA). The phylogenetic analyses were conducted by the neighbor-joining method using Mega 6 software. The reliability of the phylogenetic tree obtained for the VP2 region was evaluated by running 1,000 replicates in the bootstrap test.

3 | Results

3.1 | PCR detection and genotype analysis

The PCR results showed that all 44 samples from domestic dogs were positive for CPV-2. A 1775-bp fragment covering the full-length of VP2 was amplified and sequenced from all samples. All of the VP2 genes were typed according to the key amino acid residues at positions 87, 101, 103, 297, 300, 305, 426 and 555. Detailed information from the 44 samples is provided in Table 1. Among the 44 CPV-positive samples, 31 CPVs were classified as CPV-2c (70.4%), 8 as new CPV-2a, 4 as new CPV-2b (9.1%), and 1 as CPV-2.

3.2 | Phylogenetic analysis

A phylogenetic tree was constructed using the neighbor-joining method with MEGA 6 based on the full-length VP2 nucleotide sequences obtained in this study, along with sequences retrieved from GenBank. As shown in Figure 1, all of the CPV-2c isolates were roughly divided into two clusters. The first cluster includes European and American isolates of CPV-2c. The second cluster consists of all 31 CPV-2c strains isolated in this study and other CPV-2c strains isolated from China and other countries previously. This shows that the Chinese CPV-2c isolates are obviously distinct from the prototypical CPV-2c strains from European countries such as Germany, Greece, Italy, and so on. Meanwhile, the 31 CPV-2c isolates in Jilin Province in this study were far from the earliest CPV-2c strains (GU380303, GU380305) isolated in Jilin Province in 2009. These newly identified CPV-2c strains are closely related to local CPV-2c isolates in cluster II, such as Anhui (MK518005), Henan (MF467229), Sichuan (MH476587), Shandong (MK268682), and so on. Sequence comparisons also revealed 98.7%–100% nucleotide similarity in VP2 between these CPV-2c subtype strains

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and local CPV-2c isolates. However, the similarities of the nucleotides among the CPV-2c strains identified in this study and reported abroad or in the original Jilin isolates (GU380303, GU380305) were 98.8%–99.4% and 98.8%–99.3%, respectively. These results indicate that the CPV-2c isolates from China were likely to be derived from the local adaption of the original CPV-2c isolates rather than introduction from other countries. Of even more concern, all strains from recent epidemics clustered in a separate group that is far from the vaccine strains.

3.3 | Analysis of amino acid mutations

In this study, the amino acid mutations of the VP2 protein were identified, and the amino acid sequences of 44 isolates were compared with reference strains (Table 2). The results showed that 43 isolates had Phe267Tyr and Tyr324Ile mutations, and all new CPV-2a and new CPV-2b strains identified in this study had the Thr440Ala substitution. The substitutions of Ala5Gly and Gln370Arg were found in all CPV-2c subtype isolates, which have been reported to be specific to the CPV-2c virus (Geng et al., 2015). Of the 31 CPV-2c strains, only one showed a unique substitution on Arg481Lys. Residue 481 is located in the greatest variable GH loop (loops 3 and 4), comprising aa 267–498 of the VP2 protein (Agbandje, Parrish*, & Rossmann, 1995).

4 | Discussion

In this study, 44 rectal swabs or intestinal tissue samples were collected from several animal hospitals in Changchun City and Liaoyuan City, Jilin Province. A total of 44 dogs were confirmed to be infected with CPV-2 by PCR. Then, the VP2 genes of the 44 strains of CPV-2 identified in this study were sequenced and compared with the GenBank database. The subtypes were classified with reference to different amino acids at positions 297 and 426. The results showed that 1 strain of CPV-2, 8 strains of new CPV-2a, 4 strains of new CPV-2b and 31 strains of CPV-2c were found in this study. These results showed that the CPV-2c subtype was the main epidemic strain of CPV-2 in the Jilin area, accompanied by the coexistence of the new CPV-2a and new CPV-2b subtypes.

