Predominance of canine parvovirus 2b in Japan: An epidemiological study during 2014-2019.

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Research Article

Keywords: pathogenicity, vaccines, vaccination, mutations, Canine parvovirus-2 (CPV-2)

Posted Date: April 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-424846/v1>

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Version of Record: A version of this preprint was published at Archives of Virology on August 13th, 2021. See the published version at <https://doi.org/10.1007/s00705-021-05200-0>.

Abstract

Canine parvovirus-2 (CPV-2) is an important pathogen of domestic dogs and wild canids. In Japan, CPV-2 infection remains one of the most common infection diseases among dogs. We analyzed samples collected between 2014 and 2019 to determine the antigenic variants of CPV-2 among dogs in Japan. Our results demonstrated that the CPV-2b variant was predominant. The CPV-2c variant was not found among our samples. Our findings demonstrate that the distribution of CPV-2 antigenic variants in Japan was more similar to the distribution in Australia compared with that of neighboring countries in Asia.

Introduction

Canine parvovirus-2 (CPV-2) is an important pathogen of domestic dogs and wild canids. CPV-2 is small non-enveloped virus with a single-stranded DNA genome, and classified into the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus*, species *Carnivore protoparvovirus-1* [1]. The CPV-2 genome encodes for 4 proteins: NS1, NS2, VP1, and VP2. NS1 and NS2 are non-structural proteins that regulate replication of the viral genome, while VP1 and VP2 are structural proteins that constitute capsids of the virion [2, 3]. Amino acid residue in VP2 defines the antigenic variants of CPV-2 [3]. CPV-2 infection is characterized by gastroenteritis-like symptoms such as fever, anorexia, diarrhea, and vomiting [4]. Unvaccinated adult dogs usually have subclinical infection [5].

CPV-2 was identified in the late 1970s in dogs [6]. CPV-2 is genetically similar to other carnivore parvoviruses, and presumably derived from feline panleukopenia virus (FPLV) [7]. Within a few years after the outbreak of CPV-2 infection across the United States and Australia in 1978, CPV-2 infection was identified in dogs across a number of countries. In the 1980s, two antigenic variants of CPV-2, namely CPV-2a and CPV-2b, emerged [8]. In 2000, a third variant, CPV-2c, was detected in dogs in Italy [9, 10]. These three antigenic variants have a different amino acid in residue 426 of VP2 protein (CPV-2a: N, CPV-2b: D, CPV-2c: E) [8, 10, 11]. Epidemiological studies over the past 20 years have identified these three antigenic variants in the dog population. However, the first CPV-2 (original CPV-2) has virtually disappeared in the dog population. Studies suggest that there is no difference among the antigenic variants in terms of pathogenicity [12]. However, the licensed CPV2 vaccine is thought to be less effective in protecting dogs against CPV-2c [13].

An epidemiological study by Soma *et al.* [14] demonstrated that CPV-2b was the dominant antigenic variant among dogs in Japan between 2009 and 2011. However, a recent study demonstrated that other antigenic variants are more common in other Asian countries that are geographically close to Japan. For example, the dominant antigenic variant among dogs in Taiwan has shifted from CPV-2b to CPV-2c [15, 16]. Similarly, an epidemiological study in Laos and Vietnam demonstrated that over 90% of infected dogs had the CPV-2c variant [17, 18]. CPV-2c was identified in various countries in Asia except in Japan [15–22]. Collectively, these studies suggest that the composition of antigenic variants in Japan is rare compared to other neighboring countries. However, the data about CPV-2 antigenic variants among dogs in Japan has not been updated in the last 10 years. Therefore, the distribution of CPV-2 antigenic variants in Japan may have shifted in recent years.

In the present study, we analyzed samples collected between 2014 and 2019 to determine the antigenic variants of CPV-2 among dogs in Japan.

Materials And Methods

Samples

Fecal samples were collected from dogs with diarrhea symptoms between 2014 and 2019. These samples were sent to Marupi Lifetech Co., Ltd., (Osaka, Japan) by veterinary clinics in Japan.

