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Genetic Characterization of Canine Parvovirus Isolates From Beijing of China Between 2009 and 2011

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Abstract: In order to investigate the new strains circulating in Beijing, 24 positive samples of CPVs were isolated from Beijing of China between 2009 and 2011 and the sequences of the main capsid protein VP2 were obtained. These sequences were analyzed in comparison with the sequences of 29 other strains of CPV from GenBank. Two types of CPV, including CPV-2 and CPV-2a were detected and CPV-2a (with Ala297 mutation) was predominant in Beijing and 4 strains CPV-2a types were found to carry the amino acid substitution Ala440. The unique Ile324 mutation in the VP2 of Beijing CPV isolate was detected as compared with a Tyr324 in the VP2 of the reference CPV strains. A phylogenetic tree was constructed from the *VP2* genes. These results showed that most of the isolated Beijing strains classified in a cluster Chinese field isolates which were distinct from American and Italian isolates.

Key words: Canine parvovirus, *VP2* gene, phylogenetic analysis, amino acid, Beijing, China

INTRODUCTION

Canine Parvovirus (CPV) was first recognized in 1978 (Kapil *et al*., 2007). CPV is a non-enveloped virus with a single stranded DNA genome of approximately 5.2 kb which encodes two nonstructural proteins (NS1 and NS2) and two structural proteins (VP1 and VP2) (Cotmore and Tattersall, 1987; Reed *et al*., 1988). VP2 is the major capsid protein containing the main antigenic determinants and also having an important ability to self-assemble, forming immunocompetent Virus Like Particles (VLPs) (Lopez de Turiso *et al*., 1992).

In the late 1970s and early 1980s, the most widely accepted hypothesis was the emergence of CPV type 2 from a variant of Feline Panleukopenia Virus (FPLV) or from a closely related virus infecting another carnivore (Horiuchi *et al*., 1988; Truyen *et al*., 1998). Some biological properties of the virus particularly its rate of nucleotide substitution determined the continuous appearance of new variants with high sanitary impact on the dog populations (Shackelton *et al*., 2005). Since, the emergence of CPV type 2, two new antigenic types, designated CPV-2a and CPV-2b had arisen and almost completely replaced CPV type 2 viruses (Parrish *et al*., 1985). More recently, a third antigenic type CPV-2c was reported for the 1st time in Italy and then in numerous other parts of the world (Kapil *et al*., 2007; Buonavoglia *et al*., 2001; Nakamura *et al*., 2004; Vieira *et al*., 2008; Perez *et al*., 2007; Decaro *et al*., 2006; Calderon *et al*., 2009; Nandi *et al*., 2010). Since then, gene sequencing and genetic analyses had been applied frequently and many new CPV variants throughout the world had been detected such as the new CPV-2a, new CPV-2b, CPV-2c (a) and CPV-2c (b) (Ohshima *et al*., 2008; Ikeda *et al*., 2000).

The high sanitary impact of CPV at international level makes it important to perform the precise identification of new emerging strains and to understand its evolution in the field (Decaro *et al*., 2009; Battilani *et al*., 2002). In this study, researchers extend the study of the CPV evolution (Calderon *et al*., 2009, 2011) focusing in the analysis of 24 *VP2* gene sequences of fecal samples between the years 2009 and 2011.

MATERIALS AND METHODS

Preparation of samples: About 30 fecal samples were collected from dogs in Beijing between 2009 and 2011. The fecal samples were manipulated according to the methods that had been described before (Mochizuki *et al*., 1993).

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PCR amplification of *VP2* gene: Genomic DNAs were extracted from the fecal samples using the Axygen kit (California, USA) and were used as a template to amplify the full length VP2 fragment by PCR. The sense strand primer (5'- C TAG GGA TCC ATG AGT GAT GGA GCA GTT -3') and the antisense strand primer (5'- C CGC CTC GAG TTA ATA TAA TTT TCT AGG T -3'). The templates were denatured at 94°C for 2 min followed by 30 cycles of PCR (45 sec at 94°C, 45 sec at 58°C and 72°C for 2 min) and a final extension at 72°C for 10 min.

