Full-length genome characterization of canine parvovirus strains circulating in Nigeria

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Title: Full-length genome characterization of canine parvovirus strains circulating in Nigeria

Short running title: Analysis of CPV strains circulating in Nigeria

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Summary

Canine parvovirus type 2 (CPV-2) emerged suddenly in the late 1970s as pathogen of dogs, causing a severe and often fatal gastroenteric disease. The original CPV-2 was replaced by three antigenic variants, CPV-2a, CPV-2b and CPV-2c, which to date have gained a worldwide distribution with different relative proportions. All previous studies conducted in Africa were based on partial VP2 gene sequences. The aim of this study was to provide a full-length genome analysis of CPV strains collected in Nigeria, Africa. Rectal swab samples (n = 320) were collected in 2018 and tested by means of an immunochromatographic assay. Among the 144 positive samples, 59 were selected for further analyses using different molecular assays. The results revealed a high prevalence of CPV-2c (91.5%) compared to the CPV-2a variant (8.5%). The VP2 gene sequence showed a divergence from the strains analysed in 2010 in Nigeria and a closer connection with CPV strains of Asian origin. The non-structural genes analysis evidenced amino acid changes never previously reported. The molecular analysis based on genomic sequences evidenced a geographical pattern of distribution of the analysed strains, suggesting a potential common evolutionary origin with CPV of Asian origin. This study represents the first CPV molecular characterization including all the encoding gene sequences conducted in the African continent and contributes to define the actual geographical spread of the CPV variants worldwide.

Keywords: Canine parvovirus; Protoparvovirus; Nigeria; genome analysis; NS1; molecular characterization

Introduction

Canine parvovirus (CPV) is a small (about 25 nm in diameter), non-enveloped DNA virus, which emerged suddenly in the late 1970s as pathogen of dogs, causing severe and often fatal epizootics of gastroenteritis worldwide (Decaro & Buonavoglia, 2012; Decaro et al., 2007a). CPV was recently included, together with feline panleukopenia virus (FPLV), mink enteritis virus (MEV) and raccoon parvovirus (RaPV), in the unique specie *Carnivore protoparvovirus 1*, within the *Protoparvovirus* genus (family *Parvoviridae*, subfamily *Parvovirinae*) (Cotmore et al., 2019; 2014). Its genome consists of a 5200-nucleotide (nt) DNA molecule containing two large open reading frames (ORFs) encoding for two nonstructural (NS1 and NS2) and two structural (VP1 and VP2) proteins through alternative splicing of the same mRNAs (Decaro & Buonavoglia, 2012).

Soon after its emergence, the original CPV-2 was replaced by two antigenic variants, CPV-2a and CPV-2b (Parrish et al., 1985; 1991), and in 2001 a third antigenic variant was described (Buonavoglia et al., 2001). To date, all three CPV variants are worldwide distributed, with different relative proportions according to the year and country of collection (Miranda & Thompson, 2016; Amrani et al., 2016; Woolford et al., 2017).

In the African continent, CPV has been described in South Africa and Namibia (Dogonyaro et al., 2013; Steinel et al., 1998), Zambia (Kapiya et al. 2019), Mozambique (Figuiredo et al., 2017), Ghana (Folitse et al., 2017), Morocco (Amrani et al, 2016), Cape Verde (Costanheira et al., 2014), Nigeria (Chollom et al., 2013) and Tunisia (Touhiri et al., 2009). In Nigeria, only recently the molecular analyses based on the partial VP2 gene sequence of CPV strains described the circulating CPV variants (Dogonyaro et al., 2013; Apaa et al., 2016; Fagbohun and Omobowale, 2018). Unfortunately, all previous studies conducted in Africa lack a comprehensive sequence analysis including all the viral

genome, thus preventing a more in-depth knowledge on the origin and evolution of the circulating CPV strains. The aim of this study was to analyse the complete genome sequence of CPV strains recently collected in Nigeria, and to compare the obtained sequences with worldwide related sequences.

Materials and Methods

Samples were collected between January and June 2018 from 320 dogs of different breeds with clinical signs of gastroenteritis and suspected of CPV infection or apparently healthy. Rectal swabs were collected in Nigeria from four cities placed in three states, namely Plateau state (Jos), Benue state (Makurdi), Nassarawa state (Lafia) and in the Federal Capital Territory (Abuja). Samples were submitted from eight private veterinary clinics, two from each state, and from kennels/breeders in the same areas. Details are reported in Supplementary Table 1.