CPV-2 was discovered in the United States in the late 1970s. In China, CPV-2 was first reported to spread among dogs in 1983, and several variants (including CPV-2a, CPV-2b, new CPV-2a, new CPV-2b and CPV-2c) have cocirculating in recent years. In 2010, CPV-2c was detected for the first time in Jilin Province, China (Chiang, Wu, Chiou, Chang, & Lin, 2016). In 2014, the CPV-2c strain emerged in Shandong province (Zhao et al., 2017). From 2014 to 2015, 14 CPV-2c-positive samples (out of 95 samples) in Heilongjiang province and 18 CPV-2c-positive samples (out of 43 samples) in Beijing were found, and several strains were isolated (Geng et al., 2015; J. Wang et al., 2016) . It was reported in 2018 that CPV-2c had spread to Henan, Guangxi and Jiangsu provinces (Wu et al., 2018). Eleven CPV-2c samples (out of 24 samples) were reported in Sichuan in 2019 (Zhuang et al., 2019). The isolation rate of CPV-2c has increased year by year. It can be seen that in the past 10 years, CPV-2c has gradually replaced the old genotypes and become the new major epidemic genotype.

VP2 is the main antigenic determinant of canine parvovirus and is related to the host immune response. A small number of mutations in the VP2 gene may lead to a change in pathogenicity (Lin et al., 2014). To trace the evolution of antigenic variants, the mutations at 5, 267, 324 and 370 of VP2 were analyzed. Compared with the classical European original CPV-2c isolate G7/97 in 1997, all of the strains of CPV-2c identified in this study had aa substitutions of Ala5Gly, Phe267Tyr, Tyr324Ile, and Gln370Arg, while the earliest Chinese Jilin isolate 08/09 (GU380305) in 2009 did not have any amino acid mutations (Table 2). In contrast, the original Jilin isolate 06/09 (GU380303) showed a mutation of Tyr324Ile. Another early Jilin isolate 06/09 (GU380303) harbored the F267Y mutation. The Phe267Tyr mutation was also reported in 2013 in the Chinese CPV-2c isolates G15 (KF482471) and G1 (KF482468). The mutation of residue 267 may not affect the antigenicity of CPV because residue 267 is not exposed on the capsid surface (Decaro et al., 2006). However, the Tyr324Ile mutation, which affects the binding to the canine transferrin receptor, could lead to a change of the host range of canine parvovirus (Hueffer et al., 2003). In 2014, the amino acid substitution Gln370Arg appeared in Chinese isolates WANGQING-1, YANJI-1 and HRB-A6, and it

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might be involved in a required conformational change, or mediate an effect on receptor binding through the neighboring residues (Guo et al., 2013). In 2014 and 2015, a novel mutation at residue 5, substituting glycine for the highly conserved alanine present in all CPV-2c strains, was isolated in Beijing (J. Wang et al., 2016). Residue 5 is one of the surface and core residues of the antigen site. Therefore, the Ala5Gly mutation may change its antigenicity and immunogenicity (Li, Tang, Chen, Niu, & Liu, 2019). So far, these four amino acid mutations have simultaneously appeared in CPV-2c strains recently isolated from China and they are also present in the 31 CPV-2c strains identified in this study.

In the present study, we detected a mutation of Arg481Lys that has not been reported previously and its potential functional consequence remains to be determined. Residue 481 is located in the GH loop comprising aa 267-498 of VP2 protein (Agbandje & Rossmann, 1995). The residues inserted in the GH loop have the greatest variability, and the residues inserted in the GH loop have been proven to bind to neutralizing antibodies in CPV. Therefore, the substitution of amino acid 481 may result in changes in its antigenicity and immunogenicity. Further study is needed for confirmation.

According to the phylogenetic tree of VP2, the earliest Chinese CPV-2c strains were clustered in the same branch with the European original CPV-2c isolates, while the Chinese CPV-2c isolated in recent years forms a monophyletic cluster that is obviously different from the foreign strains in their evolutionary relationship. This consistent with the results of previous phylogenetic analysis (Zhao et al., 2017; Zhuang et al., 2019). The Chinese CPV-2c strains may be derived from foreign strains and subsequently evolved locally during distribution. It may be that in the development of the pet industry, the pet dog transportation trade has also broken the original regional barriers of CPV and led to the spread and prevalence of foreign CPV-2c strains in China. It has also been reported that CPV migration is likely to spread between geographically close countries through the movement of infected animals or mechanical vectors (Hoelzer et al., 2008).

