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A novel antigenic variant of *Canine parvovirus* from a Vietnamese dog

Brief Report

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Summary. Nine isolates of *Canine parvovirus* (CPV) were obtained from Vietnamese dogs and cats. One canine isolate showed a unique antigenic property which indicates a novel antigenic variant of CPV-2b when examined with hemagglutination inhibition tests using our monoclonal antibodies, 21C3 and 19D7, which were recently developed. This isolate had an amino acid substitution of residue 426, Asp to Glu, and the same substitution has recently been found in CPV from Italian dogs. This study first showed that such substitution caused an antigenic difference demonstrable by monoclonal antibodies and that a similar evolution may have occurred in CPV in Vietnam.

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Canine parvovirus (CPV) is a small, non-enveloped virus that possesses single-stranded DNA. The CPV capsid is composed of two structural proteins, VP1 and

VP2, which are translated from alternatively spliced mRNAs [20]. VP2 is a main component of capsid and amino acid substitutions in VP2 cause antigenic changes of CPV [18, 21].

As CPV has shown several antigenic and host range changes since its emergence, it is thought to be an interesting model of viral evolution. CPV or CPV-2 suddenly emerged in dogs in the late 1970's and rapidly spread worldwide [6]. After the emergence of CPV-2, two new antigenic variants, designated CPV-2a and CPV-2b, have arisen consecutively [17, 19]. These two variants have almost completely replaced CPV-2 and have been distributed worldwide [7, 8, 17, 22]. Five conserved amino acid differences in VP2 are observed between CPV-2 and CPV-2a [19]. CPV-2b has two additional substitutions in VP2 of residue 426 Asn (Asn-426) to Asp and Ile-555 to Val [19]. Asp-426 is an important substitution that distinguishes CPV-2b from the other antigenic types, including the related feline panleukopenia virus (FPLV) and mink enteritis virus (MEV) [19].

Recently, another antigenic change was observed. We isolated CPV-2a- and CPV-2b-related viruses from domestic and leopard cats in Vietnam [10, 11, 13]. Three isolates from leopard cats were shown to be a new antigenic type by the absence of reactivity with several monoclonal antibodies (MAbs) [11]. They were designated CPV-2c and further divided into CPV-2c(a) and CPV-2c(b) by variation of residue 426, which distinguishes CPV-2b from CPV-2a. CPV-2c viruses have the substitution of Gly-300 to Asp, which is thought to be responsible for the characteristic antigenicity of them.

The emergence of CPV-2c indicates that CPV is still evolving in Vietnam. Therefore, it is important to research on field isolates in this area. In this study, we isolated CPV from rectal swab samples of dogs and cats collected in Vietnam and determined genetic and antigenic properties of the isolates. Interestingly, one canine isolate showed a unique antigenic property which indicates a novel antigenic variant of CPV-2b.

Eighty-six rectal swab samples from domestic dogs and 40 rectal swab samples from domestic cats were collected in Ho Chi Minh City and Hanoi in Vietnam in 2002. Samples were suspended in Dulbecco's modified Eagle's medium and filtrated through Millipore filter (pore size 0.22 μm). Samples were inoculated onto Crandell feline kidney (CRFK) cells, Madin-Darby canine kidney (MDCK) cells, or the thymic lymphoma cell line 3201, followed by blind passages one to three times until cytopathic effects (CPEs) were observed. The isolates were propagated in CRFK or MDCK cells and used for antigenic and sequence analyses.

