PREDOMINANCE OF CANINE PARVOVIRUS TYPE *2B* IN DOGS OF ULAANBAATAR CITY

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ABSTRACT

Canine parvovirus is a highly contagious virus that causes fatal disease acute hemorrhagic enteritis and myocarditis in dogs. The aim of this work is to detect canine parvovirus 2 (CPV-2) by standard polymerase chain reaction (PCR). Viral DNA was isolated from faecel samples of 36 puppies with suspicious symptoms for parvovirus infection and used as template in standard PCR. 23 samples were of CPV-2b serotype, 9 samples of CPV-2a serotype but 4 samples were neither 2b and nor 2a. We used two different primer sets, one specific both serotypes CPV-2a and CPV-2b and one specific only for CPV-2b. This allowed us to differentiate serotypes from each other. The further extension of this work will be essential for the epidemiology, viral evolution and phylogenetic studies of the mongolian domestic canine, cats and wild carnivores.

KEY WORDS: canine parvovirus (CPV), bloody diarrhea, hemorrhagic enteritis, myocarditis, polymerase chain reaction (PCR)

INTRODUCTION

Canine parvovirus 2 (CPV-2) is a highly contagious virus, that causes fatal disease, characterized by vomiting and hemorrhagic gastroenteritis in dogs of all age (1), and mycarditis and subsequent heart failure in pups of less than 3 months of age (2). Canine parvovirus 2, so named to differentiate it from CPV-1, the genetically and antigenically distinct minute virus of canines, is a small, nonenveloped, single-stranded DNA virus (1). Canine parvovirus 2, along with *Feline panleukopenia virus* (FPV), Raccoon parvovirus (RPV), and Blue fox parvovirus (BFPV), comprise the Feline parvovirus (FPV) subgroup of the genus *Parvovirus*. CPV-2 emerged in 1978 as the cause of a new disease in dogs throughout the world, when it rapidly spread in domestic dog populations as well as wild dogs with high morbidity (100%) and frequent mortality up to 10% (1, 2).

The main source of infection is the feces of infected dogs containing large numbers of virus particles (109 virus particles/g of faeces) that excreted in the faeces.

Between 1979 and 1981 the original (1978) strain of the virus (CPV-2) had been replaced by a genetically and antigenically variant strain termed CPV-2a (5). The two viruses differ in 5-6 amino acids, which constitute two different neutralizing antigenic sites on the surface of the capsid.

In 1984, a further antigenically variant virus was detected which differ in only a single epitope,

designated as CPV-2b (6).The CPV-2, a non-enveloped virus with an approximate diameter of 20 nm, is a member of the genus Parvovirus of the family Parvoviridae.

MATERIALS AND METHODS

Fecal samples were collected from 36 dogs came to Veterinary hospital (Ulaanbaatar, Sukhbaatar district) between March-June/ 2013 that showing symptoms of fever, diarrhoea or hemorrhagic diarrhoea and vomition, clinically suspected for CPV infections. The faecal samples were collected by rectal disposable swabs, and directly transferred in ice to the laboratory of Veterinary School for strategic studies and scientific researches, stored at (-20°C) until the DNA was extracted. A commercially available inactivated vaccine was used as a positive control of CPV and a stool sample from a healthy dog processed similarly was used as a negative control. The viral DNA was extracted from fecal samples using Genomic DNA extraction kit (Intron Biotech, Korea) according to the manufacturer's protocol.

The PCR was standardized for the primer set CPV-2ab and CPV-2b, as designed under the scientific standards in primer designing with slight modifications. The details of primers are given in Table 1.

The PCR reaction mixture contained 100 μM dNTPs, 10 pmol of each primer, 1X PCR reaction mixture containing 12.5 mM $MgCl_2$ and 2 μL of processed sample as source of template DNA. Amplification was performed in a thermocycler (*Applied Biosystems*). 1 μL of DNA polymerase (1 IU/μL) was added to above reaction mixture after initial denaturation was done at 95°C for 5 min in the thermocycler. The cyclic condition was denaturation at 95°C for 1 min, primer annealing at 55°C for 2 min and extension at 72°C for 0.5 min. The cyclic condition was repeated for 30 times and a final extension at 72°C was given for 10 min. After PCR, amplified products were analyzed on 1.0% agarose gel containing ethidium bromide to a final concentration of 0.5 μg/mL. 10 μL of the amplified product was loaded into the well and run along with 100 bp to 1 Kbp DNA ladder in 1X TBE electrophoresis buffer at 5 volts/cm^2 and the progress of mobility was monitored by migration of dye. At the end of the electrophoresis, the gel was visualized under the UV transilluminator.