An additional finding is that all of the CPV-2 strains collected here in this study were separated from the vaccine strains except the strain CPV-CC-33, which may be a vaccine-like strain. Furthermore, CPV-2c is becoming the most predominant strain in China. However, current commercial vaccines available in China are based on CPV-2. Therefore, special concerns regarding the efficacy of the current vaccines have been raised. Although previous studies have claimed that vaccination with CPV-2 type vaccine can cross-protect against challenge with virulent CPV-2a, CPV-2b, and CPV-2c (Siedek, Schmidt, Sture, & Raue, 2011; Spibey, Greenwood, Sutton, Chalmers, & Tarpey, 2008), some evidence suggests that dogs with a complete vaccination program still suffer from infection with CPV-2c or CPV-2c variants (Decaro et al., 2008; Faz et al., 2019). CPV-2c viruses have been isolated not only from unvaccinated but also from vaccinated dogs (J. Wang et al., 2016). Considering the inability of vaccine strains to provide adequate protection against field viruses, further and extensive epidemiological investigations are required in the future, and the CPV vaccines needed to be updated by replacing the original type 2 with the CPV variants currently circulating.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical Statement

The author confirms that the ethical polices of the journal, as noted on the journal‘s author guidelines page, have been adhered to. No ethical/welfare authority approval was required.

Data availability statement

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All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figure Legends

Phylogenetic analysis based on the VP2 protein sequences of CPV-2 isolates. A phylogenetic tree was constructed based on the complete VP2 protein sequences using the neighbor-joining method with 1000 bootstrap replicates with MEGA 6.0 software package. Solid triangle $(^{)}, circle(*), diamond(*), and square()indicates the CPV-2c, new CPV-2a, new CPV-2b and new CPV-2 strains identified in this study, respectively. Others were retrieved from GenBank. The strains are shown as "strain name", "ac

Table 1 Summary of CPV genotypes in this study

No.	Strain	Place of collection	GenBank no.	Genetic type
1	CPV-CC-3	Changchun,China	MN810918	CPV-2c
2	CPV-CC-5	Changchun,China	MN810917	CPV-2c
3	CPV-CC-6	Changchun,China	MN810916	CPV-2c
4	CPV-CC-7	Changchun,China	MN810915	CPV-2c
5	CPV-CC-8	Changchun,China	MN810914	CPV-2c
6	CPV-CC-10	Changchun,China	MN810913	CPV-2c
7	CPV-CC-11	Changchun,China	MN810912	CPV-2c
8	CPV-CC-12	Changchun,China	MN810911	CPV-2c
9	CPV-CC-21	Changchun,China	MN810910	CPV-2c
10	CPV-CC-22	Changchun,China	MN810909	CPV-2c
11	CPV-CC-24	Changchun,China	MN810908	CPV-2c
12	CPV-CC-25	Changchun,China	MN810907	CPV-2c
13	CPV-CC-29	Changchun,China	MN810904	CPV-2c
14	CPV-CC-30	Changchun,China	MN810903	CPV-2c
15	CPV-CC-31	Changchun,China	MN810902	CPV-2c
16	CPV-CC-34	Changchun,China	MN810899	CPV-2c
17	CPV-CC-35	Changchun,China	MN810898	CPV-2c
18	CPV-CC-36	Changchun,China	MN810897	CPV-2c
19	CPV-CC-37	Changchun,China	MN810896	CPV-2c
20	CPV-CC-39	Changchun,China	MN810894	CPV-2c
21	CPV-CC-41	Changchun,China	MN810893	CPV-2c
22	CPV-CC-42	Changchun,China	MN810892	CPV-2c
23	CPV-CC-43	Changchun,China	MN810891	CPV-2c
24	CPV-CC-44	Changchun,China	MN810890	CPV-2c
25	CPV-CC-46	Changchun,China	MN810889	CPV-2c
26	CPV-CC-820	Changchun,China	MN810885	CPV-2c
27	CPV-LY-1	Liaoyuan,China	MN810883	CPV-2c
28	CPV-LY-3	Liaoyuan,China	MN810881	CPV-2c
29	CPV-LY-5	Liaoyuan,China	MN810879	CPV-2c
30	CPV-LY-6	Liaoyuan,China	MN810878	CPV-2c
31	CPV-LY-8	Liaoyuan,China	MN810876	CPV-2c
32	CPV-CC-2	Changchun,China	MN810919	New CPV-2a
33	CPV-CC-26	Changchun,China	MN810906	New CPV-2a
34	CPV-CC-32	Changchun,China	MN810901	New CPV-2a
35	CPV-CC-729	Changchun,China	MN810886	New CPV-2a
36	CPV-CC-1103	Changchun,China	MN810884	New CPV-2a
37	CPV-LY-2	Liaoyuan,China	MN810882	New CPV-2a
38	CPV-LY-4	Liaoyuan,China	MN810880	New CPV-2a
39	CPV-LY-7	Liaoyuan,China	MN810877	New CPV-2a
40	CPV-CC-27	Changchun,China	MN810905	New CPV-2b