Rectal swabs were tested using an in-clinic assay for detection of CPV antigen (SensPERT® Canine Parvovirus Test Kit, VetAll Laboratories, Gyeonggi-do, Korea), according to the manufacturer's instructions. Among the positive samples, 59 rectal swabs were selected and submitted for molecular analyses, where they were stored at −80 °C until use.

Viral DNA was extracted from 200 µl of swab homogenate, obtained as previously described (Purpari et al., 2018), using the DNeasy Blood & Tissue Kit (Qiagen S.p.A., Milan, Italy), according to the manufacturer's instructions. The presence of CPV DNA was confirmed using a primer pair (Supplementary Table 2) in a PCR protocol amplifying a 700-bp fragment of the VP2 gene (Touihri et al., 2009), using the commercial kit GoTaq® G2 DNA Polymerase (Promega Italia s.r.l., Milan, Italy), as

previously described (Mira et al., 2018a). Amplicons were checked after electrophoresis on a 3% agarose gel supplemented with ethidium bromide.

Ten microliters of each amplicon were digested with 5 unit (U) of restriction endonuclease *Mbo*II (New England BioLabs® Inc., Ipswich, U.S.A.) in a 50-µl reaction mix consisting of 5 µl of NEBuffer and 34 µl of nuclease-free water, under the following reaction conditions: incubation at 37°c for 2 hours and inactivation at 65°C for 20 min. The profile was determined by electrophoresis on a 3% agarose gel supplemented with ethidium bromide.

Specimens from each city of collection (n = 4 from Makurdi; n = 11 from Jos; n = 8 from Abuja and n = 5 from Lafia), representing dogs with different age, vaccinal and clinical status, were selected to determine the VP2 gene sequence (Table 1). The VP2 gene sequence was determined using a primers pair (Supplementary Table 2), which allows for amplification of a 1745-bp fragment (Battilani et al., 2001; Mokizuki et al., 1996), using the commercial kit GoTaq® G2 DNA Polymerase (Promega Italia s.r.l., Milan, Italy), as previously described (Purpari et al., 2018) with minor modifications (thermal conditions: 1 min for the annealing step). After electrophoresis on agarose gel supplemented with ethidium bromide, positive amplicons were purified with Illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Amersham, Buckinghamshire, UK) and submitted to BMR Genomics srl (Padova, Italy) for direct Sanger sequencing with 6.4 pmol of the reverse primer used for amplification and of two additional internal primers (Supplementary Table 2). According to an overlapping strategy, sequences were assembled and analysed using BioEdit ver 7.2.5 software (Hall, 1999).

By excluding the VP2 gene sequence with complete nt identities, 8 CPV DNAs from different cities of collection were further selected for a second-round sequence analysis amplifying a long genome

sequence encompassing both major ORFs (Table 1). Sequence analyses were carried out using primers pairs described by Pérez et al. (2014), using the commercial kit GoTaq® G2 DNA Polymerase (Promega Italia s.r.l., Milan, Italy), as previously described (Mira et al., 2018a) with minor modifications (Mira et al., 2019a). After electrophoresis on agarose gel, amplicons were purified and submitted for direct Sanger sequencing with a set of primers, as previously described (Mira et al., 2018b). Sequences were assembled, analysed and submitted to nBLAST program (Zhang et al., 2000) to search related sequences into public domain databases. These sequence data were submitted to the DDBJ/EMBL/GenBank databases under accession numbers MK895483-90.

Phylogenetic analyses based on the full-length NS1 and VP2 gene sequences and on the whole genome encompassing the two ORFs were conducted using the best-fit model of nt substitution with MEGA version X software (Kumar et al., 2018), inferred with the maximum-likelihood method based on the Tamura 3-parameter (T92) and Hasegawa-Kishino-Yano (HKY) models, with discrete Gamma distribution (five rate categories) (G) and invariant sites (I) (bootstrap 1,000 replicates), the best-fitting models after the model test analyses (VP2: T92+G; NS1: HKY+G; whole genome: HKY+G+I).

RNA was extracted from samples using the QIAamp Viral RNA Mini Kit (Qiagen S.p.A.), according to the manufacturer's instructions. Extracted DNA/RNA were amplified using a set of gel-based or real-time (RT-)PCR assays useful for the detection of canine distemper virus (CDV) (Elia et al., 2006), canine adenovirus (CAdV) types 1 and 2 (Dowgier et al., 2016), canine herpesvirus (CaHV-1) (Decaro et al., 2010), canine coronavirus (CCoV) (Decaro et al., 2004) and canine rotavirus (CRoV) (Freeman et al., 2008).

