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Full Length Research Paper

Molecular characterisation of canine parvovirus strains circulating in China

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Canine parvovirus (CPV) was first isolated at 1978 in the USA. Analysis of CPV isolates by monoclonal antibodies and restriction enzymes have shown that after the first emergence of CPV (CPV-2) it evolved to give rise to new antigenic types, which were designated CPV types 2a, 2b and 2c. These new types have replaced the original CPV type 2, although the proportions of each of the new antigenic types vary in different countries. In China, CPV infections were first observed in 1982, however, there has been no information concerning the antigenic types of CPV prevailing in China now. In this study, we designed a PCR assay to type canine parvovirus strains in fecal samples collected from symptomatic dogs from 2006 to 2009. Our data showed that the CPV prevalent strain is mostly 2b, the proportion of CPV-2a is very low, no CPV-2c and CPV-2 were observed.

Key words: Canine parvovirus; polymerase chain reaction; molecular characterization.

INTRODUCTION

Canine parvovirus (CPV) was first identified in 1978 in the USA (Appel et al., 1979) and was designated CPV type 2 (CPV-2) to distinguish it from a previously recognized parvovirus of dogs known as minute virus of canines (Binn et al., 1970). After its emergence, CPV-2 spread globally, and now CPV viruses are endemic in most populations of domestic and wild canids (Parrish et al., 1988). Analysis of CPV isolates by monoclonal antibodies and restriction enzymes have shown that a new antigenic strain, designated CPV type 2a (CPV-2a), became widespread around 1979 and that it replaced the original strain during 1980 to 1981 in the USA (Parrish et al., 1985). Later examination of canine isolates identified another antigenic variant, designated CPV type 2b (CPV-2b) that emerged around 1984, and after 1986 replaced CPV-2a in many parts of the USA (Parrish et al., 1991). Now, CPV-2c has emerged first in Italy (Buonavoglia et al., 2001). The same pattern of spread of CPV-2, and the emergence and subsequent replacement by the new antigenic strains were also shown by antigenic analysis of isolates from Denmark, Germany, France, Spain, Japan, Australia, Italy and Africa although the proportions of CPV-2a, CPV-2b and CPV-2c strains differ (Parrish et al.,1988; Mochizuki et al., 1993; Ybanez et al., 1995; Truyen et al., 1996; Buonavoglia et al., 2001, Steinel et al.,1998). In China, CPV infections were first observed as sporadic cases during 1982 (Liang et al., unpublished data). Subsequently, widespread outbreaks of canine haemorrhagic enteritis with high morbidity and mortality occurred over the whole country, although there has been no information concerning the antigenic types of CPV prevailing in China. In this study, we developed a polymerase chain reaction (PCR) assay to type CPV strains based on the genetic markers of the three antigenic variants and used this assay to characterize the CPV strains prevalent in China.

MATERIALS AND METHODS

Samples

Faecal specimens (n = 30) collected from vaccinated and unvaccinated diarrhoeic dogs from years 2006 to 2009 and tested positive to CPV-2 by commercial immune colloidal gold assay kit (bioindist, South Korea) were recruited in this study, These samples

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Abbreviations: CPV, Canine parvovirus; **PCR,** polymerase chain reaction; **PBS,** phosphate-buffered saline.

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came from young dogs of 1 to 17 months. As negative controls, 2 faecal samples from CPV-2 negative dogs were used, and CPV live vaccine for positive controls.

DNA preparation

The samples were homogenised (10%, w/v) in phosphate-buffered saline (PBS) and clarifed by high-speed centrifugation (8000×g for 5 min). DNA preparation was carried out rapidly by boiling the supernatants for 10 min and subsequent chilling on ice. In order to remove the residual inhibitors of the polymerase activity, the DNA extracts were reduced to effective concentrations by diluting 1:10 in distilled water prior to PCR amplifications (Decaro et al., 2005).

