NOTE *Virology*

Molecular epidemiological and phylogenetic analyses of canine parvovirus in domestic dogs and cats in Beijing, 2010–2013

Jing WU[1)#], Xin-Tao GAO[1)#], Shao-Hua HOU[1)], Xiao-Yu GUO[1)], Xue-Shong YANG[2)], Wei-Feng YUAN[1)], Ting XIN[1)], Hong-Fei ZHU[1)] and Hong JIA[1)]*

[1)]*Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China*
[2)]*Beijing GuanZhong Animal Hospital, Beijing, China*

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ABSTRACT. Fifty-five samples (15.62%) collected from dogs and cats were identified as canine parvovirus (CPV) infection in Beijing during 2010–2013. The nucleotide identities and aa similarities were 98.2–100% and 97.7–100%, respectively, when compared with the reference isolates. Also, several synonymous and non-synonymous mutations were also recorded for the first time. New CPV-2a was dominant, accounting for 90.90% of the samples. Two of the 16 samples collected from cats were identified as new CPV-2a (12.5%), showing nucleotide identities of 100% with those from dogs. Twelve samples (15.78%) collected from completely immunized dogs were found to be new CPV-2a, which means CPV-2 vaccines may not provide sufficient protection for the epidemic strains.
KEY WORDS: Beijing, canine parvovirus, molecular epidemiology

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Canine parvovirus type 2 (CPV-2) is the etiological agent of an epizootic severe gastroenteritis of dogs [2, 10, 38]. It has genomic substitution rates similar to those of RNA viruses, with values of about 10^{-4} substitutions per site per year, and host-immunity pressure may contribute to the progressive emergence of CPV-2 antigenic variants [34]. During 1979–1981, an antigenic variant, CPV-2a, was found. In 1984, a second variant, CPV-2b, was identified [23, 27, 28]. These antigenic types differ from the original CPV-2 in the VP2 gene, with five distinct amino acid differences that are mostly in the VP2 domain interacting with the host-cell transferrin receptor (TfR) [4, 8, 29]. During the last few decades, CPV-2a/2b with the Ser297Ala mutation was designated as new CPV-2a/2b [11, 23, 26]. Currently, new CPV-2a and new CPV-2b appear to have replaced the prototype CPV-2a and CPV-2b and to have become the predominant types, and they appear to be co-circulating in many countries [13, 16, 24, 26, 40, 41]. Another antigenic variant having an amino acid substitution (Asp426Glu), named as CPV-2c, was first reported in Italy in 2000 [6], and it is the most predominant variant in Italy, Germany, Uruguay and Argentina currently [10–12, 25, 39]. CPV-2 is considered the main pathogen responsible for acute gastroenteritis in dogs. However, CPV variants can also infect feline hosts [5, 35]. In Germany, CPV was previously detected in only approximately 10% of feline samples [37], but in Vietnam and Taiwan, reports estimated that up to approximately 80% of diseased cats were infected with CPV [19]. Some reports previously found that new-type CPV-2a and CPV-2b have been the predominant types in some provinces of China since the 1990s [42], while the epidemic situation of CPV variants in the domestic cat in mainland of China is still unknown.

In the present study, to clarify the evolution of CPV-2, which has recently been considered to be epidemic in China, and compare the epidemic isolates with the vaccine strain, the VP2 gene sequences of CPV-2 detected in Beijing from 2010 to 2013 were analyzed and compared with strains from China, Korea and other areas throughout the world. A total of 352 samples (blood, feces and secretions) from domestic dogs and domestic cats with diarrhea or bloody diarrhea were collected during 2010–2013, and subjected PCR. The primers used for PCR are shown in Table 1. A 1752 bp fragment covering the full-length sequence of the VP2 gene was successfully amplified from 55 samples (15.63%); among them, BJ-E13-2012 and BJ-E53-2012 collected from cats were identified as new CPV-2a (12.5%) and showed nucleotide identities of 100% with CPV strains collected from domestic dogs, but their infectiousness and pathogenicity in cats and dogs still need a further study. The sequences were submitted to GenBank. The GenBank accession numbers are KF803589 to KF803643. Detailed information on the origin and accession numbers of the CPV-positive samples is shown in Table 2. The resulting sequences were aligned with CPV reference isolates retrieved from the GenBank database. Sequences comparisons showed no deletion or shift in any sequences of the detected samples, and the nucleotide identities and deduced aa sequences identities were 98.2–100% and 97.7–100%, respectively, when compared with the CPV reference isolates.