Results

Among the collected 320 samples, 144 rectal swabs tested positive for CPV by in-clinic assay (40%). Relative positive samples were registered for each city of collection in Table 2. The presence of CPV DNA was confirmed in the selected samples (n = 59) using the conventional PCR assay. The same samples tested negative for CDV, CAdVs, CaHV-1, CCoV and CRoV by gel-based or real-time (RT)-PCR assays. Based on the RFLP analysis, 54 CPV-positive samples (91.5%) were typed as CPV-2c (yielding 3 fragments of 56, 275 and 369 bp, respectively) and 5 (8.5%) as a different CPV type (CPV-2, CPV-2a or CPV-2b) (yielding 2 fragments of 331 and 369 bp, respectively).

The RFLP untyped CPV strains (n = 5) and the CPV-2c strains (n = 23) from different geographic areas were subjected to sequence analyses to screen the VP2 gene sequence. According to aa residues 297 and 426, the sequence analysis confirmed the RFPL assay results for samples typed as CPV-2c (426-Glu) and typed the other 5 samples as "new CPV-2a" (297-Ala, 426-Asn). The VP2 sequences showed 99.77-100% and 99.98-100% reciprocal nt identities among the CPV-2a and CPV-2c strains, respectively. Four synonymous changes were observed in the VP2 sequence of the CPV-2a strains (nts: a639g, c969t, a1074t, a1275g). One non-synonymous (nt: c14g; aa: A5G) and four synonymous (nts: a318g, t1014c, g1083t, t1629c) changes were observed in the CPV-2c strains. VP2 synonymous changes are reported in Supplementary Table 3.

Compared to CPV sequences previously analysed in Nigeria, the VP2 sequences of the analysed CPV-2a strains showed 99.60-99.25% (calculated on 1750 nts), 99.87% (on 787 nts) and 99.34-98.72% (on 459 and 470 nts) nt identities with strains collected in 2010, 2014 and 2017 (Dogonyaro et al., 2013; Apaa et al., 2016; Fagbohun and Omobowale, 2018), respectively. Compared to the CPV-2a strains collected in 2010, three non-synonymous changes (Y267F, I324Y, A440T) were observed (Table 3).

Compared to the CPV-2c sequence previously analysed in Nigeria (acc.no. MH795059), the VP2 sequences of CPV-2c strains showed 99.36% of nt identity (on 466 nts).

Compared to CPV sequence previously analysed in other African countries, the VP2 sequences of CPV-2a strains showed 99.81% (on 529 nts), 99.61% (on 515 nts) and 99.85-99.05% (on 1745 and 1583 nts) sequence identities with strains collected in 2017 in Zambia (GenBank accession number LC409284), 2010 in Mozambique (acc. no. KU523911), 2010 and 1996 in South Africa (acc. no. HQ602976-8 and AJ007500), respectively. Compared to the CPV-2a strains previously collected in other African countries, seven non-synonymous changes (K265T, F267Y, S/N297A, Y324I, A424V, T440A) were observed (Table 3). Compared to the CPV-2c sequence previously analysed in other African countries (Zambia, 2017; acc.no. LC409284; Kapiya et al., 2019), the VP2 sequences of CPV-2c strains showed 100% (on 529 nts).

By the second-round sequence amplifications, the nearly complete genome sequences including both ORFs (4269 nt) of 8 CPV strains were obtained. The complete genome sequences of CPV-2a strains showed 99.27-99.34% nt identities with CPV strains collected in China in 2017 (acc. no. MH476580, MH476590, MH476591) and in Uruguay in 2011 (acc. no. KM457139). The complete genome sequences of CPV-2c strains showed 99.88-99.92% nt identities with CPV strains of Asian origin (China, 2017, acc. no. MG013488; Thailand, 2016, acc. no. MH711902; Italy, 2017, acc. no. MF510157).

Sequence analysis revealed aa changes previously described mainly in CPV-2a/2c strains of Asian origin (NS1: 60V, 544F, 545V, 630P - NS2: 60V, 151N, 152V - VP2: 5A/G, 267Y, 297A, 324I, 370R) (Tables 4 and 5). Additional changes at aa residues 164L, 351K, 584K, 586V, 596A and 517I of the CPV-2a and CPV-2c NS1 protein, respectively, were also found (Table 5). While changes 351K and 596A had been detected in CPV sequences mainly from South and North Americas, changes 164L,

584K, 586V and 517I had not been previously reported.

Amino acid change I60V in NS1 also lies at the same residue in the NS2-encoding sequence. Additional four amino-acid changes in the NS2-enconding sequences were observed: D93E, T94A, D151N and M152V (Table 5). These changes resulted in silent mutations in the corresponding encoded NS1 protein.