Detection of CPV-2 in samples

Viral DNA was extracted from the samples using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. PCR to diagnose CPV-2 infection was performed as described by Senda *et al.* [23] using AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific, Fostercity, CA, U.S.A.). The sequences of the primer pair (Pabs-Pabas) are presented in Table.1.

Sequencing and Phylogenetic analysis of VP2

The DNA that was extracted as described above was used to determine the nucleotide sequences of the VP2 gene. The full length VP2 gene sequence was generated using four overlapping PCR amplicons using the sets of primers. The sequences of the primer pairs are

presented in Table 1. The reaction conditions for PCR were set according to the published protocols for each primer. The reaction conditions for the primer pairs 2304F-3148R were as follows: DNA was denatured at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 1 min, primer annealing at 52°C for 1 min, and 72°C for 1 min with the final extension at 72°C for 10 min. Nucleotide sequences were determined by a commercial service (Fasmac Co., Ltd., Kanagawa, Japan). Multiple sequence alignment of the translated amino acid sequences was performed using MAFFT (version 7.475). The aligned VP2 sequences were used for construction of a phylogenetic tree using the maximum likelihood method based on the Jones-Taylor-Thornton (JTT) model in MEGA-X [25].

Results

PCR was performed to detect the CPV-2 gene in fecal samples from dogs with diarrhea symptoms that were collected by Marupi Lifetech Co., Ltd., (Ikeda, Japan) between 2014 and 2019. The Pabs-Pabas primer pair was used for PCR to specifically identify the VP2 gene as it represents the CPV-2 antigenic variant. A total of 206 samples were provided to the study team. The following samples were excluded from the study: 1) 43 samples in which the CPV2 gene was not detected using the F4-R4 and 555F-555R primer pairs (antigenic variant strain and vaccine strain were indistinguishable), and 2) 5 samples that had the vaccine strain of CPV2 based on its amino acid sequence (only the original CPV-2 is used as vaccine in Japan). Thus, 158 samples were used to determine the antigenic variants of CPV-2 based on residue 426 of VP2. Our results demonstrated that the sample consisted of 12 (7.6 %) CPV-2a, 146 (92.4 %) CPV-2b, and 0 (0.0 %) CPV-2c. Table S1 summarizes the information of all 158 samples with GenBank accession numbers.

Among the 12 strains of CPV-2a, 9 strains (dominant CPV-2a) had the following for VP2: L⁸⁷, T¹⁰¹, A²⁹⁷, G³⁰⁰, Y³⁰⁵, and I³²⁴/Y³²⁴ (Table 2). In comparison to dominant CPV-2a, VP2 of CPV2-82-VP2 and CPV2-83-VP2 had shifted from N²⁹⁷ to K²⁹⁷, P⁴³⁵ to A⁴³⁵, and Y⁵⁷³ to C⁵⁷³. Furthermore, residue 297 of VP2 was serine in CPV2-7-VP2 compared with alanine in dominant CPV-2a.

Among the 146 strains of CPV-2b, 116 strains (dominant CPV-2b) had the following for VP2: L⁸⁷, T¹⁰¹, A²⁹⁷, G³⁰⁰, Y³⁰⁵, and Y³²⁴ (Table 2). Other CPV-2b strains had different amino acid sequences compared with dominant CPV-2b. In the 18 CPV-2b strains, several residues had phenylalanine instead of serine. Furthermore, 4 strains (CPV2-94-VP2 to CPV2-97-VP2) had amino acid changes (N³²¹→K³²¹, Y⁵⁷⁰→E⁵⁷⁰, and A⁵⁷³→R⁵⁷³) that were similar or the same as the CPV-2b strain contained in CPV-2 vaccines not available in Japan.