Mutations in the VP2 protein are associated with the CPV

*CORRESPONDENCE TO: JIA, H., Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, No. 2 Yuanmingyuan South Street, Haidian District, Beijing 100193, P. R. China.
e-mail: zhuhongfei65@126.com
#These authors contributed equally to this work.

Table 1. The primers used for PCR detection of CPV

Primer	Sequence (5′→3′)	Positions (in VP2 gene)	Fragment length
CPV-1F	TTAAAGACTGTTTCAGAATCTGC	448–470	746 bp
CPV-1R	AATCTCTCAGGTGTTTCTCCTGTT	1170–1193	
CPV-2F	GGCGAATTCATGAGTGATGGAGCAGTTC	1–19	1,752 bp
CPV-2R	CGCCTCGAGATATAATTTTCTAGGTGCT	1733–1752	

genotypes, so the nucleotide sequences were translated into aa sequences. All samples, except one original CPV2a (BJ-A45-2010), were identified as "new CPV-2a/2b" reported worldwide and were identified as "new CPV-2a/2b" [9, 20, 22]. Previous research has shown residue 297 is under strong positive selection [30, 35]. Also, the 297Ala variant can not be distinguished serologically and does not change the viral antigenic type, but it potentially has a marked influence on host adaptation. Only four samples (BJ-A63-2010, BJ-B6-2011, BJ-B16-2011 and BJ-B43-2011) with Asn426Asp and ser297Ala mutations were characterized as new CPV-2b (7.27%). New CPV-2a accounted for 90.9%, while no CPV-2c was detected. Five samples (BJ-A2-2010, BJ-A49-2010, BJ-A68-2010, BJ-A69-2010 and BJ-A108-2010) also present Met at 87 position (9.09%), and six samples (BJ-A2-2010, BJ-A45-2010, BJ-A50-2010, BJ-A68-2010, BJ-A69-2010 and BJ-A108-2010) present Ile at 101 position (10.91%), which characterized as new CPV-2a like. Four samples presented Val139Ile mutation (7.27%). There are 24 samples presented Phe267Tyr mutation (43.64%). The Phe267Tyr change also has been described in Asiatic CPV-2b from Vietnam [25] and in CPV-2a from Thailand [32] and China [31, 35]. Residue 267 is not exposed on the capsid surface [1], so substitutions in this position may not affect the antigenicity of the virus. Fifty four samples presented Ala300Gly mutation (98.18%). Tyr324Ile mutation was first detected in China and Korea in 2004 [20, 35], and reported among the CPV 2a/2b isolates in China [17, 35]. Previous studies have shown that residue 324 is subject to strong positive selection in all parvoviruses of carnivores [17]. Residue 324 is likely to have had an effect on the parvovirus host range [35]. It is adjacent to residue 323, and together with residue 93, affects TfR binding [18]. In this study, fifty one samples presented Tyr324Ile mutation (92.73%) indicated that strain with mutation Tyr324Ile is the major epidemic strain. Thr440Ala has been described in CPV-2a and CPV-2b strains from China, Korea, India, Italy, Brazil and Uruguayan [3, 22, 30, 31] and in CPV-2c strains from the United States [21] and Argentina [7]. The 440 residue is important, because it is located at the top of the three-fold spike, the main antigenic site of the virus [36]. This residue is undergoing positive selection and has evolved in different populations independently, which explains its world-wide presence in unrelated CPV-2 populations [12]. In this study, seventeen samples presented Thr440Ala mutation, which accounted for 30.91%. And, all samples presented Tyr at 305 position and Val at 555 position. Also, Ser192Phe mutation in BJ-A2-2010 was just previously reported in SC02-2011. Asp375Asn mutation in BJ-A45-2010 was just previously reported in CPV-b, GZ0201 and JL0201. Previous report has indicated that residue 375 is associated with the ability of CPV to hemagglutinate or alter pH dependence of hemagglutination [15, 36], so BJ-A45-2010 may have different coagulation feature with others. While the mutations, Pro202Thr, Ile219Vla, Ala347Thr, Gln386Lys, Pro187Gln, Ser188 Gln and Val308Ile, present in this study were interesting, because it has not been detected previously in any other strains. Pro202Thr mutation was firstly reported in BJ-A2-2010, and Ile219Vla, Ala347Thr and Gln386Lys mutations were firstly reported in BJ-A45-2010. Pro187Gln and Ser188 Gln mutations were firstly reported in BJ-A69-2010. Also, Val308Ile mutation was firstly reported in BJ-D6-2012 and BJ-D14-2012. While, Asp427His, The445Asn and Pro-512His which appeared in Nanjing strains of 2009–2012 [41] and Thr442Ala and Gln370Arg reported in the giant panda strain B11 [15] were not present in this study. The detail mutation sites, types and rates in VP2 genes were shown in Table 3. The affects of these mutations to virus itself, which appeared in this study, need our further research.