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41	CPV-CC-38	Changchun,China	MN810895	New CPV-2b
42	CPV-CC-517	Changchun,China	MN810888	New CPV-2b
43	CPV-CC-721	Changchun,China	MN810887	New CPV-2b
44	CPV-CC-33	Changchun,China	MN810900	CPV-2

Table 2 Amino acid substitutions in VP2 of CPV detected in this study

Strain name	Genetic type	Amino acid sites in the VP2 gene 5	Amino acid sites in the VP2 87
Reference strains			
CPV-b(M38245/1996/USA)	CPV-2	A	M
CPV-15(M24003/1984/USA)	CPV-2a	-	L
CPV-39(M74849/1984/USA)	CPV-2b	-	L
CPV-435(AY742953/2005/USA)	New CPV-2a	-	L
CPV-436(AY742955/2005/USA)	New CPV-2b	-	L
G7/97(FJ005196/1997/Germany)	CPV-2c	-	L
08/09(GU380305/2009/China)	CPV-2c	-	L
G1(KF482468/2009/China)	CPV-2c	-	L
06/09(GU380303/2009/China)	CPV-2c	-	L
WANGQING-1(KP749851/2014/China)	CPV-2c	-	L
BJ14-8(KT162005/2014/China)	CPV-2c	G	L
This study			
CPV-CC-33	CPV-2	-	-
CPV-CC-2	New CPV-2a	-	L
CPV-CC-26	New CPV-2a	-	L
CPV-CC-32	New CPV-2a	-	L
CPV-CC-729	New CPV-2a	-	L
CPV-CC-1103	New CPV-2a	-	L
CPV-LY-2	New CPV-2a	-	L
CPV-LY-4	New CPV-2a	-	L
CPV-LY-7	New CPV-2a	-	L
CPV-CC-27	New CPV-2b	-	L
CPV-CC-38	New CPV-2b	-	L
CPV-CC-517	New CPV-2b	-	L
CPV-CC-721	New CPV-2b	-	L
CPV-CC-3	CPV-2c	G	L
CPV-CC-5	CPV-2c	G	L
CPV-CC-6	CPV-2c	G	L
CPV-CC-7	CPV-2c	G	L
CPV-CC-8	CPV-2c	G	L
CPV-CC-10	CPV-2c	G	L
CPV-CC-11	CPV-2c	G	L
CPV-CC-12	CPV-2c	G	L
CPV-CC-21	CPV-2c	G	L
CPV-CC-22	CPV-2c	G	L
CPV-CC-24	CPV-2c	G	L
CPV-CC-25	CPV-2c	G	L
CPV-CC-29	CPV-2c	G	L
CPV-CC-30	CPV-2c	G	L
CPV-CC-31	CPV-2c	G	L
CPV-CC-34	CPV-2c	G	L

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CPV-CC-35	CPV-2c	G	L
CPV-CC-36	CPV-2c	G	L
CPV-CC-37	CPV-2c	G	L
CPV-CC-39	CPV-2c	G	L
CPV-CC-41	CPV-2c	G	L
CPV-CC-42	CPV-2c	G	L
CPV-CC-43	CPV-2c	G	L
CPV-CC-44	CPV-2c	G	L
CPV-CC-46	CPV-2c	G	L
CPV-CC-820	CPV-2c	G	L
CPV-LY-1	CPV-2c	G	L
CPV-LY-3	CPV-2c	G	L
CPV-LY-5	CPV-2c	G	L
CPV-LY-6	CPV-2c	G	L
CPV-LY-8	CPV-2c	G	L