Hemagglutination inhibition (HI) tests were performed for antigenic analysis of Vietnamese isolates as previously described [12]. The HI titer was determined as the reciprocal of the highest dilution that completely inhibited viral hemagglutination. MAbs A3B10, B6D5, B4E1, A4E3, C1D1, B4A2 [16], and P2-215 [9] were previously reported elsewhere. MAbs 2G5, 21C3, 19D7, and 20G4 were generated in our previous work [15]. The viruses used as reference strains in the antigenic analysis were FPLV TU-1 [14], MEV-2 M-1 [11], CPV-2 CPV-b

[16] and Cp49 [2], CPV-2a CPV-31 [17] and 97-003 [11], CPV-2b CPV-39 [17] and 97-008 [11], CPV-2c(a) V139 [10], and CPV-2c(b) V203 [10]. In addition to Vietnamese isolates, recent isolates from Japanese dogs and cats [unpublished] were also analyzed.

For sequence analysis, the VP2 gene was amplified by PCR with the primer sets reported previously [11]. After the amplified DNA fragments were purified from the agarose gel, they were used for the sequencing reaction with a Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.). The samples were resolved on an automated DNA sequencer (model 3100-Avant; Applied Biosystems). The sequences of the VP2 genes of the previously published isolates were obtained from the DDBJ database. Phylogenetic analysis was carried out using GENETYX-MAC ver 12.0 (Genetyx Co., Tokyo, Japan). Pair-wise genetic distances were calculated by using the Jukes-Cantor method. Phylogenetic trees were constructed by using the neighbor-joining method and bootstrap analysis was performed with 1000 trials.

Eight isolates from dogs and one isolate from a cat were obtained (Table 1). HNI-3-4 was isolated and propagated in MDCK cells. HCM-8 and HNI-4-1 were isolated in 3201 cells and propagated in CRFK cells. The other six isolates were obtained and propagated in CRFK cells. Seven of the eight isolates obtained from Vietnamese dogs were classified into CPV-2b type by HI assay (Table 1), suggesting that CPV-2b viruses were predominant in the dog population in this area. CPV-2b viruses were isolated from Vietnamese cats in our previous study [10, 11, 13] and also in this study (HNI-1-18) (Table 1). The isolates from Vietnamese dogs were not phylogenetically separated from those from Vietnamese cats (Fig. 1). These data suggest that transmission of CPV-2b between dogs and cats has occurred in Vietnam.

The sequence analysis of the VP2 gene confirmed the classification of these isolates and revealed some amino acid substitutions. HCM-8 had the substitutions of Pro-13 to Ser and Thr-265 to Lys (Table 2). A similar substitution, Thr-265 to Pro, was observed in Italian isolates [3, 4]. HCM-18 and HNI-2-13 had a common substitution of Phe-267 to Tyr (Table 2). Because residues 265 and 267 are not exposed on the capsid surface [1, 25], substitutions of these residues may not affect antigenicity of the viruses. All isolates have the common substitution of Ser-297 to Ala (Table 2), which has been observed in most recent CPV-2a- and CPV-2b-related isolates in Asia [11], Italy [3–5], and Germany [23].

Usually, CPV-2b has been distinguished from CPV-2a by a single MAb B4A2 [19] which recognizes CPV-2a but not CPV-2b. In addition to the MAb, we recently developed an MAb, 21C3, which distinguishes CPV-2b from CPV-2a [15], recognizing CPV-2b but not CPV-2a. By using these MAbs, we can clearly distinguish CPV-2b from CPV-2a. Both MAbs recognize the antigenic site A [15, 21], which is located on the tip of the threefold spike in the capsid structure [24]. This antigenic site A contains the residue 426, which causes antigenic changes of CPV-2a to CPV-2b by substitution of the residue from Asn to Asp. In this study, one isolate, HNI-4-1, showed a unique antigenic property different from

