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## **A Retrospective Analysis on Phylogeny and Evolution of CPV Isolates in China**

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### **ABSTRACT**

Canine parvovirus (CPV) is a widespread pathogen among canine in China. It has made great veterinary public health burden and economic loss. Three CPV-2 variations, CPV-2a, CPV-2b and CPV-2c, have emerged, however, the CPV-2 variations distribution and the dominant epidemic strain in China are still undetermined. To better understand the epidemiology of CPV in China, the CPV-VP2 gene sequences which had been submitted to GenBank from 1983 to 2011 in China were analyzed and were compared with the representative references from other countries. Phylogenetic trees based on the VP2 sequences were constructed and they showed that the Chinese CPV-2a and CPV-2b strains were close to the Korean and Vietnam strains respectively. It was found that CPV-2a had been the prevalent strain in China and most CPV isolates existed in northeast China. The VP2 protein in CPV-2a and CPV-2b isolated from China had three mutations compared to the American standard strain: 297Ser-Ala, 324Tyr-Ile and 555Ile-Val.

**Key words:** Canine parvovirus (CPV), epidemiology, phylogenetic tree, China

### **INTRODUCTION**

As analyzed in many articles, canine parvovirus (CPV) is a severe virus that causes canine acute hemorrhagic enteritis and myocarditis (Zhou *et al.*, 2009). CPV was first isolated in CPV-2 form in the US in 1978 (Appel *et al.*, 1979). With a high evolutionary rate, three CPV-2 variations have emerged since its first isolation (Shackelton *et al.*, 2005). CPV-2a was first confirmed around 1980 in the US (Parrish *et al.*, 1988). CPV-2b replaced CPV-2a in many regions of the US after 1986 and this was analyzed by a panel of monoclonal antibodies (Parrish *et al.*, 1991). In 2001, CPV-2c was first discovered in Italy and then in some other countries including the US and Brazil (Hong *et al.*, 2007; Perez *et al.*, 2007).

CPV-2a and CPV-2b have been continuously reported from north to south America as major subtypes (Castro *et al.*, 2011), while all three subtypes have been detected in almost all European countries, however, the distributions were different among different countries though geographically closed to each other (Decaro *et al.*, 2007). For instance, in Italy, CPV-2c was the main epidemic strain, while in Germany and Portugal, CPV-2b was the main strain. CPV-2a and CPV-2b coexist in the UK (Decaro *et al.*, 2007). Similar conditions existed in some Asian countries. CPV-2a was the dominant type in Korea and coexisted with CPV-2b (Yoon *et al.*, 2009; Jeoung *et al.*, 2008). In Japan, CPV-2b had been the dominant type since 1997 (Ohshima *et al.*, 2008; Doki *et al.*, 2006). In Indian, it was reported that CPV-2b was the main subtype, yet CPV-2a

at a smaller proportion (Nandi *et al.*, 2009). Recently, CPV-2c had been gradually reported in many Asian countries, firstly in Vietnam (Nakamura *et al.*, 2003) and then in Indian (Nandi *et al.*, 2010).

In China, CPV infections were first observed in 1982 and then had drawn major concerns of veterinarians (Hu *et al.*, 2011). However, the CPV-2 variations distribution and the dominant epidemic strain in China are still undetermined. Zhang *et al.* (2010a) reported that CPV-2b was the major subtype in China while Zhang *et al.* (2010b) found that CPV-2a was the predominant type in China. The discrepancy may be due to the different investigation regions and different periods. Early studies focused on Jiangsu and Zhejiang strains from 2006 to 2009 (Zhang *et al.*, 2010a), while later samples were collected from Sichuan, Yunnan and Jilin provinces from 1983 to 2008 mostly (Zhang *et al.*, 2010b).

A survey on the CPV covering most of the provinces is necessary to evaluate the dominant epidemic strains in China. The present study provided a retrospective observation on CPV distribution in China by analyzing the sequences data that had been submitted to the GenBank. The objective of this study was to investigate the predominant strain and evolution of CPV circulating in China.

## **MATERIALS AND METHODS**

All CPV strains (133) collected from 1983 to 2011 in China were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/index.html>), one strain of CPV-2a was randomly selected as representative sample in each province each year, while maximal two strains of CPV-2b in each province and each year were selected because of limited CPV-2b data. Samples were sorted according to the collecting origins and dates. Numbers of the different genotypes of CPV-2a, CPV-2b and CPV-2c were statistically analyzed.