Twelve strains were detected in dogs completely immunized with CPV-2 vaccine (Table 2). Although the effectiveness of CPV-2 vaccine against CPV-2a type has not been evaluated in China, our results showed that 12 out of 55 samples were detected as "new CPV-2a/2b" in dogs completely immunized with CPV-2 vaccine, which suggested that complete immunity may not be provided to dogs even if CPV-2 vaccines are used as previously reported [14, 15, 33, 35].

To examine the phylogenetic relationships of the 55 samples with the reference isolates, a phylogenetic tree based on the nucleotide sequence from 1 to 1752 nt of the VP2 gene was built. As shown in Fig. 1, all the samples clustered in CPV group, while separated from FPV-b. Most of them are with the closest relationship with the Chinese isolates, such as Guanzhou (JX66-690, SC02-2011), Beijing (HQ883267, Beijing; HQ883273, BJ-2010), Wuhan (FJ432717, 08-5-WH), Nanjing (EU095252, NJ-04) and so on. All sequences are with the distant relationship with Brazil, France, Japan, Vietnam, the United States and Italy isolates. However, BJ-B25-2011, BJ-B26-2011, BJ-A72-2010 and BJ-A63-2010 are with the distant relationship with others. Interestingly, BJ-A72-2010 is with the closest relationship with vaccine strains (EU914139, Pfizer vaccine 06 GQ169553, Vac 2), CPV-2 representative strain (M38245, CPV-b) and FPV representative strain (M24004, FPV-b). BJ-A63-2010 is with the closest relationship with South Korea strain (EF599097, DH326), Taiwan strain (AY869724, Taichung) and Chinese CPV 2c strain (GU380305 08-09). As the phylogenetic tree