Phylogenetic analysis of the full VP2 gene revealed that CPV-2a can be categorized as either the East-Asian CPV-2a isolate or European/American CPV-2a isolate, except for 3 strains (CPV2-7-VP2, CPV2-82-VP2, and CPV2-83-VP2) (Figs. 1 and S1). CPV2-7-VP2 could not be categorized into any of the groups. CPV2-82-VP2 and CPV2-83-VP2 were related to 4 strains of CPV-2b that are similar to the vaccine strain. All of the CPV-2bs detected in Japan were categorized in one major group.

Additionally, Japan was divided into two regions (Western and Eastern Japan) for the purpose of the analysis to determine the proportion of the antigenic variants in the two regions (Table 3). In both regions, over 90% of all samples consisted of CPV-2b. The age, sex, and vaccination status were not associated with the antigenic variant.

Discussion

CPV-2 infection is very common in dogs throughout the world. CPV-2 is categorized into 3 antigenic variants: CPV-2a, CPV-2b, and CPV-2c. These variants have different mutation frequencies and are found across the world [8–10]. The genomic substitution rate of CPV-2 is approximately 1×10^{-4} substitutions per site per year [26]. Thus, although CPV-2 is a DNA virus, the rate at which variants are produced is similar to an RNA virus. Continued mutations of the CPV-2 genome may result in the emergence of a CPV-2 antigenic variant for which the current vaccine is no longer effective. Currently, none of the known CPV-2 antigenic variants has a negative impact on the vaccine efficacy. However, given the genetic diversity of CPV-2, it is important to continue tracking the presence of CPV-2 antigenic variants in dogs.

A number of countries have performed phylogenetic analysis of CPV-2 based on the complete sequence of VP2. VP2 is the major structural protein that determines the antigenicity of CPV-2 [9, 27–29]. In Japan, phylogenetic analysis of CPV-2 based on the complete sequence of VP2 has not been performed for over 10 years [30]. In addition, there is little information available on the complete

sequence of VP2 in CPV-2 identified in Japan. We analyzed the complete sequence of VP2 to provide information about CPV-2 antigenic variants found in Japan.

Our phylogenetic analysis showed that 2 of CPV-2a and 4 of CPV-2b formed the same cluster. In other words, we demonstrated that the two antigenic variants were related to one another. Similar findings have been reported in previous studies [31]. This suggests that the classification based on the difference in 426aa of VP2 may not be accurate for CPV-2. Studies have also identified this limitation. Thus, additional studies are needed to examine the accuracy of the CPV-2 antigenic variant classification [32, 33]. In addition, the amino acid sequence for VP2 in the 4 CPV-2b that were found in the cluster was similar to the sequence found in CPV-2b vaccines used in Europe, and the United States. In Australia and the United States, the VP2 amino acid sequence of CPV-2 found in clinical samples from dogs was shown to be identical to the CPV-2b vaccine strain [32]. To our knowledge, the CPV-2 vaccine based on the CPV-2b strain was available in Japan until February 2017. However, all samples in which the CPV-2b vaccine strain-like virus was detected were collected in March 2018. Based on the information shown in Table S1, these samples likely came from pups having the same mother. However, it remains unclear as to why the CPV-2b vaccine strain-like virus was identified from these pups. Future studies are needed to examine if a similar phenomenon occurs in other samples.

Our sequence analysis demonstrated that CPV-2b was the dominant variant in Japan. Among 146 strain of CPV-2b, 116 strain had the same complete sequence for VP2. CPV-2c strains are currently circulating in East and South-East Asian countries [16–22]. However, we demonstrated that none of the dogs in Japan was infected with CPV-2c. To our knowledge, among other Asian countries that conduct regular epidemiological studies of CPV-2, Japan remains the only country in which CPV-2c has not been detected. This could be attributed to various possible factors: 1) Import of CPV-2c-infected dogs to Japan is geographically challenging as Japan is isolated from countries where CPV-2c is prevalent, and 2) it is unlikely that dogs that enter Japan are infected with CPV-2c as they are strongly recommended to be vaccinated. Interestingly, CPV-2c has not been detected in New Zealand, which is an island country like Japan [34]. CPV-2c has been identified in wild carnivores such as leopard cats and the Asian palm civet [35, 36]. Thus, it is possible that dogs in countries where CPV-2c is prevalent may frequently come in contact with these wild carnivores. The CPV-2 vaccine efficacy tends to be lower against CPV-2c compared with other antigenic variants. Thus, epidemiological studies should be conducted regularly to ensure that CPV-2c outbreaks do not occur in Japan.