Table 1. HI reactivity of MAbs against Vietnamese isolates

Isolates	Origin	Reactivity with the following MAbs											Antigenic type
		A3B10	B6D5	B4E1	A4E3	C1D1	B4A2	P2-215	2G5	21C3	19D7	20G4	
HCM-6	Dog	NT[a]	NT	NT	NT	NT	NT	NT	32,000	6,400	128,000	3,200	CPV-2b
HCM-8	Dog	12,800	640	10	128	1,280	<4	<1,000	64,000	6,400	128,000	3,200	CPV-2b
HCM-18	Dog	12,800	640	10	128	1,280	<4	<1,000	32,000	6,400	64,000	1,600	CPV-2b
HCM-23	Dog	12,800	640	10	128	1,280	<4	<1,000	64,000	6,400	128,000	3,200	CPV-2b
HNI-2-13	Dog	NT	NT	NT	NT	NT	NT	NT	32,000	6,400	64,000	1,600	CPV-2b
HNI-3-4	Dog	12,800	640	10	128	1,280	<4	<1,000	64,000	6,400	128,000	3,200	CPV-2b
HNI-3-11	Dog	NT	NT	NT	NT	NT	NT	NT	64,000	12,800	128,000	3,200	CPV-2b
HNI-4-1	Dog	12,800	640	10	64	640	<4	<1,000	32,000	400	8,000	3,200	CPV-2b variant
HNI-1-18	Cat	NT	NT	NT	NT	NT	NT	NT	64,000	12,800	128,000	3,200	CPV-2b
FPLV		6,400	640	1,280	<4	<10	32	16,000	<1,000	<100	32,000	<100	Reference
MEV		NT	NT	NT	NT	NT	NT	NT	<1,000	<100	64,000	<100	strains[b]
CPV-2		12,800	1,280	1,280	64	<10	16	<1,000	<1,000	<100	32,000	<100	
CPV-2a		6,400	1,280	20	128	640	16	<1,000	64,000	<100	32,000	3,200	
CPV-2b		6,400	640	10	64	1,280	<4	<1,000	64,000	3,200	128,000	1,600	
CPV-2c(a)		NT	NT	NT	NT	NT	NT	NT	128,000	<100	32,000	512,000	
CPV-2c(b)		NT	NT	NT	NT	NT	NT	NT	64,000	3,200	128,000	128,000	

[a] *NT* not tested

[b] FPLV TU-1, MEV M-1, CPV-2 CPV-b, Cp49, CPV-2a CPV-31, 97-003, CPV-2b CPV-39, 97-008, CPV-2c(a) V139 and CPV-2c(b) V203 were used as reference strains