Table 2. Detailed informations of the CPV-positive samples

Strains	GenBank ID	Sampling year	Host	Age	Clinical signs	Sample source	Vaccinated	Genetype
BJ-A1	KF803589	2010	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-A2	KF803590	2010	Domestic dog	2 months	Vomit and diarrhea	Blood	Not completely	New CPV-2a like
BJ-A45	KF803591	2010	Domestic dog	3 months	Cough and diarrhea	Feces	Not completely	CPV-2a
BJ-A48	KF803592	2010	Domestic dog	10 months	Cough and diarrhea	Secretions	Not completely	New CPV-2a
BJ-A49	KF803593	2010	Domestic dog	18 months	Recovery phase	Feces	Yes	New CPV-2a
BJ-A50	KF803594	2010	Domestic dog	2 months	Recovery phase	Feces	Not completely	New CPV-2a like
BJ-A53	KF803595	2010	Domestic dog	2 months	Recovery phase	Feces	Yes	New CPV-2a
BJ-A57	KF803596	2010	Domestic dog	2 months	Restored from diarrhea	Feces	Yes	New CPV-2a
BJ-A61	KF803597	2010	Domestic dog	4 months	Restored from diarrhea	Feces	Not completely	New CPV-2a
BJ-A63	KF803598	2010	Domestic dog	4 months	Restored from diarrhea	Feces	Yes	New CPV-2b
BJ-A64	KF803599	2010	Domestic dog	6 months	Injury	Feces	Not completely	New CPV-2a
BJ-A68	KF803600	2010	Domestic dog	6 months	Injury	Blood	Yes	New CPV-2a like
BJ-A69	KF803601	2010	Domestic dog	6 months	Diarrhea	Feces	Yes	New CPV-2a like
BJ-A72	KF803602	2010	Domestic dog	7 months	Restored from diarrhea	Feces	Not completely	New CPV-2a
BJ-A108	KF803603	2010	Domestic dog	2 months	Diarrhea and anorexia	Feces	Not completely	New CPV-2b
BJ-B4	KF803604	2011	Domestic dog	3 months	Diarrhea	Feces	Not completely	New CPV-2a
BJ-B5	KF803605	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B6	KF803606	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2b
BJ-B8	KF803607	2011	Domestic dog	2 months	Vomit	Feces	Not completely	New CPV-2a
BJ-B10	KF803608	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B11	KF803609	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Yes	New CPV-2a
BJ-B13	KF803610	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B16	KF803611	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2b
BJ-B19	KF803612	2011	Domestic dog	12 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B21	KF803613	2011	Domestic dog	3 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B22	KF803614	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B25	KF803615	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B26	KF803616	2011	Domestic dog	2 months	Cough and diarrhea	Secretions	Not completely	New CPV-2a
BJ-B28	KF803617	2011	Domestic dog	3 months	Diarrhea	Feces	Not completely	New CPV-2a
BJ-B31	KF803618	2011	Domestic dog	4 months	Vomit and bloody	Feces	Not completely	New CPV-2a
BJ-B32	KF803619	2011	Domestic dog	12 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B33	KF803620	2011	Domestic dog	2 months	Diarrhea and bloody	Feces	Yes	New CPV-2a
BJ-B34	KF803621	2011	Domestic dog	3 months	Vomit and bloody	Feces	Not completely	New CPV-2a
BJ-B38	KF803622	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B39	KF803623	2011	Domestic dog	3 months	Anorexia	Feces	Not completely	New CPV-2a
BJ-B40	KF803624	2011	Domestic dog	10 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-B41	KF803625	2011	Domestic dog	3 months	Vomit and diarrhea	Feces	Yes	New CPV-2a
BJ-B42	KF803626	2011	Domestic dog	4 months	Vomit and diarrhea	Feces	Yes	New CPV-2a
BJ-B43	KF803627	2011	Domestic dog	3 months	Vomit	Feces	Not completely	New CPV-2b
BJ-B44	KF803628	2011	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-D4	KF803629	2012	Domestic dog	2 months	Diarrhea	Feces	Not completely	New CPV-2a
BJ-D6	KF803630	2012	Domestic dog	2 months	Vomit and diarrhea	Feces	Yes	New CPV-2a
BJ-D14	KF803631	2012	Domestic dog	1 month	Cough	Feces	Not completely	New CPV-2a
BJ-D15	KF803632	2012	Domestic dog	2 months	Anorexia and Vomit	Feces	Not completely	New CPV-2a
BJ-E4	KF803633	2012	Domestic dog	2 years	Anorexia and diarrhea	Feces	Not completely	New CPV-2a
BJ-E13	KF803634	2012	Domestic cat	6 months	Health	Blood	Not completely	New CPV-2a
BJ-E14	KF803635	2012	Domestic dog	5 years	Health	Blood	Yes	New CPV-2a
BJ-E34	KF803636	2012	Domestic dog	2 months	Anorexia and diarrhea	Feces	Not completely	New CPV-2a
BJ-E53	KF803637	2012	Domestic cat	7 months	Diarrhea	Blood	Not completely	New CPV-2a
BJ-E64	KF803638	2012	Domestic dog	3 months	Vomit	Feces	Not completely	New CPV-2a
BJ-E81	KF803639	2012	Domestic dog	7 months	Diarrhea	Feces	Not completely	New CPV-2a
BJ-P21	KF803640	2013	Domestic dog	2 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-P27	KF803641	2013	Domestic dog	7 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-P33	KF803642	2013	Domestic dog	3 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a
BJ-P34	KF803643	2013	Domestic dog	4 months	Vomit and diarrhea	Feces	Not completely	New CPV-2a

Table 3. Table of statistics of mutation sites, types and rates in VP2 genes

aa sites	87	101	139	187	188	192	202	219	267	297	300	305	308	324	347	375	386	426	440	555
aa mutation	L→M	T→I	V→I	P→I P→Q	A→Q	S→F	P→T	I→K	F→Y	S→A	A→G	D→Y	V→I	Y→I	A→T	D→N	Q→K	N→D	T→A	I→V
Sample numbers	5	6	4	2	1	1	1	1	24	54	54	55	2	51	1	1	1	4	17	55
Mutation rates (%)	9.09	10.91	7.27	3.64	1.82	1.82	1.82	1.82	43.64	98.18	98.18	100	3.64	82.73	1.84	1.84	1.84	7.27	30.91	100

shows, most of the viruses isolated in China formed a large cluster and certain mutations detected in Chinese CPVs probably arose during the process of local adaptation, as indicated by previous surveys [15, 30].