The aa change K116R in the VP1 gene sequence is added to the aa changes of the VP2 gene sequence lying in the corresponding encoded VP1 protein (A148G, M230L, I244T, F410Y, S440A, A443G, D448Y, Y467I, Q513R, N518D, T583A).

Phylogenetic analysis inferred from VP2 sequences showed that the analysed strains are more closely related to the CPV strains of Asian origin rather than to strains collected in Europe or in North/South America, clustering in separate branches within the clades (Figure 1). Phylogenetic tree inferred from NS1 sequences (Figure 2) indicated that the CPV-2a strains cluster separately within the clade of CPV strains of Asian origin. Although with a low bootstrap support, the almost complete genome sequence phylogeny showed that the analysed strains clustered according to the geographical origin and the year of collection rather than to the CPV variant (Figure 3).

Discussion

CPV has still been playing a main role in inducing severe and often fatal gastroenteritis in young or non-immunized dogs. During years, CPV spread and evolution have been well documented in North and South America, Europe and Asia (Miranda and Thompson, 2016; Zhou et al., 2017). More recently, data about its spread were also obtained from Australia and Africa (Woolford et al., 2017; Kapiya et al. 2019; Figuiredo et al., 2017; Folitse et al., 2017; Amrani et al, 2016; Castanheira et al., 2014;

Chollom et al., 2013; Dogonyaro et al., 2013; Touhiri et al., 2009). Most of these studies were based on the partial or complete VP2 gene sequence, due to the involvement of the VP2 capsid protein in host switch and to its fast-evolutionary rate (Hueffer at al., 2003; Nelson et al., 2007; Shackelton et al., 2005), with limited information on other CPV encoding gene sequences. With the aim to investigate the epidemiology and evolution of CPV in Nigeria, the VP2 gene or nearly complete genome sequences including both ORFs of CPV representative strains were analysed and compared to the related strains retrieved from the GenBank database.

Early studies on detection of CPV in Nigeria were based on serological or PCR assays (Chollom et al., 2013; Babalola et al., 2016) and only recently the analysis of the partial VP2 gene sequence was included in the studies (Dogonyaro et al., 2013; Apaa et al., 2016; Fagbohun and Omobowale, 2018). These analyses conducted in Nigeria evidenced firstly the circulation of CPV-2a strains (Dogonyaro et al., 2013; Apaa et al., 2016) and only recently of CPV-2b/2c types (Fagbohun and Omobowale, 2018). In the present study based on the analysis of 59 CPV positive samples, 5 new CPV-2a and 54 CPV-2c strains have been found. These data showed a high prevalence of CPV-2c (91.5%) compared to the CPV-2a variant (8.5%), without any evidence of CPV-2b. Moreover, the divergence in the VP2 gene sequence of the CPV strains compared to those previously circulating in Nigeria suggests the potential circulation or introduction of different strains during years. Indeed, despite the limits of the length of available sequences, the CPV strains analysed in this study are prone to be related to a different origin than the previous ones. All CPV strains collected in 2010 (Dogonyaro et al., 2013) displayed divergent amino acids at specific VP2 residues (267, 324) with a closer phylogenetic connection with the CPV-2a strains previously collected in other continents such as North/South America and Europe. Differently, since 2014 the analysed circulating CPV strains showed a closer connection with CPV strains of Asian

origin. Despite some previously analysed CPV sequences lack of informative VP2 aa residues (5, 267, 324, 370), the comparison with our sequences suggested that both CPV-2a and CPV-2c strains collected in Nigeria after 2014 have a different phylogenetic origin with respect to previous analyses (Apaa et al., 2016; Fagbohun and Omobowale, 2018), suggesting a most recent introduction or evidence.

The analysis included both ORFs and allowed to gain additional information. Indeed, in this study the whole VP gene sequences were analysed, showing the VP1 and VP2 aa changes K116R and A5G, respectively. The VP1 aa change was due to a nt change in the second base of the codon (VP1 a347g) and was common to CPV strains of different origin, irrespective of the CPV antigenic variant. Moreover, the VP2 aa change was due to a nt change in the second base of the codon (VP2 c14g), but this change was common only to CPV strains of Asian origin (Wang et al., 2016; Zhuang et al., 2019; Mira et al., 2018b; Mira et al., 2019b). The potential biological relevance of these changes has not been described yet and needs to be assessed in further studies.