Primers for PCR

The sequences of all PCR primers were designed according to the VP2 capsid genes from published nucleotide sequences of CPV-2, 2a, 2b and 2c (Accession No. AY380577, DQ340434, DQ025992, AB054223). Primer A, which detects the mutual upstream sequence of VP2 gene, the forward primer (5'-GGA TGGGTGGAAATC ACAGC-3') and reverse primer (5'-ATAACC AACTCAGCTGGTC-3') located, respectively at 2954-2973 and 3780-3799 yielded a 815 bp product. Primer B (forward primer 5'-TCCAGAAGGAGATTGG ATTC-3' and reverse primer 5'-TTCTAGGTGCTAGTTGAGATT-3'), which for amplification the downstream sequence of VP2 gene, respectively located at 4012 - 4031 and 4509 - 4529 yield a 518 bp product, and the product containing an *mbo* II site for specific digestion of CPV-2c stain.

PCR reaction

The PCR assay was performed as previously described (Kong and Zhang 2003) with some modifications in the $MgCl_2$ final concentration. The most sensitive and reliable concentration of $MgCl_2$ was found to be 1.5 mM for all the primers. Briefly, the PCR was performed in a thermal cycler (Applied Biosystem, 2720 thermal cycler) for 30 cycles, each consisting of denaturation at 94℃ for 30 s, annealing at 52℃ for 2 min and polymerization at 72℃ for 1 min. PCR products were electrophoresed on 1.2% agarose gels, gels were stained with ethidium bromide and then visualized with ultraviolet light.

Restriction enzyme analysis of the PCR products (518 bp amplicon)

The restriction enzyme Mbo II was found to recognize selectively the sequence GAAGA at 4060-4064 bp for CPV-2c. Then, the amplicons (10 μl) obtained by PCR amplification with primer B were digested at 37℃ for 3 h with 5 units *Mbo* II restriction endonuclease as recommended by the manufacture (TAKARA, Da Lian, China), the 1% agarose gels were photographed under UV light after ethidium bromide staining.

DNA sequencing

Amplocons were sequenced, using an ABI prism 3730-Perkin Elmer automated sequencer, either directly or after being cloned using the T/A clone PCR product cloning Kit (Takara, DaLian, China). Sequencing was carried out twice on both strands using Big Dye terminator v3.1 as recommended by the manufacturer.

RESULTS

CPV diagnosis

Two DNA bands of the expected size (815, 518 bp), corresponding to the partial amplification of the VP2 gene, was observed by gel electrophoresis in 23 of the 30 field samples suspected of hanging CPV. Two bands of the same size were used as positive control. No amplified products were obtained from faecal samples of clinically healthy dogs used as negative controls.

Genotyping the samples: RFLP and sequence

In order to test the presence of CPV-2c in the samples, we performed a RFLP analysis using the *Mbo* II enzyme as reported by Buonavoglia et al. (2001) Our analysis indicated that no type 2c RFLP pattern were observed (Table 1). So, all the samples were sent to sequence analysis, and the results revealed that all the 23 samples were presented as CPV-2a and CPV-2b type.

The sequencing of the 23 samples revealed that only 2/23 presented an AAT codon at position 426 of the VP2 protein, characteristic of CPV-2a, while other 21 samples presented a GAT codon at the same position of VP2 protein, characteristic of CPV-2b (Table 2).

The VP2 sequence alignment of the Chinese CPV-2a indicated that the strains Ch-NJ1 and Ch-NJ3 exhibited 100% nucleotide identity with the CPV-2a from China (cpv/nj01/06) and 99.6% identical to Taiwanese isolate (Taiwan 9), it is worth noting that the two strains came from the same place, NanJiang, the capital of JiangSu province. In comparison with the Japanese CPV-2b (HNI-2-13), the Ch-YZ1, Ch-AH1, Ch-YZ2, Ch-YZ3, Ch-ZhJ and Ch-ZhJ2 had 99.4% (ten differences), Ch-BJ, Ch-XZ, Ch-AH2 and Ch-ZJ had 99.2% (thirteen differences), the Ch-HA, Ch-CZ, Ch-SQ, Ch-ZhJ2, Ch-JX had 99.1% (fifteen differences), and others had 98.9% (eighteen differences) nucleotide identity. The Ch-ZJ, Ch-CZ, Ch-BJ, Ch-YZ3 showed two amino acid changes (Phe-137 to Leu, Thr-314 to Ala), Phe-267 to Tyr were observed in Ch-LYG, Ch-NT2, Ch-SQ and Ch-TZ, Thr-440 to Ala was observed in Ch-HA and Ch-AH2, the other nucleotides changes observed among CPV-2b were synonymous (Table 2).