Sequencing and phylogenetic analysis: The VP2 fragments were directly sequenced with the dideoxy mediated chain termination method (Beijing Sunbiotech Co., Ltd.) (Calderon *et al*., 2011). Multiple sequence alignment analysis was performed with ClustalW Software. A phylogenetic and molecular evolution tree was constructed from the whole *VP2* gene nucleotide sequences of the CPV strains used in this study and other sequences obtained from GenBank database with MEGA version 4.0 with the neighbor joining method. The reliability of the phylogenetic tree obtained for the VP2 region was evaluated by running 1,000 replicates in the bootstrap test.

The GenBank accession numbers of the amino acid and nucleotide sequences of reference CPV strains were as follows: CPV-d strain (CPV-2, M23255); CPV-15 strain (CPV-2a, M24003); CPV-193 strain (CPV-2b, AY742932); Pome (CPV-2c (a), EF599098) and 56/00 (CPV-2c, FJ222821).

RESULTS AND DISCUSSION

Detection of CPV by PCR: During this study, 30 fecal samples from animals suspected of CPV infection were tested, CPV specific DNA was detected by PCR amplification of VP2 fragment (1755 bp). In all cases, PCR products were analyzed by gel electrophoresis. The results of PCR showed that 24 out of 30 (80%) is positive of which 9 out of 10 (90%) samples in 2009, 8 out of 10 (80%) samples in 2010 and 7 out of 10 (70%) samples in 2011. About 9 strains were named CP0901-CP0909 in 2009, 8 strains were named CP1001-CP1008 in 2010, 7 strains were named CP1101-CP1107 in 2011.

Amino acid analysis: Sequence comparisons showed nucleotide identities of 97.7-99.7% among the CPVs isolated in Beijing from 2009-2011. Nucleotide sequences were translated into amino acid sequences to identify the isolates as CPV-2, CPV-2a types. Critical positions of the CPV *VP2* gene products of isolates sequenced in this study were shown in Table 1. As shown, all CPV-2a strains were identified as new CPV-2a with the Ala297 mutation and Tyr→Ile at positions 324 except CP1004 strain; the CP0905 strain was identified in one strain

Table 1: Amino acid variants analysis in the *VP2* gene of CPV

	Amino acid variants positions													
Strains	87	101	297	300	305	324	345	375	418	426	435	440	555	Genetic type
CP0901	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP0902	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP0903	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP0904	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP0905	M	I	S	A	D	Y	L	N	I	N	P	T	V	CPV-2
CP0906	L	I	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP0907	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP0908	L	T	A	G	Y	I	F	D	I	N	P	A	V	CPV-2a
CP0909	L	T	A	G	Y	I	C	D	I	N	P	T	V	CPV-2a
CP1001	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1002	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1003	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1004	L	T	A	G	Y	Y	F	D	I	N	P	T	V	CPV-2a
CP1005	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1006	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1007	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1008	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1101	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1102	L	T	A	G	Y	I	F	D	I	D	P	A	V	CPV-2a
CP1103	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1104	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CP1105	L	T	A	G	Y	I	F	D	I	N	P	A	V	CPV-2a
CP1106	L	T	A	G	Y	I	F	D	I	N	P	A	V	CPV-2a
CP1107	L	T	A	G	Y	I	F	D	I	N	P	T	V	CPV-2a
CPV-d	M	I	S	A	D	Y	F	N	I	N	P	T	V	CPV-2
CPV-15	L	T	S	G	Y	Y	F	D	I	N	P	T	I	CPV-2a
CPV-193	L	T	A	G	Y	Y	F	D	I	D	P	T	V	CPV-2b
56/00	L	T	A	G	Y	Y	F	D	I	E	P	T	V	CPV-2c
Pome	L	T	A	D	Y	Y	F	D	T	N	S	A	V	CPV-2c (a)