Amino acids variations of the VP2 protein of the Chinese strains were gained by comparing the reference strain (M24003) isolated from the US in 1984 by DNAMAN software (DNAMAN 6.0.3.99) (Prajapat *et al.*, 2011). Phylogenetic trees of CPV-2a and CPV-2b based on the VP2 nucleotides of the Chinese strains were then constructed. The sequences were aligned using ClustalX 2.0 software (<http://www.clustal.org/clustal2/>) to generate the proper format and MEGA 5.0 software (<http://www.megasoftware.net/>) was used to analyze the CPV evolution in China. A neighbor-joining method (bootstrap replicates=1000) was chosen to draw the phylogenetic trees (Raj *et al.*, 2010).

## **RESULTS AND DISCUSSION**

The collected data showed that CPV-2a had a higher frequency (60/100) than CPV-2b (39/100) in China (Table 1), stating that CPV-2a had been the predominant strain, which was in accord with the previous report (Zhang *et al.*, 2010b). Most of the CPV distributed in the northeast of China (Fig. 1). The nucleotide homology of the Chinese CPV-2a and CPV-2b with the Korean and Vietnam strains was 99.49 and 99.83%, respectively. Three amino mutations were encountered in the VP2 protein compared with the US reference, displaying 297Ser-Ala, 324Tyr-Ile and 555Ile-Val.

The higher morbidity of CPV-2a might be induced by the following reasons. First, it is related to the vaccines. In China, most licensed vaccines used to protect CPV infection are produced with CPV-2 attenuated vaccine, which protection effect is not sufficient against heterologous types CPV-2a and CPV-2b (Pratelli *et al.*, 2000; Cavalli *et al.*, 2008). It was reported that the protection effect in dogs vaccinated with the CPV-2b strain was more efficient than the CPV-2 vaccine (Ohshima *et al.*, 2008). Second, CPV-2a has a higher virulence than CPV-2b which had been confirmed by the clinical observations (Moon *et al.*, 2008). With the decrease of the antibody titers,

Table 1: CPV isolates from China during 1983~2011 retrieved from GenBank

Year	Genetic type	Numbers	Origin	Gen Bank No.			
1983	CPV-2	1	Jilin(1)*	GU569943			
1986	CPV-2a	1	Jilin(1)	GU569948			
1996	CPV-2a	2	Taiwan(2)	U72697/ U72698			
1998	CPV-2a	1	Taiwan(1)	U72695			
1999	CPV-2a	1	Beijing(1)	GU569947			
2001	CPV-2a	1	Jilin(1)	GU569945			
2002	CPV-2a	4	Yunnan(1)	GU569939			
			Shandong(1)	GU569941			
			Jilin(2)	GU569946/ GU569942			
			Guizhou(2)	GU569937/ GU569944			
	CPV-2b	4	Yunnan(2)	GU569938/ GU569940			
2004	CPV-2a	1	Beijing(1)	EF011664			
2005	CPV-2a	1	Sichuan(1)	DQ903936			
	CPV-2b	3	Sichuan(1)	EF028071			
2006	CPV-2a	7	Shandong(1)	GQ857599			
			Jiangsu(1)	DQ120515			
			Shandong(2)	GQ857602/ GQ857600			
			Hubei(2)	GQ169544/ EU377537			
2007	CPV-2a	22	Jiangsu(3)	GQ169550/ GQ169551/ EU310373			
			Xinjiang(1)	EU170352			
			Taiwan(1)	EF592511			
2008	CPV-2a	20	Shandong(4)	GQ857604/GQ857606/GQ857607/ GQ857614			
			Beijing(16)	GQ169545-GQ169549,EF666059- EF666069			
			Shandong(3)	GQ857608/ GQ857605/ GQ857603			
			Beijing(1)	EU145954			
			Zhejiang(2)	EU213078/ EU213075			
			Taiwan(1)	EF592511			
2009	CPV-2a	16	Shandong(3)	GQ857611/ GQ857612/ GQ857613			
			Sichuan(2)	FJ435345/ FJ435347			
			Hubei(8)	GQ169537- GQ169544, FJ432717			
			Jilin(3)	EU441280/ FJ432718/ FJ432716			
			Henan(2)	EU441279/ EU441280			
			Taiwan(2)	FJ011097/ FJ011098			
			2010	CPV-2b	17	Shandong(2)	GQ857609/ GQ857610
						Zhejiang(4)	EU483515/ EU483517/ EU483512/ EU483510
						Taiwan(11)	FJ265775- FJ265784/ U72696
2009	CPV-2a	16	Jiangsu(10)* Jilin(6)	HM236035- HM236044 GU452713/ GU452715/GU380304/ GU380302/ GU380301/ GU380298			
	CPV-2b	21	Jiangsu(18)* Jilin(3)	HM209458-HM209465, HM236023- HM2334 GU380299/ GU380300/ GU569937			
2010	CPV-2a	2	Guangdong(1)	JF795456			
			Sichuan(1)	HQ651237			
2011	CPV-2a	1	Xian(1)	JN403045			