In summary, new CPV-2a is the prominent type of CPV in Beijing from 2010 to 2013. Few samples still present Met at 87 position, Ile at 101 position and Ala at 300 position. Most samples contained the mutation Phe267Tyr, Ala300Gly, Tyr324Ile and Thr440Ala. All samples presented Tyr at 305 position and Val at 555 position. While, Pro187Gln, Ser188 Gln, Ile219Vla, Ala347Thr, Gln386Lys and Val308Ile mutations were firstly reported in present study. Also, new CPV-2a was detected in cats with diarrhea, and CPV-2 vaccines may not provide complete immunity to dogs in China. Due to the continuing evolution of CPV, monitoring the prominent type of CPV, and detecting genetic mutation and antigenic changes, will be necessary to find new vaccines and control CPV infection in China.

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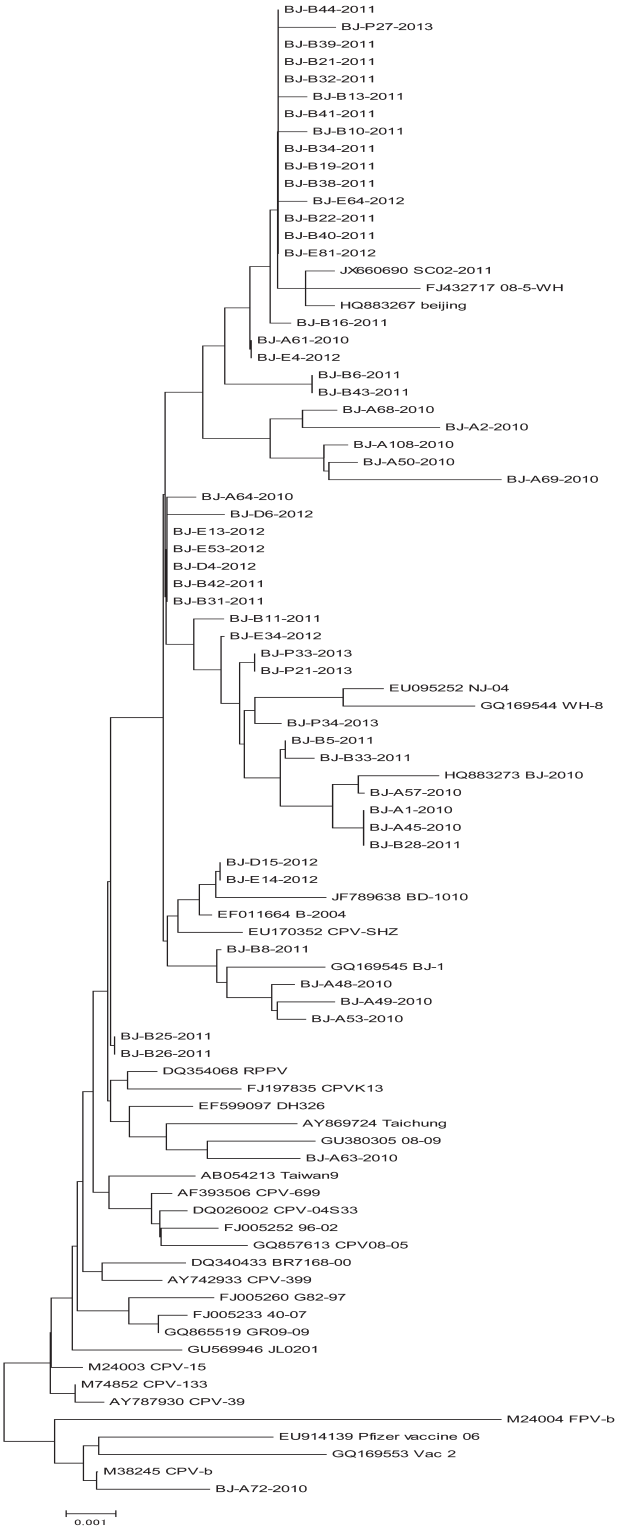

Fig. 1. Phylogenies of the VP2 gene sequences. The phylogenetic analyses were performed using the MEGA software, version 4.0 (http://www.megasoftware.net/). The neighbor-joining method was chosen to draw the nucleotide phylogenetic tree. The reliability of the phylogenetic tree obtained for the VP2 region was evaluated by running 1,000 replicates in the bootstrap test.

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