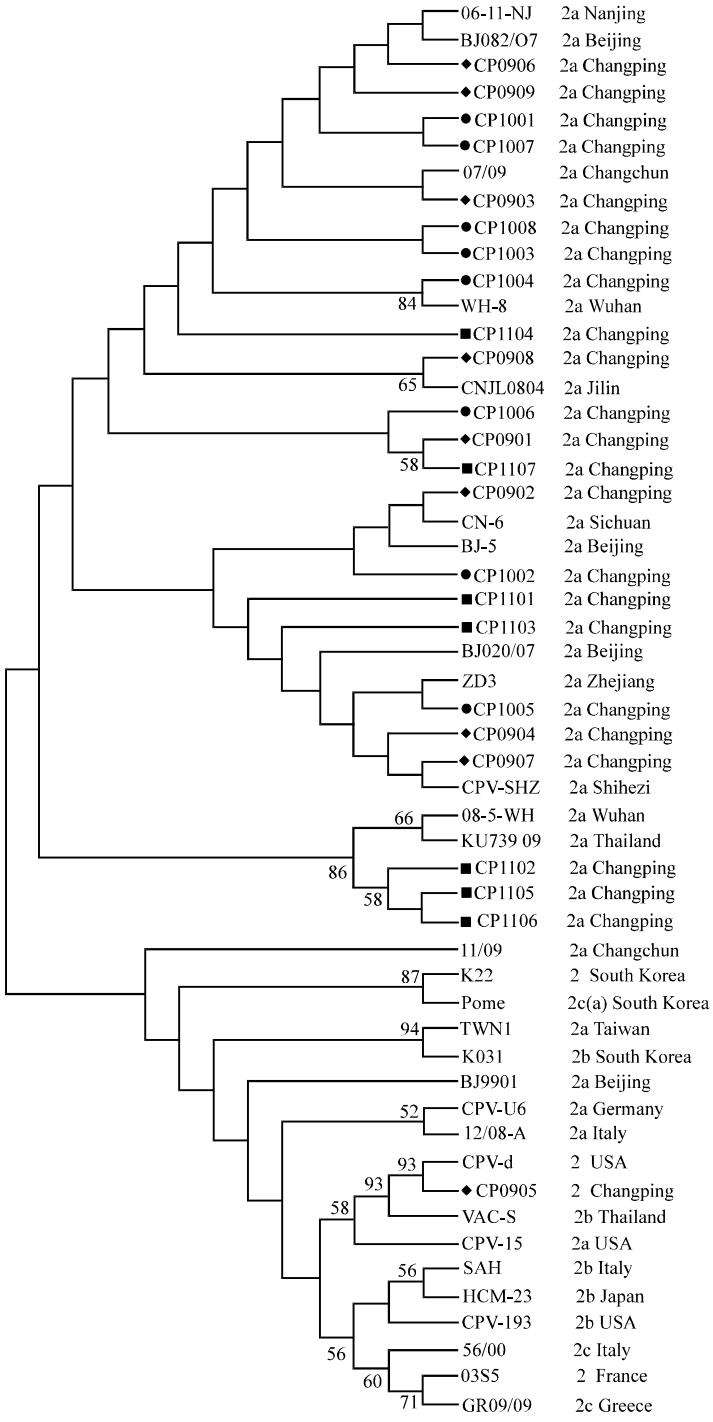

Fig. 1: Comparison of the nucleotide sequences of the *VP2* gene of CPV isolates obtained from dogs in Beijing of China and other sequences obtained from the GenBank database using the neighbor joining method and bootstrap analysis performed with 1000 trials

isolated in 2009 and CPV-d. About 23 strains of CPV isolates of new CPV-2a indicating that this type had been the predominant CPV in Beijing during 2009-2011 while new CPV-2b strain that has been reported in the South of China was not found. Neither CPV-2c (a), CPV-2c (b) (with Asp300 mutation) nor CPV-2c (with Glu426 mutation) were

found. CP0908, CP1102, CP1105 and CP1106 were found to carry the amino acid substitutions (with Ala440 mutation).

To examine the phylogenetic relationship of the Beijng isolates with representative CPVs, the phylogenetic tree based on the nucleotide sequences from 1-1,755 of the *VP2* gene were constructed. As shown in Fig. 1, most of the CPVs isolated in Beijing formed a major monophyletic cluster. CP1102, CP1105 and CP1106 of CPV type 2a were clustered close to the Wuhan 08-5-WH and Thailand KU739 09, CP0905 that had been characterized as CPV type 2 was closely related to the USA CPV-d.