As most recent Asian CPVs, the Nigerian strains displayed other three aa substitutions in the VP2 sequence (F267Y, Y324I, Q370R). While change at aa residue 324 is predominant in all three CPV variants in Asia (Zhou et al., 2017; Yi et al., 2016; Geng et al., 2015; Zhao et al., 2017), the other changes have been less frequently observed, mainly in China since 2013, and change Q370R has been detected only in CPV-2c strains (Guo et al., 2013; Geng et al., 2015; Wang et al., 2016; Mira et al., 2018b; Mira et al., 2019b; Zhuang et al., 2019). These aa substitutions are located in the greatest variable VP2 GH loop, comprised between aa 267–498, but while residue 267 is not exposed on the capsid surface (Chiang et al., 2016) and may not affect the antigenicity of CPV (Xu et al., 2015), residues 324 and 370 could have immunological implications or biological relevance. Indeed, residue 324 is subject to

positive selection (Hoelzer et al., 2008) and is adjacent to residue 323, which affects binding to the canine transferrin receptor (Hueffer and Parrish, 2003). Residue 370 is close to residues associated with the ability of CPV to hemagglutinate, altering the pH dependence of hemagglutination or affecting the canine transferrin receptor (TfR) binding that determines the canine host range (Tsao et al., 1991; Kaelber et al., 2012; Guo et al., 2013).

Among the synonymous substitution observed in the CPV-2a VP2 gene sequences, nt change a1275g has been previously described in CPV-2c strains (Decaro et al., 2009, 2013a; Amrani et al., 2016). This change, observed in the strains UV1 and UV6, was detected in the binding region of the type-2a and -2c specific probes of the minor groove binder (MGB) probe assay (Decaro et al., 2006; Decaro et al., 2005). Although this change potentially accounts for the absence of VIC fluorescence in the 2a/2b and 2b/2c assays, specific additional studies are necessary to evaluate its real implication in the characterization of this CPV-2a mutant by the MGB probe assay, as previously done for the same substitution in the CPV-2c mutants (Decaro et al., 2013b). The in-clinic assay used in this study was able to detect the CPV-2a showing this substitution, as previously observed for another rapid assay used to test also the CPV-2c mutants (Decaro et al., 2013b).

Limited studies are available on the non-structural genes (Hoelzer et al., 2008; Pérez et al., 2014) and, only recently, the analysis of the NS1 gene sequence was included in the CPV phylogenies from several countries (Mira et al., 2019a; Mira et al., 2019b; Grecco et al., 2018; Zhuang et al., 2019; Canuti et al., 2017). In this study, sequence analysis revealed aa changes previously described mainly in NS1/NS2 gene sequences of CPV-2a/2c strains of Asian origin. Additional changes were also evident, some previously reported in South/North America and others never previously observed. This divergence may suggest the same ancestral origin with the CPV strains of Asian origin but a separate

evolution, as well as a continuous adaptative process of the virus in separate environments. Indeed, some of these changes lay in the potential encoding sequence of functional domains (Mira et al., 2019a) and, particularly, residues 351, 517 and 545 are located between the α5- and α6-helices, between the β5- and α11-helices and just close to the α11-helix of the helicase domain protein sequence, respectively, as illustrated in Niskanen et al. (2010). Therefore, their role needs to be further evaluated.

The molecular analysis based on long genome sequences evidenced the geographical origin of the analysed strains rather than the clustering based only on the CPV antigenic variant. Therefore, this study supports further studies aimed to track the viral spread and elucidate the CPV evolution. Indeed, the recent evidence of specific aa changes, as well as the divergence from previous circulating strains, does not allow to rule out the possible introduction of these strains from other countries, highlighting the need for further studies on CPV whole genome in different geographic areas. This suggestion is supported by the evidence of genetic signatures typical of CPV or other canine viruses with different origins (Mira et al., 2018b; Mira et al., 2018c; Decaro et al., 2007b; Martella et al., 2006), probably connected with the trading and transport of dogs between countries and continents.

This study represents the first CPV molecular characterization including all the encoding gene sequences conducted in the African continent and contributes to define the current geographic spread of the CPV variants worldwide. The evidence of mutations that have not been detected before suggests the need for further investigations in order to determine any biological consequences and underlines the continue evolution of CPV.

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Table 1. Identification code, origin, age, vaccination and clinical status, strain and sequence information of tested dogs.