Our findings, as well as those of the previous study by Soma *et al.* [14], indicate that the dominant antigenic variant of CPV-2 in Japan has been CPV-2b for approximately 20 years since 2000. This is a rare phenomenon compared to other countries. Furthermore, VP2 of the dominant CPV-2b identified in the present study had the same amino acid sequence as CPV-2b identified in 2003 in Japan (AB128923; mistakenly registered as “CPV-2a” in Genbank). This suggests that the dominant CPV-2b in Japan underwent little to no antigenic drift for over 17 years. This phenomenon indicates that the vaccine used in Japan, which is based on the original CPV-2 sequence, may not be effective against dominant CPV-2b. Thus, it is important to determine whether dogs that have been vaccinated for CPV-2 have sufficient neutralizing antibodies against dominant CPV-2b, and consider the presence of maternally-derived antibodies at the time of vaccination. Recent studies in Australia indicate that the dominant antigenic variant is shifting from CPV-2a to CPV-2b [32]. While it remains unclear whether CPV-2b becomes dominant, it is important to continue monitoring the data from Australia.

In the present study, we analyzed samples collected from 2014 to 2019 in Japan and demonstrated that CPV-2b was the dominant antigenic variant. CPV-2 infections remain one of the most common infections among dogs in Japan. In order to eliminate CPV-2 infections, a number of steps need to be taken including improved vaccination, development of an appropriate vaccination protocol, and appropriate management of dogs. Moreover, in order to prevent CPV-2c outbreaks in Japan, it is important to ensure that dogs and cats that are imported to Japan have received vaccination against CPV-2 and to identify possible CPV-2 infections among wild carnivores. Epidemiological studies are important and should be performed regularly to prevent novel CPV-2 epidemics.

Declarations

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

This research did not use human participants or other animals.