CPV has a nucleotide substitution rate that is similar to that of RNA viruses (Nakamura *et al*., 2004). The *VP2* gene encodes the major structural protein of parvoviruses (Hong *et al*., 2007). It determines the antigenicity of the parvovirus and its host specificity (Parrish *et al*., 1991). In the *VP2* gene there are five amino acid differences at residue 87, 101, 300, 305 and 555 between CPV-2 and CPV-2a and five amino acid differences at residues 87, 101, 300, 305 and 426 between CPV-2 and CPV-2b (Buonavoglia *et al*., 2001; Decaro *et al*., 2006). The significant differences between CPV-2a and CPV-2b are the substitution of two amino acids in the major antigenic VP2 capsid protein that is Asn426 in 2a (Asp426 in 2b) and Ile555 in 2a (Val555 in 2b) (Martella *et al*., 2004). Recently, CPV-2c is a new CPV mutant that has a glutamate substitution at the 426th residue of VP2 protein (Shackelton *et al*., 2005; Hoelzer *et al*., 2008; Parrish *et al*., 1988).

New CPV-2a strains appear to have replaced the prototype CPV-2a strains and become the predominant type in many countries (Nakamura *et al*., 2004; Ohshima *et al*., 2008; Chinchkar *et al*., 2006; Martella *et al*., 2005; Meers *et al*., 2007). It has been reported that mutation Ala297 does not change the viral antigenic type (Truyen *et al*., 2000) so the Ala297 variant cannot be distinguished serologically. However, the emergence and spread of this variant indicates that the Ala297 mutation potentially has had a marked influence on the process of continuing host adaptation and previous research has shown that site 297 is under strong positive selection (Pereira *et al*., 2007). The analysis has showed that CP0908, CP1102, CP1105 and CP1106 were found to carry the amino acid substitution Ala440. It is interesting to point out that this mutation was found in samples collected from the years 2009-2011 indicating its recent appearance in the Beijing dog population.

In this study, one strain of CPV isolates in 2009 was found to be CPV-2 type which had a close relationship with typical strain CPV-d indicated by the phylogenetic tree. Since, CPV-2 viruses are no longer considered to circulate in dog populations world wide, it is likely that the virus detected in the dog was CPV-2 vaccine virus (Parrish *et al*., 1991). Researchers found one mutation among the CPV-2a isolates: Tyr→Ile at position 324 except CP1004 strain. Residue 324 is adjacent to residue 323 which affects canine transferrin receptor binding, together with residue 93 (Horiuchi *et al*., 1994; Hueffer and Parrish, 2003; Llamas-Saiz *et al*., 1996; Parker and Parrish, 1997; Strassheim *et al*., 1994).

In summary, new CPV-2a is the prominent type of CPV in Beijing. The variant firstly detected in China and Korea, most isolates contained the mutation Ile324 which probably arose around 2004 (Jeoung *et al*., 2008). CPV-2b/2c which had displayed an exceptional ability to spread rapidly throughout the canine population in European countries (Decaro *et al*., 2007), Asia (Nakamura *et al*., 2004) and America (Kapil *et al*., 2007; Perez *et al*., 2007; Hong *et al*., 2007) had not been detected in Beijing.

CONCLUSION

From this study, researchers concluded that due to the continuing evolution of CPV, monitoring of field CPV isolates and detection of genetic mutation and antigenic changes would be necessary to control CPV infection in Beijing. At present, the CPV variation is mainly due to the use of the vaccine under the conditions of strong immune pressure CPV occurs the antigenic drift. The CPV variation happens quickly because of using vaccine first in USA. In comparison, the vaccine is used late in China, the small immune pressure, facilitates slight CPV variation. And other viruses have a variety of prototype, all kinds of prototype is the common stability existence. However, CPV is the new antigenic type gradually replaced the old. Over time, CPV will happen variation situation which needs further study and attention.

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