ID code	City	Age	Vaccination status	Remarks	Strain	Sequence	Acc. no.
YV 8	Makurdi	12 weeks	Vaccinated	Clinical	CPV-2a	ORF1/2	MK895484
UV 1	Abuja	6 months	Not vaccinated	Subclinical	CPV-2a	ORF1/2	MK895483
UV 6	Abuja	10 weeks	Not vaccinated	Subclinical	CPV-2a	ORF1/2	MK895485
RKB 2	Abuja	5 weeks	Not vaccinated	Clinical	CPV-2a	VP2	-
LTG 3	Lafia	12 weeks	Not vaccinated	Apparently healthy	CPV-2a	VP2	-
PB 3	Makurdi	6 weeks	Vaccinated	Clinical	CPV-2c	VP2	-
YV 2	Makurdi	6 weeks	Vaccinated	Clinical	CPV-2c	ORF1/2	MK895486
JOE 2	Makurdi	7 weeks	Vaccinated	Apparently healthy	CPV-2c	ORF1/2	MK895488
NV 19	Jos	5 months	Vaccinated	Clinical	CPV-2c	VP2	-
NC	Jos	10 weeks	Vaccinated	Clinical	CPV-2c	ORF1/2	MK895489
YA 3	Jos	6 weeks	Vaccinated	Clinical	CPV-2c	VP2	-
NV 10	Jos	10 weeks	Vaccinated	Apparently healthy	CPV-2c	VP2	-
NV 4	Jos	10 weeks	Vaccinated	Apparently healthy	CPV-2c	VP2	-
CVC 14	Jos	6 months	Not vaccinated	Clinical	CPV-2c	VP2	-
PSV 21	Jos	11 weeks	Not vaccinated	Clinical	CPV-2c	ORF1/2	MK895490
CVC 1	Jos	2 years	Not vaccinated	Clinical	CPV-2c	VP2	-
CVC 21	Jos	12 weeks	Not vaccinated	Clinical	CPV-2c	VP2	-
PSV 15	Jos	8 weeks	Not vaccinated	Apparently healthy	CPV-2c	VP2	-
PSV 5	Jos	8 weeks	Not vaccinated	Apparently healthy	CPV-2c	VP2	-
VC 5	Abuja	8 weeks	Vaccinated	Clinical	CPV-2c	VP2	-
EV 8	Abuja	5 months	Vaccinated	Clinical	CPV-2c	ORF1/2	MK895487
MV 1	Abuja	8 weeks	Vaccinated	Apparently healthy	CPV-2c	VP2	-
EV 3	Abuja	7 weeks	Vaccinated	Apparently healthy	CPV-2c	VP2	-
EV 5	Abuja	9 weeks	Not vaccinated	Clinical	CPV-2c	VP2	-
RF 3	Lafia	7 weeks	Vaccinated	Clinical	CPV-2c	VP2	-
N 1	Lafia	9 weeks	Vaccinated	Clinical	CPV-2c	VP2	-
LO 11	Lafia	2 years	Vaccinated	Apparently healthy	CPV-2c	VP2	-
V 4	Lafia	18 weeks	Not vaccinated	Clinical	CPV-2c	VP2	-

Table 2. Number and prevalence of CPV positive samples.

State	City	Total samples	Positive samples	Negative samples	Prevalence (%)
Plateau	Jos	80	54	26	65.70
Benue	Makurdi	80	35	45	43.75
Nassarawa	Lafia	80	23	57	28.75
Federal Capital Territory	Abuja	80	32	48	40.00
Total		**320**	**144**	**176**	**45**

Table 3. VP2 non-synonymous changes of CPV strains analysed in this study and of CPV strains from Africa previously analysed.