References

1. Cotmore SF, Agbandje-McKenna M, Canuti M, Chiorini JA, Eis-Hubinger AM, Hughes J, Mietzsch M, Modha S, Ogliastro M, Penzes JJ, Pintel D, Qiu J, Soderlund-Venermo M, Tattersall P, Tijssen P (2019) ICTV virus taxonomy profile: Parvoviridae. *J Gen Virol* 100:367–368
2. Tu M, Liu F, Chen S, Wang M, Cheng A (2015) Role of capsid proteins in parvoviruses infection. *Virol J* 12:114. 10.1186/s12985-015-0344-y. doi
3. Li G, Ji S, Zhai X, Zhang Y, Liu J, Zhu M, Zhou J, Su S (2017) Evolutionary and genetic analysis of the VP2 gene of canine parvovirus. *BMC Genom* 18:534. doi:10.1186/s12864-017-3935-8
4. Goddard A, Leisewitz AL (2010) Canine parvovirus. *Vet Clin North Am Small Anim Pract* 40:1041–1053
5. Freisl M, Speck S, Truyen U, Reese S, Proksch AL, Hartmann K (2017) Faecal shedding of canine parvovirus after modified-live vaccination in healthy adult dogs. *Vet J* 219:15–21
6. Appel MJ, Scott FW, Carmichael LE (1979) Isolation and immunisation studies of a canine parvovirus-like virus from dogs with haemorrhagic enteritis. *Vet Rec* 105:156–159
7. Truyen U, Evermann JF, Vieler E, Parrish CR (1996) Evolution of canine parvovirus involved loss and gain of feline host range. *Virology* 215:186–189
8. Miranda C, Thompson G (2016) Canine parvovirus: the worldwide occurrence of antigenic variants. *J Gen Virol* 97:2043–2057
9. 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 2 in Italy. *J Gen Virol* 82:3021–3025
10. Decaro N, Buonavoglia C (2012) Canine parvovirus—a review of epidemiological and diagnostic aspects, with emphasis on type 2c. *Vet Microbiol* 155:1–12
11. Zhou P, Zeng W, Zhang X, Li S (2017) The genetic evolution of canine parvovirus—A new perspective. *PLoS One* 12:e0175035. doi:10.1371/journal.pone.0175035
12. Moon HS, Lee SA, Lee SG, Choi R, Jeoung SY, Kim D, Hyun C (2008) Comparison of the pathogenicity in three different Korean canine parvovirus 2 (CPV-2) isolates. *Vet Microbiol* 131:47–56
13. Decaro N, Desario C, Elia G, Martella V, Mari V, Lavazza A, Nardi M, Buonavoglia C (2008) Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. *New Microbiol* 31:125–130
14. Soma T, Taharaguchi S, Ohinata T, Ishii H, Hara M (2013) Analysis of the VP2 protein gene of canine parvovirus strains from affected dogs in Japan. *Res Vet Sci* 94:368–371
15. Chiang SY, Wu HY, Chiou MT, Chang MC, Lin CN (2016) Identification of a novel canine parvovirus type 2c in Taiwan. *Virol J* 13:160. doi:10.1186/s12985-016-0620-5
16. Lin YC, Chiang SY, Wu HY, Lin JH, Chiou MT, Liu HF, Lin CN (2017) Phylodynamic and genetic diversity of canine parvovirus type 2c in Taiwan. *Int J Mol Sci* 18:2703. doi:10.3390/ijms18122703
17. Vannamahaxay S, Vongkhamchanh S, Intanon M, Tangtrongsup S, Tiwananthagorn S, Pringproa K, Chuammitri P (2017) Molecular characterization of canine parvovirus in Vientiane, Laos. *Arch Virol* 162:1355–1361
18. Hoang M, Lin WH, Nga BTT, Chiou MT, Lin CN (2019) Molecular epidemiology of canine parvovirus type 2 in Vietnam from November 2016 to February 2018. *Virol J* 16:52. doi:10.1186/s12985-019-1159-z
19. Moon BY, Jang J, Kim SH, Kim YH, Lee HK, So B, Park CK, Lee KK (2020) Genetic characterization of canine parvovirus type 2c from domestic dogs in Korea. *Transbound Emerg Dis* 67:1645–1653
20. Wang J, Lin P, Zhao H, Cheng Y, Jiang Z, Zhu H, Wu H, Cheng S (2016) Continuing evolution of canine parvovirus in China: isolation of novel variants with an Ala5Gly mutation in the VP2 protein. *Infect Genet Evol* 38:73–78
21. Temuujin U, Tserendorj A, Fujiki J, Sakoda Y, Tseren-Ochir EO, Okamatsu M, Matsuno K, Sharav T, Horiuchi M, Umemura T, Chultemdorj T (2019) The first isolation and identification of canine parvovirus (CPV) type 2c variants during 2016–2018 genetic surveillance of dogs in Mongolia. *Infect Genet Evol* 73:269–275
22. Charoenkul K, Tangwangvivat R, Janetanakit T, Boonyapisitsopa S, Bunpapong N, Chaiyawong S, Amonsin A (2019) Emergence of canine parvovirus type 2c in domestic dogs and cats from Thailand. *Transbound Emerg Dis* 66:1518–1528
23. Senda M, Parrish CR, Harasawa R, Gamoh K, Muramatsu M, Hirayama N, Itoh O (1995) Detection by PCR of wild-type canine parvovirus which contaminates dog vaccines. *J Clin Microbiol* 33:110–113