DISCUSSION

As surprising as the emergence and sudden dispersion that CPV-2 experimented in the 1970s, were the changes, including an extended host range that the virus further underwent in a few years (Shackelton et al., 2005; Truyen, 2006). The great virus variability, in part consequence of its high mutation rate similar to the one observed in fast evolving RNA viruses (Shackelton et al., 2005) has raised concern about its potential negative impact on the health of domestic dogs and wildlife species.

Table 1. Chinese CPV stain analysed in the present study.

Sample	Vaccines	Course	Procedure	Race	Cpv
Ch-YZ1	Complete	Died	YangZhou	Undefined	2b
Ch-NJ1	Incomplete	Recovered	NanJing	Pekinese	2a
Ch-AH1	Complete	Recovered	AnHui	Poodle	2b
Ch-YZ2	Complete	NA	YangZhou	Undefined	2b
Ch-NT1	Complete	Recovered	NanTong	ChowChow	2b
Ch-TZ	NA	NA	TaiZhou	German shepherd	2b
Ch-NJ2	Complete	Recovered	NanJing	Collie	2b
Ch-YC	Complete	Died	YanCheng	Collie	2b
Ch-YZ3	Complete	Recovered	YangZhou	Golden retriever	2b
Ch-NJ3	Complete	Died	NanJing	Shar Pei	2a
Ch-BJ	Complete	Recovered	BeiJing	Saint Bernard	2b
Ch-XZ	None	Recovered	XiZhang	Tibetan Mastiff	2b
Ch-LYG	Incomplete	NA	LianYungang	ChowChow	2b
Ch-NT2	Complete	Recovered	NanTong	Poodle	2b
Ch-AH2	Complete	Recover	AnHui	Bull dog	2b
Ch-SH	Incomplete	Died	ShangHai	German shepherd	2b
Ch-JX	Complete	Recovered	JiaXing	Saint Bernard	2b
Ch-ZJ	Complete	Recovered	ZhenJiang	Bull dog	2b
Ch-ZhJ	Complete	NA	ZhengJiang	Collie	2b
Ch-HA	NA	Died	HuaiAn	Labrador retriever	2b
Ch-CZ	Complete	Recovered	ChangZhou	Great Pyrenees	2b
Ch-SQ	Incomplete	Recovered	SuQian	Pekinese	2b
Ch-ZhJ2	Complete	Died	ZhengJiang	Chihuahua	2b

Faecal samples were collected from vaccinated and unvaccinated puppies with haemorrhagic enteritis. Dogs were assisted in veterinary clinics located in different Chinese departments. Some dogs presented a complete vaccination plan; others had not received the complete series (at least three doses) of vaccines and other did not receive any vaccines at all. Genotype characterization was performed by DNA sequencing and restriction fragment length polymorphism (RFLP). NA: non-available date.

Although the new variant CPV-2c had been detected first in Italy (Buonavoglia et al., 2001) , subsequent occurrence in other European countries and South America (Schacketlton et al., 2005; Decaro et al., 2006, 2007; Ruben et al., 2007), no CPV-2c was detected in the present study. More striking than the simple presence is the high frequency (21 out of 23) that CPV-2b achieved in China. This fact, together with its occurrence in different localities (Table 1) is indicative that type 2b is currently the prevalent field CPV circulating in the country. Although we know that CPV appeared in China around 1982 (Liang et al., unpublished data), there is no data about CPV genotypes existing in the country in previous years that allow the comparative analysis of the evolution of CPV variants over time. We can hypothesize that the Chinese CPV-2b replaced previous circulating CPV, as occurred in USA. This result, together with our own findings, indicates that the spreading ability of the type 2b is associated with an important replacement capacity that could eventually lead to the elimination of CPV-2a.