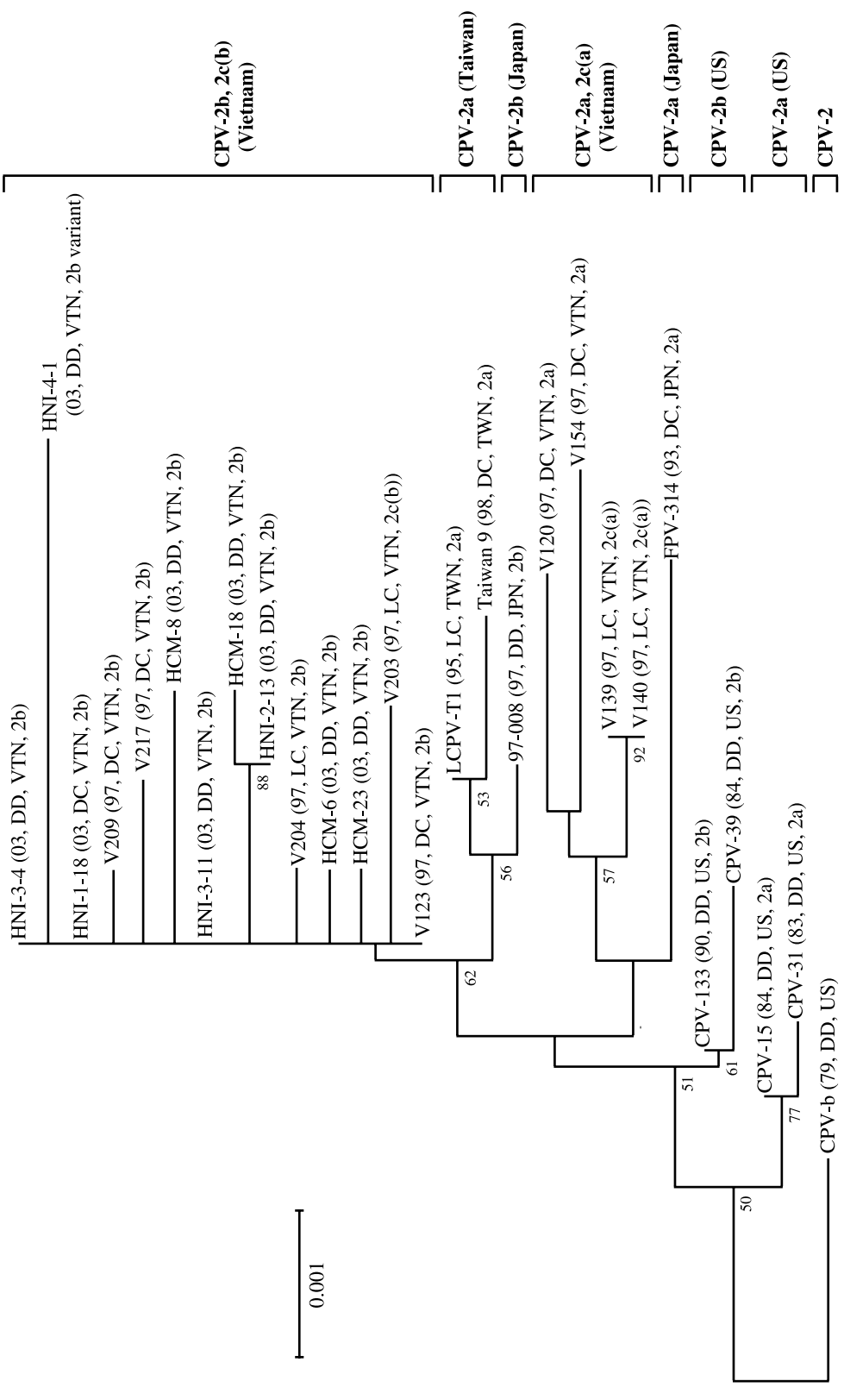

Fig. 1. Phylogenetic analysis based on the complete VP2 nucleotide sequences of CPV isolates. Sequences of the VP2 gene of the previously published isolates were obtained from the DDBJ database; CPV-2 CPV-b (Accession number: M38245), CPV-2a CPV-31 (M24000), CPV-15 (M24003), FPV-314 (D78585), Taiwan9 (AB054213), LCPV-T1 (AB054214), V120 (AB054215) and V154 (AB054217), CPV-2b CPV-39 (M74849), CPV-133 (M74852), 97-008 (AB115504), V123 (AB054218), V209 (AB054219), V217 (AB054220) and V204 (AB054221), CPV-2c(a) V139 (AB054222) and V140 (AB054223), CPV-2c(b) V203 (AB054224). The trees were constructed by using the neighbor-joining program and bootstrap values were indicated. The number in parentheses means the year when the strain was isolated. *DD*, domestic dog; *DC*, domestic cat; *LC*, leopard cat; *JPN*, Japan; *VTN*, Vietnam; *TWN*, Taiwan