24. Mochizuki M, Ohshima T, Une Y, Yachi A (2008) Recombination between vaccine and field strains of canine parvovirus is revealed by isolation of virus in canine and feline cell cultures. *J Vet Med Sci* 70:1305–1314
25. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549
26. 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 U S A* 102:379–384
27. Amrani N, Desario C, Kadiri A, Cavalli A, Berrada J, Zro K, Sebbar G, Colaianni ML, Parisi A, Elia G, Buonavoglia C, Malik J, Decaro N (2016) Molecular epidemiology of canine parvovirus in Morocco. *Infect Genet Evol* 41:201–206
28. Mira F, Purpari G, Lorusso E, Di Bella S, Gucciardi F, Desario C, Macaluso G, Decaro N, Guercio A (2018) Introduction of Asian canine parvovirus in Europe through dog importation. *Transbound Emerg Dis* 65:16–21
29. Chen Y, Wang J, Bi Z, Tan Y, Lv L, Zhao H, Xia X, Zhu Y, Wang Y, Qian J (2021) Molecular epidemiology and genetic evolution of canine parvovirus in East China, during 2018-2020. *Infect Genet Evol* 90:104780. doi:10.1016/j.meegid.2021. 104780
30. Ohshima T, Hisaka M, Kawakami K, Kishi M, Tohya Y, Mochizuki M (2008) Chronological analysis of canine parvovirus type 2 isolates in Japan. *J Vet Med Sci* 70:769–775
31. Battilani M, Modugno F, Mira F, Purpari G, Di Bella S, Guercio A, Balboni A (2019) Molecular epidemiology of canine parvovirus type 2 in Italy from 1994 to 2017: recurrence of the CPV-2b variant. *BMC Vet Res* 15:393. doi:10.1186/s12917-019-2096-1
32. Kwan E, Carrai M, Lanave G, Hill J, Parry K, Kelman M, Meers J, Decaro N, Beatty JA, Martella V, Barrs VR (2020) Analysis of canine parvoviruses circulating in Australia reveals predominance of variant 2b and identifies feline parvovirus-like mutations in the capsid proteins. *Transbound Emerg Dis* In press. doi:10.1111/tbed.13727
33. Voorhees IE, Lee H, Allison AB, Lopez-Astacio R, Goodman LB, Oyesola OO, Omobowale O, Fagbohun O, Dubovi EJ, Hafenstein SL, Holmes EC, Parrish CR (2019) Limited intrahost diversity and background evolution accompany 40 years of canine parvovirus host adaptation and spread. *Journal of virology* 94:e01162–e01119. doi:10.1128/JVI.01162-19
34. Ohnaiser SA, Hills SF, Cave NJ, Passmore D, Dunowska M (2015) Canine parvoviruses in New Zealand form a monophyletic group distinct from the viruses circulating in other parts of the world. *Vet Microbiol* 178:190–200
35. Ikeda Y, Mochizuki M, Naito R, Nakamura K, Miyazawa T, Mikami T, Takahashi E (2000) Predominance of canine parvovirus (CPV) in unvaccinated cat populations and emergence of new antigenic types of CPVs in cats. *Virology* 278:13–19
36. Xinyu T, Min CS, Yifan W, Lien SM, Chan A, Hui TX, Lee B, Yelin W, Chia-Da H, Oh S, Fernandez CJ, Taoqi H (2019) Canine parvovirus-2c (CPV-2c) infection in wild Asian Palm Civets (*Paradoxurus hermaphroditus*) in Singapore. *J Wildl Dis* 55:965–969

Tables

Table 1. Primer sequences used in this study.