The results of the investigation of the 23 CPV strains indicate that in the area examined the CPV-2b variant is more popular than the CPV-2a variant. A similar epidemiological pattern has been reported in the USA and UK (Ybañez et al., 1995; Greenwood et al., 1996; Davies, 2008); in Germany and in Spain the two variants appear to be distributed about equally; in contrast, CPV-2a appears to be more common in Australia. The reasons for the different worldwide distribution of the CPV variants are unclear, and they will probably remain obscure until the mechanisms whereby CPV has evolved are defined. Beside the CPV-2b, we also identified the Chinese samples, two CPV-2a strains characterized by the presence of an Asn426 residue, which had 100% identity with the reported sequence of nj01/06 strain in China. Probably, the CPV-2a strain represents a relic of the original types that were predominant in China before CPV-2b appearance, furthermore, this speculation is in accordance with the trend circulating in neighbouring countries as Japan (Doki et al., 2006). To our knowledge, no reports about CPV-2c until now in Asia except Vietnam (Nakamura et al., 2004). So, it is evident that further studies will be needed to obtain additional evidence on the CPV behaviour in China in the next years.

Table 2. Amino acid differences in whole VP2 sequences of Chinese strains of CPV (type 2a: Ch-NT1 and Ch-NJ3, type 2b: others) with CPV-2a from Japan (V154, AB054217), CPV-2b from Japan (LCPV V204, AB054221).

Strains	Place of amino acid site													Gene type
	87	93	103	137	267	297	300	314	323	426	440	562	564	
CPV-b	M	N	A	F	F	S	A	T	N	N	T	V	G	CPV-2
V154	L	N	A	F	F	A	G	T	N	N	T	V	G	CPV-2a
LCPV-V204	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-YZ1	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-NJ1	L	N	A	F	F	A	G	T	N	N	T	V	G	CPV-2a
Ch-AH1	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-YZ2	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-NT1	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-TZ	L	N	A	F	Y	A	G	T	N	D	T	V	G	CPV-2b
Ch-NJ2	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-YC	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-YZ3	L	N	A	L	F	A	G	A	N	D	T	V	G	CPV-2b
Ch-NJ3	L	N	A	F	F	A	G	T	N	N	T	V	G	CPV-2a
Ch-BJ	L	N	A	L	F	A	G	A	N	D	T	V	G	CPV-2b
Ch-XZ	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-LYG	L	N	A	F	Y	A	G	T	N	D	T	V	G	CPV-2b
Ch-NT2	L	N	A	F	Y	A	G	T	N	D	T	V	G	CPV-2b
Ch-AH2	L	N	A	F	F	A	G	T	N	D	A	V	G	CPV-2b
Ch-SH	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-JX	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-ZJ	L	N	A	L	F	A	G	A	N	D	T	V	G	CPV-2b
Ch-ZhJ	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b
Ch-HA	L	N	A	F	F	A	G	T	N	D	A	V	G	CPV-2b
Ch-CZ	L	N	A	L	F	A	G	A	N	D	T	V	G	CPV-2b
Ch-SQ	L	N	A	F	Y	A	G	T	N	D	T	V	G	CPV-2b
Ch-ZhJ2	L	N	A	F	F	A	G	T	N	D	T	V	G	CPV-2b

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REFERENCES

Appel MJ, Scott FW, Carmichael LE (1979). Isolation and immunization studies of a canine parvo-like virus from dogs with haemorrhagic enteritis. Vet. Rec. 105(8): 156-159.