Table 1

Details of primer sets

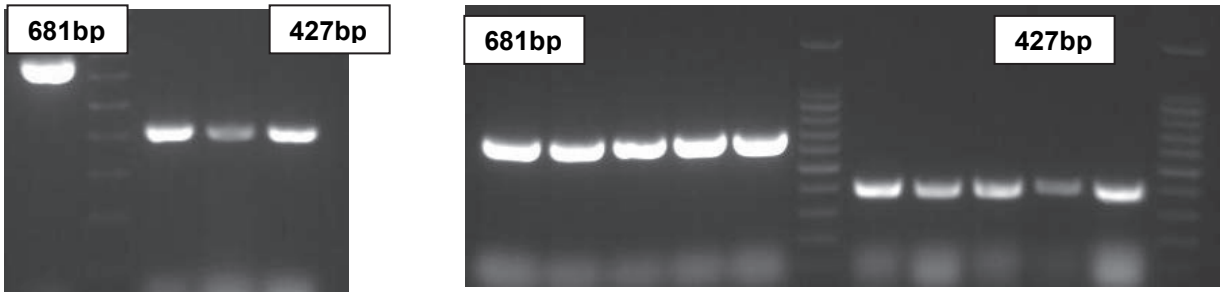
Forward and reverse primers	Primer sequence	Position in genome	Annealing temperature and product size
НПВ-2ab (F)	5'-GAAGAGTGGTTGTAAATAATT-3'	3025-3045	55 C
НПВ-2ab (R)	5'-CCTATATAACCAAAGTTAGTA-3'	3685-3706	681bp
НПВ -2b (F)	5'-CTTTAACCTTCCTGTAACAG-3'	4043-4062	55 C
НПВ -2b (R)	5'-CATAGTTAAATTGGTTATCTA-3'	4449-4470	427bp

RESULTS AND DISCUSSION

In the present study, PCR was carried out on 36 fecal samples collected from CPV suspected dogs and used as template to amplify the VP2 structural gene of CPV genome. Of the 36 fecal samples from suspected cases of CPV infections, 23 were found to be positive by CPV-2b primer set, whereas all of them are amplified by CPV-2ab primer set. The CPV-2ab primer set amplified portion of VP2 gene of both CPV-2a and CPV-2b variants (3025 to 3706 nucleotide position of CPV genomic DNA) to yield a product size of 681bp (Fig.1).

The CPV-2b primer pair amplified specific portion VP2 gene of only CPV-2b (4043 to 4470 nucleotide position of CPV genomic DNA) to yield a product size of 427bp and thereby differentiate between CPV-2a and CPV-2b (Fig. 1). So the results showed that out of 36 samples 23 were of CPV-2b variant, while 9 were CPV-2a strain.

But 4 samples showed an amplicon with neither CPV-2ab nor CPV-2b primers which indicate these samples don't have this serotypes or no CPV.



Picture 1. Picture of PCR amplicons and 100bp standard

Table 2

PCR results, + indicate presence of a PCR band , - indicate absence of a PCR band

sample	CPV-2b	CPV-2ab	sample	CPV-2b	CPV-2ab
1	+	+	19	-	-
2	+	+	20	+	+
3	+	+	21	-	+
4	+	+	22	+	+
5	+	+	23	+	+
6	-	-	24	+	+
7	-	-	25	+	+
8	-	+	26	+	+
9	+	+	27	-	+
10	+	+	28	-	+
11	+	+	29	+	+
12	-	+	30	-	+
13	-	-	31	+	+
14	+	+	32	+	+
15	-	+	33	+	+
16	-	+	34	+	+
17	-	+	35	+	+
18	+	+	36	+	+

Canine parvovirus infections have been emerged as the most important killer disease of pups in recent time as it causes vomiting, myocarditis and hemorrhagic gastroenteritis (9). Although adult dogs show less severe symptoms of gastroenteritis, and dogs serve as a source of infection. Due to its immunosuppressive nature, CPV decreases the animal's ability to fight against infections (10). After emergence of the CPV-2, two more mutants, namely CPV-2a and CPV-2b, have been reported and completely replaced the original strain (CPV2) around the world (4). Decaro (11) identified different variants of CPV circulating in the dog population in Spain. Truyen (12) studied that CPV-2a and CPV-2b have almost completely replaced the original CPV2 in the canine population in Germany. Pereira (8) reported that the predominant strain found in Brazil during 1980 was CPV-2a and CPV-2b during 1990-1995. Wang (13) reported both antigenic types CPV-2a and CPV-2b prevailing in Taiwan. Battilani (14) showed that both antigenic types 2a and 2b co-exist in canines in Italy.

The commercial available rapid diagnostic kits for CPV can't distinguish the virus serotypes but often used for rapid routine diagnosis. Ts. Ariunaa analyzed more than 100 canine faeces with CPV-rapid diagnostic test, 2011 which resulted in high infection rate (15).

This study is the first one carried out CPV-specific PCR in Mongolia, thus, the PCR technique can be adopted to diagnose rapidly, reproducibly and accurately the CPV infection. Further, different antigenic variants of CPV can also be differentiated by employing PCR with different combination of primer sets.

From the present study, it is inferred that CPV-2b is more prevalent in the dog population in Ulaanbaatar city as revealed in PCR based diagnosis. So, necessary measures should be taken to control the disease in dogs by incorporating the indigenous strain of CPV in the preparation of vaccine.

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