\*These samples contain only part of the VP2 gene, therefore which were not compared with the reference strain (M24003) in amino acid substitution in Table 2

the animals are at higher risk of infection (Munir *et al.*, 2008; Avizeh *et al.*, 2007). Diseases of the digest tract are serious in the canine (Ali Shabestari *et al.*, 2008). Unrestricted movements of the canine may play a role in the transmission of CPV (Verma *et al.*, 2008; Nandi and Kumar, 2011).



Fig. 1: Distributions of CPV-2a and CPV-2b in China during 1983~2011. Note: A, Beijing; B, Shandong; C, Jiangsu; D, Jilin; E, Sichuan; F, Yunnan, G, Guizhou; H, Xinjiang; I, Guangdong; J, Taiwan; K, Shanxi

Distribution of CPV in China showed geographical correlation. CPV-2a was first reported in Jilin, 1986, a northeast province in China and showed a 99.49% nucleotides homology. CPV-2a was then found along the east coast, from Beijing, Shandong to Jiangsu (Fig. 1). CPV-2b was first collected in Yunnan, 2002 and had a 99.83% nucleotides homology with the Vietnam strains. In Korea, CPV-2a was first reported in 1982 (Yoon *et al.*, 2009). In Vietnam, CPV-2b was first found in 1997. Provinces of Jilin and Yunnan were adjacent to Korea and Vietnam (Fig. 1). Geographical transmission of the CPV was suspected to happen among China, Korean and Vietnam.

CPV-2a and CPV-2b infections had been found in many provinces of China, however, the distribution was severely unbalanced. Most CPVs distributed in the northeast of China, except Xinjiang province (Fig.1), therefore, more attentions should be paid to protect the CPV infection in the eastern areas. Climate in the northeast of China is dry and cold, which may play an important role in the high rate of the CPV infection (Abubakar *et al.*, 2008; Ghazy *et al.*, 2007).

Compared with the US reference strain (M24003), almost all Chinese CPV-2a (except one collected in 1986) and all CPV-2b isolated from 1986 to 2011, showed high frequent amino substitution at the site 297 with a mutation Ser-Ala (Table 2) which was quite different from the rest part of the world, but showed high identity to Brazil strains (Pereira *et al.*, 2007). The wide spread of this mutation indicated that the 297 Ser-Ala mutation of CPV was under strong positive selection to make it more adaptable to the canine population in China, which had been reported previously (Zhang *et al.*, 2010a). The same also happened at position 324Tyr-Ile which was adjacent to residue 323 (had an effect on the host rang) (Hoelzer *et al.*, 2008).