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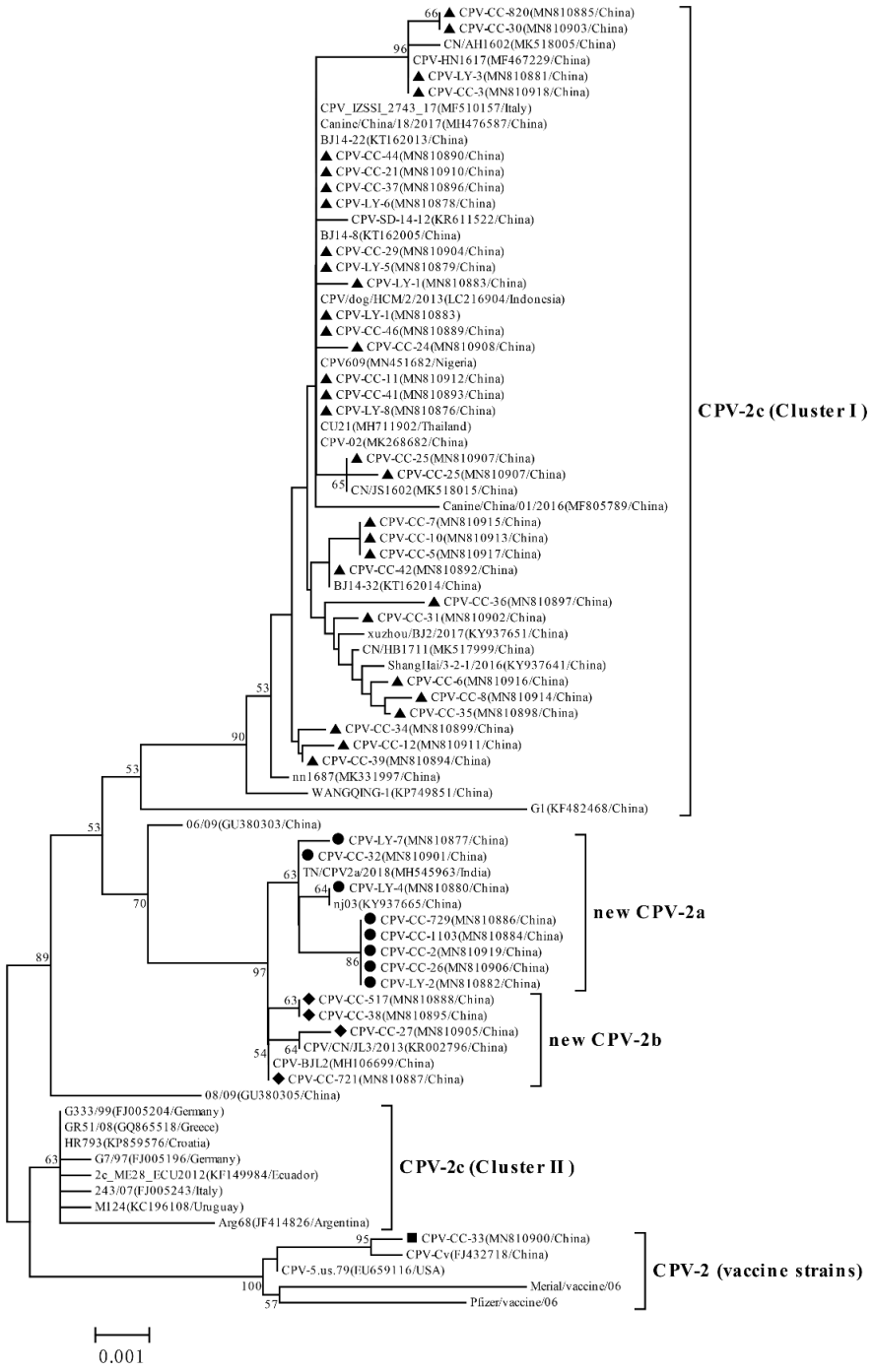

Figure 1.

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