Genetic analysis of canine parvovirus vaccine strains in Korea

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(Accepted: August 26, 2009)

Abstract: After the original identification of canine parvovirus (CPV) type 2 (CPV-2) in 1978, new antigenic variants such as CPV-2a, CPV-2b and CPV-2c have become widespread in the most countries. In this study, the genetic analysis of canine parvovirus was investigated in a total of 13 CPV vaccines, which have been licensed in Korea since late 1980s, and a field isolate of CPV from a dog with CPV infection clinical symptom. The partial VP2 gene of CPV was amplified and sequenced from 13 vaccine strains and one field isolate. The results showed that of the 13 vaccine strains, 10 strains belong to the CPV-2, 2 strains to CPV-2b, the remaining and one isolate to CPV-2a type, respectively. Several mutations of amino acids were detected at residues of the critical region of the commercial vaccine strains. These data suggest that new type of vaccines containing CPV-2a or CPV-2b/c type may be required for the better prevention of new CPV infection in dog population in Korea, because CPV-2 contained in most licensed vaccines has been replaced by antigenic variants designated CPV-2a or CPV-2b/c in the worldwide dog population.

Keywords: canine parvovirus, genetics, Korea, phylogeny

Introduction

Infection of canine parvovirus (CPV) is one of the most fatal viral diseases, showing hemorrhagic enteritis, leukopenia, nausea and myocarditis in puppies over the age of 2 months [1]. CPV belongs to a member of the genus Parvovirus of the family Paroviridae along with the feline panleukopenia virus (FPV) and mink enteritis virus (MEV) and contains negative single strand DNA about 5.2 kb in length. Since CPV was identified in late 1970s in dogs, new CPV has been spread out worldwide and named as CPV-2 type to distinguish it from CPV prototype (CPV-1). An antigenic variant of CPV-2 type was identified by using specific monoclonal antibodies [18]. In the early 1980s, two CPV-2 variants characterizing that amino acid residue at positions 297 and 426 of VP2 gene changed from serine to alanine and from asparagine to aspartic acid respectively emerged and were named as CPV-2a and CPV-2b [2, 9, 14, 17]. Currently, CPV-2a is the main genotype circulating in the dog population in India, Germany and Korea, while CPV-2b is detected commonly in USA, Taiwan and Japan [6, 10, 14, 16]. Recently, a new antigenic type (CPV-2c) was reported in several countries including Italy, Spain, Germany, Vietnam, Japan and USA [6, 8, 11]. The CPV-2c was first detected as a genetic variant of CPV-2b and of which amino acid residue at position 426 of VP2 gene changed from aspartic acid to glutamic acid in Italian dogs [3]. Many dogs infected with CPV-2c had mucoid yellow diarrhea, hemorrhagic diarrhea, leucopenia, and lymphopenia [5, 11].

The non-enveloped capsid of CPV is composed of VP1, VP2 and VP3, respectively. Among these proteins, VP2 is the main capsid protein and induces protective antibody. Since substitutions of a few amino acids in VP2 are liable for the antigenic feature and the control of host range, the deduced amino acid sequence of VP2 may make us to differentiate between variants such as CPV-2 and CPV-2a/b/c. At this stage, CPV subtyping in commercial CPV vaccine strains has not been identified. Thus, we investigated nucleotide sequence analysis of important partial VP2 gene of CPVs in thirteen commercial vaccines available in
Korea as well as in a field isolate.

**Materials and Methods**

**Virus isolation**

Fecal samples were collected from puppies (N = 7) aged 70 days in Chungju province of Korea in 2009. The dogs showed the typical symptoms of CPV infection with nausea and hemorrhagic diarrhea and four of them died. For the virus isolation, the fecal samples were checked with one step CPV antigen test kit (Anigen, Korea) for CPV. Two samples showing strong positive reaction were filtered using 0.45 μm filter and inoculated into A72 cells (A72, derived from canine fibroblast cell) grown in alpha minimum essential medium with 10% fetal bovine serum. The cells were incubated at CO₂ incubator for 7 days and cytopathic effects (CPE) were observed in the cells. The supernatant of the cells was screened by hemagglutination test and the cells fixed with cold aceton were tested by indirect fluorescent assay (IFA) using CPV specific monoclonal antibody (Jeno Biotech, Korea). The isolate was propagated on A72 cells and the viral titer was measured by IFA.

**Vaccines**

The commercial CPV vaccines produced by Korean animal vaccine companies and used in this study were as follows: Greencross DHPPL (Green cross, Korea); Daesung DHPPL (Daesung, Korea); Canishot DHPPL (ChoongAng, Korea); Komipherm DHPPL (Komipherm, Korea); Himn vac DHPPL (KoreaBNP, Korea). The eight CPV vaccines imported from several countries such as USA, France, Czech and Japan were as follows: Bayovac DHPPL (Bioveta, Czech); Canine-9 DHPPL (Kyōto biken, Japan); Canvac DHPPL (Dyntec, Czech); Duramune Max P (Fort Dodge, USA); Eurican DHPPL2 (Meril, France); Nobivac DHPPiP (Intervet, Netherlands); Quantum dog DA2PPv (Schering-Plough, USA); Vanguard plus (Pfizer, USA). Twelve commercial vaccine strains employed in this study contained modified live virus. The other Daesung DHPPL was an inactivated virus vaccine.

**DNA extraction and PCR condition**

Viral DNA was extracted from thirteen commercial CPV vaccines using a DNA extraction kit (Bioneer, Korea) according to the manufacturer’s instructions. A PCR using specific primers (Table 1), which amplify an important portion of VP2 region of CPV, was carried out for the gene amplification. The PCR was performed in PCR premix (Bioneer, Korea) containing 5 μL of denatured DNA, 1 μL of each primer (30 pmol) and 43 μL of distilled water, for a 50 μL final volume. The cycling profile was as follows: denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 1 min; and a final extension at 72°C for 5 min. PCR products were visualized using electrophoresis on 1.5% agarose gel containing ethidium bromide. Purified PCR products were ligated with the pGEM-T easy vector (Promega, USA).

**Sequencing and phylogenetic analysis**

After cloning the VP2 gene of CPV, sequencing reactions of the purified recombinant plasmid DNA were performed with ABI PRISM 7900/ Big Dye Terminator Cycle Sequencing Kit (Perkin-Elmer, USA). The phylogenetic analysis was carried out on nucleotide sequence data of 793 base pair in VP2 gene from CPV vaccine and reference CPV strains. Phylogenetic tree and homology analysis were obtained using neighbor-joining methods of the DNASTar software program (DNASTar, USA) and DNASIS software program (Hitachi, Japan).

**Results**

**Identification of isolate**

Of the two fecal samples inoculated into A72 cells, one was selected. Following second blind passage of the isolate, the cells infected with the isolate were checked by IFA test. CPV-specific fluorescence appeared in the nucleus of the infected cells (data not

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**Table 1.** List of the oligonucleotide primers used for polymerase chain reaction against canine parvovirus

<table>
<thead>
<tr>
<th>Primer designated</th>
<th>Oligonucleotide sequence (5'-3')</th>
<th>Target gene</th>
<th>Size of amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPVDF</td>
<td>GAG CAT TGG GCT TAC CAC CA</td>
<td>VP2</td>
<td>793 bp</td>
</tr>
<tr>
<td>CPVDR</td>
<td>GGA TTC CAA GFA TGA GAG GC</td>
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