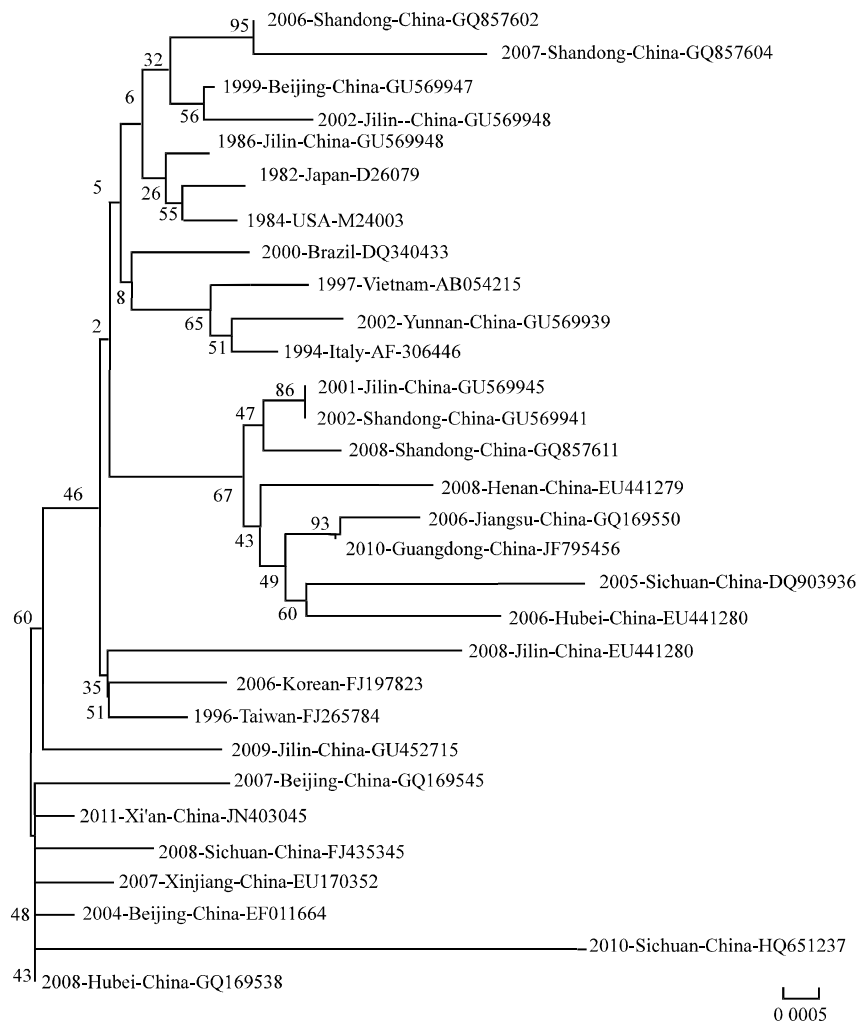


Fig. 2: Phylogenetic tree of CPV-2a in China during 1986-2010

On the contrary, a substitute Asp-Asn at position 375 found both in CPV-2a and CPV-2b was first reported in 1986, but disappeared after 2004. Not only CPV-2a isolated from China since 1986, but also the reference strains from other Asian countries (except Vietnam) revealed a mutation at position 555ile-Val compared with the US strain, which was a reversion to Feline Panleukopenia Virus (FPV) and CPV-2. This might be related to the vaccine pressure produced by CPV-2. In comparison with the US reference, 267phe-Tyr was encountered in the Chinese CPV-2a and CPV-2b isolates (Table 2).

In order to further investigate the phylogenies of CPV-2a and CPV-2b in China, phylogenetic trees based on the VP2 nucleotide sequences from 1 to 1,755 derived from the GenBank had been constructed. CPV-2a and CPV-2b isolated from China showed 99.63% and 99.66% nucleotide identities in comparison with the other countries including the US, Japan, Korea, Italy and Brazil, respectively. The phylogenetic tree showed that most CPV-2a isolated before 2001 fell into two clusters (Fig. 2). The CPV-2b isolated from Zhejiang and Jilin provinces formed a cluster and the

Table 2: Amino acid substitution in the VP2 gene of CPV-2a derived from the GenBank during 1986-2011