Strain	Acc. No.	Type	Country	Year	VP2 amino acids										Reference
					265	267	297	324	370	401	413	424	426	440	
IZSSI_PA1464/19_idUV1	MK895483	CPV-2a	Nigeria	2018	T	Y	A	I	Q	I	D	V	N	A	This study
IZSSI_PA1464/19_idYV8	MK895484	CPV-2a	Nigeria	2018	-	-	-	-	-	-	-	-	-	-	
IZSSI_PA1464/19_idUV6	MK895485	CPV-2a	Nigeria	2018	-	-	-	-	-	-	-	-	-	-	
IZSSI_PA1464/19_idYV2	MK895486	CPV-2c	Nigeria	2018	-	-	-	-	R	-	-	-	E	T	
IZSSI_PA1464/19_idEV8	MK895487	CPV-2c	Nigeria	2018	-	-	-	-	R	-	-	-	E	T	
IZSSI_PA1464/19_idJOE2	MK895488	CPV-2c	Nigeria	2018	-	-	-	-	R	-	-	-	E	T	
IZSSI_PA1464/19_idNC	MK895489	CPV-2c	Nigeria	2018	-	-	-	-	R	-	-	-	E	T	
IZSSI_PA1464/19_idPSV21	MK895490	CPV-2c	Nigeria	2018	-	-	-	-	R	-	-	-	E	T	
3-10NGR2010	HQ602990	CPV-2a	Nigeria	2010	-	F	-	Y	-	-	-	-	-	-	Dogonyaro et al., 2013
19-10NGR2010	HQ602992	CPV-2a	Nigeria	2010	-	F	-	Y	-	-	-	-	-	T	
23-10NGR2010	HQ602994	CPV-2a	Nigeria	2010	-	F	-	Y	-	-	-	-	-	T	
15-10NGR2010	HQ602995	CPV-2a	Nigeria	2010	-	F	-	Y	-	-	-	-	-	T	
NG21	KX192407	CPV-2a	Nigeria	2014						-	-	-	-	-	Apaa et al., 2016
NG32	KX192416	CPV-2a	Nigeria	2014				-	-	-	-	-	-	-	
NG35	KX192418	CPV-2a	Nigeria	2014				-	-	-	-	-	-	-	
NGA1-2a	MH795055	CPV-2a	Nigeria	2017						-	-	-	-	-	Fagbohun and Omobowale, 2018
NGA7-2a	MH795061	CPV-2a	Nigeria	2017						-	-	-	-	-	
NGA2-2b	MH795056	CPV-2b	Nigeria	2017						K	N	-	D	T	
NGA5-2c	MH795059	CPV-2c	Nigeria	2017					-	-	-	-	E	T	
CPV-Africa 3	AJ007498	CPV-2a	South Africa	1996	-	F	S	Y	-	-	-	-	-	T	Steinel et al., 1998
CPV-Africa 9	AJ007500	CPV-2b	South Africa	1996	-	F	S	Y	-	-	-	-	D	T	
63-10SA 2010	HQ602978	CPV-2a	South Africa	2010	K	F	N	Y	-	-	-	A	-	-	Dogonyaro et al., 2013
100-10SA 2010	HQ602977	CPV-2a	South Africa	2010	-	F	-	-	-	-	-	-	-	T	
72-10SA 2010	HQ602976	CPV-2b	South Africa	2010	-	F	N	Y	-	-	-	-	D	T	
MZ_CPV36/2010	KU523909	CPV-2a	Mozambique	2010							-	A	-	-	Figueiredo et al., 2017
MZ_CPV38/2010	KU523911	CPV-2b	Mozambique	2010							-	-	D	T	
CPV-2c-Lus2	LC409276	CPV-2a	Zambia	2017							-	-	-	-	Kapiya et al., 2019
CPV-2c-Lus3	LC409277	CPV-2b	Zambia	2017							-	-	D	T	
CPV-2c-Lus11	LC409284	CPV-2c	Zambia	2017							-	-	E	T	

Dark gray cells indicate that fragment of encoding sequences are not available. "-" same amino acid as in the first row.

Table 4. VP2 non-synonymous changes of analysed CPV strains described in this study.

CPV variant	Strain	Country (year)	Acc.n.	VP2 amino acids											
				5	87	101	267	297	300	305	324	370	375	426	440
CPV-2[a]	CPV-b	U.S.A. (1978)	M38245	A	M	I	F	S	A	D	Y	Q	N	N	T
CPV-2[a]	CPV-15	U.S.A. (1984)	M24003	-	L	T	-	-	G	Y	-	-	D	-	-
CPV-2b[a]	CPV-39	U.S.A. (1984)	M74849	-	L	T	-	-	G	Y	-	-	D	D	-
CPV-2c[a]	288-01	Italy (2001)	MF177239	-	L	T	-	A	G	Y	-	-	D	E	-
CPV-2a[a]	TN/CPV2a/2018	India (2018)	MH545963	-	L	T	Y	A	G	Y	I	-	D	-	A
CPV-2c[a]	Canine/China/23/2017	China (2017)	MH476592	G	L	T	Y	A	G	Y	I	R	D	E	-
CPV-2a	IZSSI_PA1464/19_idUV1	Nigeria (2018)	MK895483	-	L	T	Y	A	G	Y	I	-	D	-	A
CPV-2a	IZSSI_PA1464/19_idYV8	Nigeria (2018)	MK895484	-	L	T	Y	A	G	Y	I	-	D	-	A
CPV-2a	IZSSI_PA1464/19_idUV6	Nigeria (2018)	MK895485	-	L	T	Y	A	G	Y	I	-	D	-	A
CPV-2c	IZSSI_PA1464/19_idYV2	Nigeria (2018)	MK895486	-	L	T	Y	A	G	Y	I	R	D	E	-
CPV-2c	IZSSI_PA1464/19_idEV8	Nigeria (2018)	MK895487	-	L	T	Y	A	G	Y	I	R	D	E	-
CPV-2c	IZSSI_PA1464/19_idJOE2	Nigeria (2018)	MK895488	G	L	T	Y	A	G	Y	I	R	D	E	-
CPV-2c	IZSSI_PA1464/19_idNC	Nigeria (2018)	MK895489	G	L	T	Y	A	G	Y	I	R	D	E	-
CPV-2c	IZSSI_PA1464/19_idPSV21	Nigeria (2018)	MK895490	-	L	T	Y	A	G	Y	I	R	D	E	-

[a]Reference strains retrieved from GenBank. "-" same amino acid as in the first row.