Table 2. Amino acid variation in VP2 protein

Isolates	Accession no.	Amino acid at position												FPV-type
		13	80	87	93	265	267	297	300	305	323	426	555	
HCM-6	AB120720	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
HCM-8	AB120721	Ser	Arg	Leu	Asn	Lys	Phe	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
HCM-18	AB120722	Pro	Arg	Leu	Asn	Thr	Tyr	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
HCM-23	AB120723	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
HNI-2-13	AB120724	Pro	Arg	Leu	Asn	Thr	Tyr	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
HNI-3-4	AB120725	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
HNI-3-11	AB120726	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
HNI-4-1	AB120727	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Gly	Tyr	Asn	Glu	Val	CPV-2b variant
HNI-1-18	AB120728	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Gly	Tyr	Asn	Asp	Val	CPV-2b
FPV-b CU4[a]	M24004	Pro	Lys	Met	Lys	Thr	Phe	Ser	Ala	Asp	Asp	Asn	Val	FPLV
MEV-d Johnson	M24001	Pro	Lys	Met	Lys	Thr	Phe	Ser	Val	Asp	Asp	Asn	Val	MEV
CPV-b	M38245	Pro	Arg	Met	Asn	Thr	Phe	Ser	Ala	Asp	Asn	Asn	Val	CPV-2
CPV-31	M24000	Pro	Arg	Leu	Asn	Thr	Phe	Ser	Gly	Tyr	Asn	Asn	Ile	CPV-2a
CPV-39	M74849	Pro	Arg	Leu	Asn	Thr	Phe	Ser	Gly	Tyr	Asn	Asp	Val	CPV-2b
V139	AB054222	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Asp	Tyr	Asn	Asn	Val	CPV-2c(a)
V203	AB054224	Pro	Arg	Leu	Asn	Thr	Phe	Ala	Asp	Tyr	Asn	Asp	Val	CPV-2c(b)

[a]FPLV FPV-b CU4, MEV MEV-d Johnson, CPV-2 CPV-b, CPV-2a CPV-31, CPV-2b CPV-39, CPV-2c(a) V139, and CPV-2c(b) V203 were used as reference strains

Table 3. Antigenic characterization of recent parvovirus isolates from Japanese cats and dogs by HI tests

Year of isolation	Isolates				
	FPLV	CPV-2	CPV-2a	CPV-2b	CPV-2b variant
2001	494[a], 495	ND[b]	ND	OM-4, 1074D, 1080D	ND
2002	501, 502, 2045C	ND	2054D	OM-9, OM-10	ND
2003	ND	ND	ND	03-003, MD03-007, MD03-008, MD03-021	ND

[a]Isolates 494, 495, 501, 502, and 2045C were obtained from cats and the other isolates were obtained from dogs

[b]*ND* not detected

typical CPV-2b strains by HI assay. This isolate had no reactivity against MAb B4A2, showing the CPV-2b phenotype (Table 1). However, the reactivity of this isolate against MAb 21C3 was 8 to 32 times lower than that of the other CPV-2b isolates (Table 1). Furthermore, its reactivity against MAb 19D7 [15] was also 8 to 16 times lower than that of the other CPV-2b isolates (Table 1). MAb 19D7 reacts with all antigenic types and recognizes an epitope in the same antigenic site as MAb 21C3 does [15]. Sequence analysis revealed that the residue 426 of the isolate is Glu (Table 2) and suggested that this unique amino acid at the position is responsible for the characteristic antigenicity of the isolate. In addition, this variant is phylogenetically located in the cluster of Vietnamese CPV-2b isolates (Fig. 1). These results indicate that HNI-4-1 is a novel antigenic variant of CPV-2b. Buonavoglia et al. [5] have also reported the Glu-426 variants, strains 56/00 and 136/00, in Italian isolates. They detected these variants by RFLP assay but not by HI assay because CPV-2b-specific MAb (such as MAb 21C3) was not available [5]. Our study first showed that such substitution caused an antigenic difference which could be demonstrated by MAbs.

Glu-426 variants have been overlooked in antigenic analysis using the usual MAbs [5]. Therefore, it is important to re-examine CPV isolates classified into CPV-2b using MAb 21C3. Although we could not detect the Glu-426 variant in recent Japanese CPV-2b isolates (Table 3), a similar evolution may have occurred in CPV-2b. The evidence that Glu-426 variants are independently detected in the distinct areas, Vietnam and Italy, supports this assumption. Further monitoring of field isolates will provide us important information for research on the evolution of CPV.

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