GenBank No.	Origin	Year isolated	Amino acid at position												
			97	139	256	267	286	297	300	305	324	375	422	426	555
M24003	USA	1984	A	V	R	F	G	S	G	Y	Y	D	L	N	I
D26079	Japan	1982	-	-	-	-	-	-	-	-	-	-	-	-	V
AF306446	Italy	1994	-	-	-	-	-	-	-	-	-	-	-	-	V
AB054215	Vietnam	1997	-	-	-	-	-	-	-	-	-	-	-	-	-
DQ340433	Brazil	2000	-	I	-	-	-	A	-	-	-	-	-	-	V
FJ197823	Korean	2006	-	-	-	-	-	-	-	-	-	-	-	-	V
M74849	USA	1984	-	-	-	-	-	-	-	-	-	-	-	D	V
DQ340409	Brazil	1985	-	-	-	-	-	-	-	-	-	-	-	D	V
AB115504	Japan	1995	-	-	-	-	-	A	-	-	-	-	-	D	V
AF306449	Italy	1994	-	-	-	-	-	-	-	-	-	-	-	D	V
AB054219	Vietnam	1997	-	-	-	-	-	A	-	-	-	-	-	D	V
EU009205	Korean	2007	-	-	-	-	-	A	-	-	-	-	-	D	V
EF592511	Taiwan	2007	-	-	-	-	-	A	-	-	-	-	-	D	V
FJ265784	Taiwan	2008	-	-	-	-	-	A	-	-	-	-	-	D	V
U72698	Taiwan	1996	-	-	-	-	-	A	-	-	-	-	-	-	V
U72695	Taiwan	1998	-	-	-	-	-	A	-	-	-	-	-	-	V
FJ011097	Taiwan	2008	-	-	-	-	-	-	-	-	-	N	-	-	V
GU569948	Jilin	1986	-	-	-	-	-	-	-	-	-	N	-	-	V
GU569947	Beijing	1999	-	-	-	-	-	A	-	-	-	N	-	-	V
GU569945	Jilin	2001	-	-	-	-	-	A	-	-	-	N	-	-	V
GU569946	Jilin	2002	-	-	-	-	-	A	-	-	-	N	-	-	V
GU569941	Shandong	2002	-	-	-	-	-	A	-	-	-	N	-	-	V
GU569939	Yunnan	2002	-	-	-	-	-	A	-	-	-	N	-	-	V
EF011664	Beijing	2004	-	-	-	-	-	A	-	-	I	-	-	-	V
DQ903936	Sichuan	2005	-	-	-	-	R	A	-	-	-	-	-	-	V
GQ169544	Hubei	2006	-	I	-	Y	-	A	-	-	-	-	-	-	V
GQ857602	Shandong	2006	-	-	-	-	-	A	-	-	-	-	-	-	V
GQ169550	Jiangsu	2006	-	-	-	-	-	A	-	-	I	-	-	-	V
GQ169545	Beijing	2007	-	I	-	-	-	A	-	-	I	-	-	-	V
GQ857604	Shandong	2007	-	-	-	-	R	A	-	-	-	-	-	-	V
EU170352	Xinjiang	2007	-	-	-	-	-	A	-	-	I	-	-	-	V
EU441279	Henan	2008	-	-	-	-	-	A	-	-	-	-	P	-	V
GQ169538	Hubei	2008	-	-	-	-	-	A	-	-	I	-	-	-	V
EU441280	Jilin	2008	-	-	-	-	-	A	A	D	-	-	-	-	V
GQ857611	Shandong	2008	-	-	-	-	-	A	-	-	-	-	-	-	V
FJ435345	Sichuan	2008	T	-	-	-	-	A	-	-	I	-	-	-	V
GU452715	Jilin	2009	-	-	-	-	-	A	-	-	I	-	-	-	V
JF795456.1	Guangdong	2010	-	-	-	-	-	A	-	-	I	-	-	-	V
HQ651237	Sichuan	2010	-	-	-	-	-	A	-	-	-	-	-	-	v
JN403045	Xi'an	2011	-	-	-	-	-	A	-	-	-	-	-	-	v
GU569937	Guizhou	2002	-	-	-	Y	-	A	-	-	-	N	-	D	V
GU569944	Guizhou	2002	-	-	-	Y	-	A	-	-	-	N	-	D	V
GU569938	Yunnan	2002	-	-	K	Y	-	A	-	-	-	N	-	D	V
GU569940	Yunnan	2002	-	-	-	-	-	A	-	-	-	-	-	D	V
EF028071	Sichuan	2005	-	-	-	-	-	A	-	-	-	-	-	D	V
DQ120515	Jiangsu	2005	-	-	-	-	-	A	-	-	-	-	-	D	V