Primer	Sequence (5'-3')	Position ¹⁾	Reference
2304F	GCCAGGAGAGGTAAGGGTGT	2304-2323	This study
3148R	TAAACCAAACCTCCCAAGCA	3129-3148	
Pabs	GAAGAGTGGTTGTAAATAATT	3025-3045	[23]
Pabas	CCTATATAACCAAAGTTAGTAC	3685-3706	
F4	CATACATGGCAAACAAATAGAGCATTGGGC	3615-3644	[24]
R4	ATTAGTATAGTTAATTCCTGTTTTACCTCC	4095-4124	
555F	AGGAAGATATCCAGAAGGA	4003-4022	[9]
555R	GGTGCTAGTTGATATGTAATAAACA	4561-4585	

¹⁾ Positions are based on complete codes of CPV2 790312 (Accession No. M38245)

Table.2 Amino acid substitutions in the VP2 between dominant CPV2b and other CPV2.

amino acid position of VP2	87	156	192	221	291	297	321	324	328	350	373	376	396	426	435	445	540	570	573	575	579
dominant CPV-2b	L	S	S	S	L	A	N	Y	A	Q	D	P	E	D	P	T	R	K	Y	K	A
CPV2-4-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	P
CPV2-5-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	P
CPV2-7-VP2	*	*	*	*	V	S	*	I	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-14-VP2	*	*	*	*	*	*	*	I	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-15-VP2	*	*	*	*	*	*	*	I	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-16-VP2	*	*	*	*	*	*	*	I	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-67-VP2	*	*	*	*	*	*	*	*	*	*	*	Q	*	*	*	*	*	*	*	*	*
CPV2-82-VP2	*	*	*	*	*	*	K	*	*	*	*	*	*	N	A	*	*	*	C	*	*
CPV2-83-VP2	*	*	*	*	*	*	K	*	*	*	*	*	*	N	A	*	*	*	C	*	*
CPV2-94-VP2	*	*	*	*	*	*	K	*	*	*	*	*	*	*	*	*	*	E	*	R	*
CPV2-95-VP2	*	*	*	*	*	*	K	*	*	*	*	*	*	*	*	*	*	E	*	R	*
CPV2-96-VP2	*	*	*	*	*	*	K	*	*	*	*	*	*	*	*	*	*	E	*	R	*
CPV2-97-VP2	*	*	*	*	*	*	K	*	*	*	*	*	*	*	*	*	*	E	*	R	*
CPV2-104-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*	*
CPV2-112-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-117-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-143-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-152-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-181-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-184-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*
CPV2-120-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-158-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-159-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-160-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-195-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-197-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-201-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-202-VP2	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-141-VP2	*	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-142-VP2	*	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-149-VP2	*	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-164-VP2	*	F	F	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*	*	*	*
CPV2-178-VP2	*	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	P
CPV2-198-VP2	M	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-199-VP2	*	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-204-VP2	*	F	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-205-VP2	*	F	F	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CPV2-161-VP2	*	*	*	*	*	*	*	*	*	*	*	*	D	*	*	*	*	*	*	*	*
CPV2-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*

176-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	R	*	*	*
CPV2-188-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	R	*	*	*
CPV2-189-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	R	*	*	*
CPV2-203-VP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	*	*	*

The asterisk indicate identity to dominant CPV-2b VP2 sequence.

Table.3 CPV2 antigenic variants according to sex, age, and vaccination among dogs in Japan.

Region	Western Japan			Eastern Japan		
Virus	-	CPV2a	CPV2b	-	CPV2a	CPV2b
Total	73	4	69	85	8	77
Sex						
male	34	1	33	33	6	27
female	24	1	23	15	1	14
unknown	15	2	13	37	1	36
Age						
1m	9	0	9	5	0	5
2m	47	4	43	42	7	35
3m	4	0	4	9	1	8
>4m	1	0	1	0	0	0
unknown	12	0	12	29	0	29
Vaccine						
Vaccinated	60	4	56	56	8	48
unknown	13	0	13	29	0	29

Figures

Figure 1

Phylogenetic analysis of VP2 of CPV2. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jones-Taylor-Thornton (JTT) model in MEGA-X. The tree with the highest log likelihood (-4497.89) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 684 amino acid sequences. CPV-2a detected in this study is highlighted in blue, and CPV-2b detected in this study is highlighted in red. Figure S1 provides a higher resolution of the phylogenetic tree.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Fig.S1.pdf](#)
- [TableS1.xlsx](#)