Binn LN, Lazar BC, Eddy GA, Kajima M (1970). Recovery and characterization of a minute virus of canines. Infect. Immun. 1(5): 503-508.

Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D, Bozzo G, Elia G, Decaro N, Carmichael L (2001). Evidence for evolution of canine parvovirus type 2c in Italy. J. Gen. Virol. 82(12): 3021-3025.

Decaro N, Martella V, Desario C, Bellacicco A L, Camero M, Manna L, d'Aloja D, Buonavoglia C (2006). First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. J. Vet. Med. B Infect. Dis. Vet. Public Health, 53(10): 468-472.

Decaro N, Desario C, Elia G, Campolo M, Lorusso A, Mari V, Martella V, Buonavoglia C (2007). Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: A clinical and laboratory diagnostic dilemma. Vaccine, 25(7): 1161-1166.

Decaro N, Elia G, Martella V, Desario C, Campolo M, DiTrani L, Tarsitano E, Tempesta M, Buonavoglia C (2005). A real-time PCR assay for rapid detection and quantitation of canine parvovirus type2 DNA in the feces of dogs. Vet. Microbiol. 105: 19-28.

Davies M (2008). Canine parvovirus strains identified from clinically ill dogs in the United Kingdom. Vet. Rec. 163(18): 543-544.

Greenwood NM, Chalmers WSK, Baxendale W, Thompson H (1996). Comparison of isolates of canine parvovirus by monoclonal antibody and restriction enzyme analysis. Vet. Rec. 138 (20): 495-496.

Kong Q, Zhang YM (2003). Isolation and identification of canine parvovirus. Journal of Northwest Science Technology University. 31(3): 54-58 (in chinese).

Mochizuki M, Harasawa R, Nakatani H (1993). Antigenic and genomic variabilities among recently prevalent parvoviruses of canine and feline origin in Japan. Vet. Microbiol. 38(1-2): 1-10.

Parrish CR, Have P, Foreyt WJ, Evermann JF, Senda M, Carmichael LE (1988). The global spread and replacement of canine parvovirus strains. J. Gen. Virol. 69(5): 1111-1116.

Parrish CR, O'Connell PH, Evermann JF, Carmichael LE (1985). Natural variation of canine parvovirus. Science, 230 (4729): 1046-1048.

Parrish CR, Aquadro CF, Strassheim ML, Evermann JF, Sgro JY, Mohammed HO (1991). Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. J. V. 65 (12): 6544-6552

Ruben P, Lourdes F, Valeria R, Leticia M, Ignacio L, Martín H (2007). First detection of canine parvovirus type 2c in South Amercia. Vet. Microbiol. 124 (1-2): 147-152.

Steinel A, Venter EH, Van Vuuren M, Parrish CR, Truyen U (1998). Antigenic and genetic analysis of canine paroviruses in southern Africa. Onderstepoort J. Vet. Res. 65(4): 239-242.

Shackelton LA, Parrish CR, Truyen U, Holmes EC (2005). High rate of viral evolution associated with the emergence of carnivore parvovirus. Proc. Natl. Acad. Sci. USA, 102(2): 379-384.

Truyen U (2006). Evolution of canine parvovirus-a need for new vaccines? Vet. Microbiol. 117(1): 9-13.

Truyen U, Evermann JF, Vieler E, Parrish CR (1996). Evolution of canine parvovirus involved loss and gain of feline host range. Virology, 215(2): 186-189.

Ybañez RR, Vela C, Cortés E, Simarro I, Casal JI (1995). Identification of types of canine parvovirus circulating in Spain. Vet. Rec. 136(7): 174-175.

Nakamura M, Tohya Y, Miyazawa T, Mochizuki M, Phung HT, Nguyen NH, Huynh LM, Nguyen LT, Nguyen PN, Nguyen PV, Nguyen NP, Akashi H (2004). A novel antigenic variant of canine parvovirus from a Vietnamese dog. Arch. Virol. 149(11): 2261-2269.