Table 2: Continued

GenBank No.	Origin	Year isolated	Amino acid at position												
			97	139	256	267	286	297	300	305	324	375	422	426	555
EU213078	Zhejiang	2007	-	-	-	Y	-	A	-	-	I	-	-	D	V
GQ857603	Shandong	2007	-	-	-	Y	-	A	-	-	-	-	-	D	V
GQ857605	Shandong	2007	-	-	K	Y	-	A	-	-	-	-	-	D	V
GQ857608	Shandong	2007	-	-	K	Y	R	A	-	-	-	-	-	D	V
EU145954	Beijing	2007	-	-	-	-	-	A	-	-	-	-	-	D	V
GQ857609	Shandong	2008	-	-	-	-	-	A	-	-	-	-	-	D	V
GQ857610	Shandong	2008	-	-	-	-	-	A	-	-	I	-	-	D	V
EU483517	Zhejiang	2008	-	-	-	-	-	A	-	-	I	-	-	D	V
EU483515	Zhejiang	2008	-	-	-	-	-	A	-	-	-	-	-	D	V
GU380299	Jilin	2009	-	-	-	Y	-	A	-	-	-	-	-	D	V
GU380300	Jilin	2009	-	-	-	Y	-	A	-	-	-	-	-	D	V
GU569937	Jilin	2009	-	I	-	Y	-	A	-	-	-	N	-	D	V

-: Indicated the Amino acids identical to the reference number M24003. A: Alanine; D: Aspartic acid; F: Phenylalanine; G: Glycine; I: Isoleucine; K: Lysine; L: Leucine; N: Asparagine; P: Proline; R: Arginine; S: Serine; T: Threonine; V: Valine; Y: Tyrosine

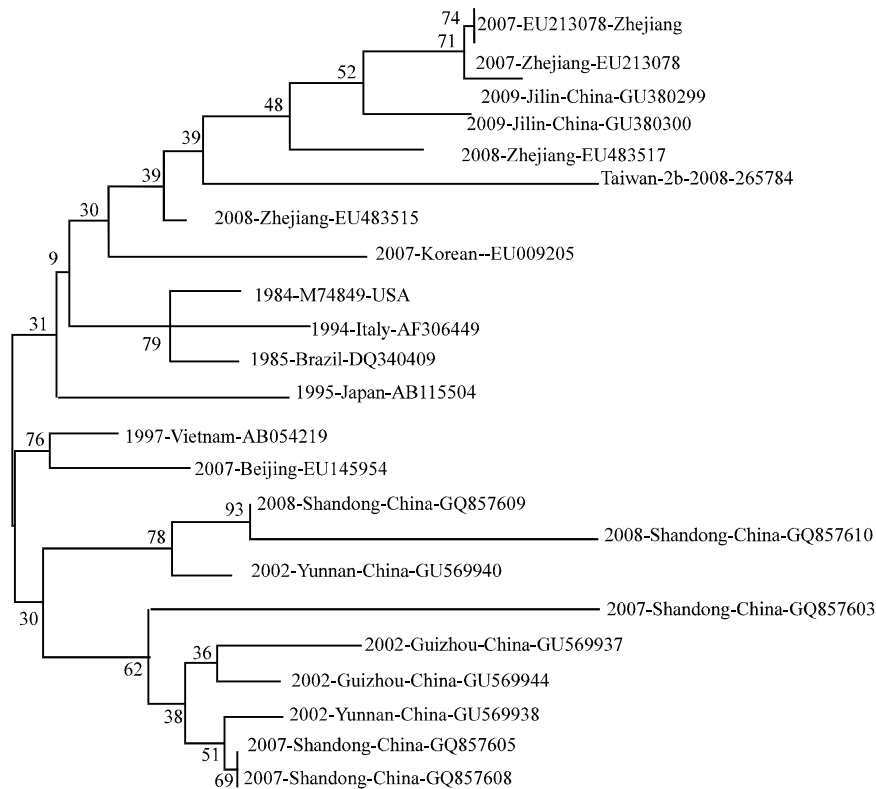


Fig. 3: Phylogenetic tree of CPV-2b in China during 2002-2009

Shandong, Yunnan and Guizhou provinces form another cluster (Fig. 3). Both clusters showed a close relationship to the Vietnam CPV-2b strain.



## CONCLUSIONS

Based on our analysis, CPV-2a was the main epidemic strain in China, with a geographic distribution characteristic. CPV-2a and CPV-2b isolated from China possessed three main mutations compared with foreign strains, displaying 297Ser-Ala, 324Tyr-Ile and 555Ile-Val. Phylogenetic tree showed that the Chinese CPV-2a were close to the Korean isolates and CPV-2b were close to the Vietnam strains.

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