Table 5. NS1 and NS2 non-synonymous changes of analysed CPV strains described in this study.

CPV variant	Strain	Country (year)	Acc.n.	NS1 amino acids										NS2 amino acids				
				60	164	351	517	544	545	584	586	596	630	60	93	94	151	152
CPV-2[a]	CPV-b	U.S.A. (1978)	M38245	I	S	N	V	Y	E	T	A	V	L	I	D	T	D	M
CPV-2a[a]	CPV-15	U.S.A. (1984)	AY787926	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPV-2b[a]	CPV-39	U.S.A. (1984)	AY787930	-	-	-	-	F	-	-	-	-	-	-	-	-	-	V
CPV-2c[a]	288-01	Italy (2001)	MF177239	-	-	-	-	-	-	-	-	-	-	-	-	A	N	V
CPV-2a[a]	TN/CPV2a/2018	India (2018)	MH545963	-	-	-	-	F	V	-	-	-	-	-	-	-	N	V
CPV-2c[a]	Canine/China/23/2017	China (2017)	MH476592	V	-	-	-	F	V	-	-	-	P	V	-	-	N	V
CPV-2a	UV1	Nigeria (2018)	MK895483	-	-	K	-	F	-	K	V	A	-	-	E	A	N	V
CPV-2a	YV8	Nigeria (2018)	MK895484	-	-	K	-	F	-	K	V	A	-	-	E	A	N	V
CPV-2a	UV6	Nigeria (2018)	MK895485	-	L	K	-	F	-	K	V	A	-	-	E	A	N	V
CPV-2c	YV2	Nigeria (2018)	MK895486	V	-	-	-	F	V	-	-	-	P	V	-	-	N	V
CPV-2c	EV8	Nigeria (2018)	MK895487	V	-	-	-	F	V	-	-	-	P	V	-	-	N	V
CPV-2c	JOE2	Nigeria (2018)	MK895488	V	-	-	I	F	V	-	-	-	P	V	-	-	N	V
CPV-2c	NC	Nigeria (2018)	MK895489	V	-	-	-	F	V	-	-	-	P	V	-	-	N	V
CPV-2c	PSV21	Nigeria (2018)	MK895490	V	-	-	-	F	V	-	-	-	P	V	-	-	N	V

[a]Reference strains retrieved from GenBank. "-" same amino acid as in the first row.

Figure 1. Maximum-likelihood tree based on 49 full-length VP2 gene sequences of canine parvovirus type 2 strains (bootstrap 1000 replicates; bootstrap values greater than 65 are shown). Black dots (●) and black triangles (▲) markings indicate CPV-2a and CPV-2c strains from Nigeria, respectively, analysed in this study. Each sequence is indicated with virus type (FPLV: feline panleukopenia virus - CPV: canine parvovirus) or variant (CPV-2, CPV-2a, CPV-2b, CPV-2c), country and year of collection, accession number and strain/isolate name. The scale bar indicates the estimated numbers of nucleotide substitutions per site.

Figure 2. Maximum-likelihood tree based on 49 full-length NS1 gene sequences of canine parvovirus type 2 strains (bootstrap 1000 replicates; bootstrap values greater than 65 are shown). Black dots (●) and black triangles (▲) markings indicate CPV-2a and CPV-2c strains from Nigeria, respectively, analysed in this study. Each sequence is indicated with virus type (FPLV: feline panleukopenia virus - CPV: canine parvovirus) or variant (CPV-2, CPV-2a, CPV-2b, CPV-2c), country and year of collection, accession number and strain/isolate name. The scale bar indicates the estimated numbers of nucleotide substitutions per site.

Figure 3. Maximum-likelihood tree based on 49 complete genome sequences of canine parvovirus type 2 strains (bootstrap 1000 replicates; bootstrap values greater than 65 are shown). Black dots (●) and black triangles (▲) markings indicate CPV-2a and CPV-2c strains from Nigeria, respectively, analysed in this study. Each sequence is indicated with virus type (FPLV: feline panleukopenia virus - CPV: canine parvovirus) or variant (CPV-2, CPV-2a, CPV-2b, CPV-2c), country and year of collection, accession number and strain/isolate name. The scale bar indicates the estimated numbers of nucleotide

substitutions per site.

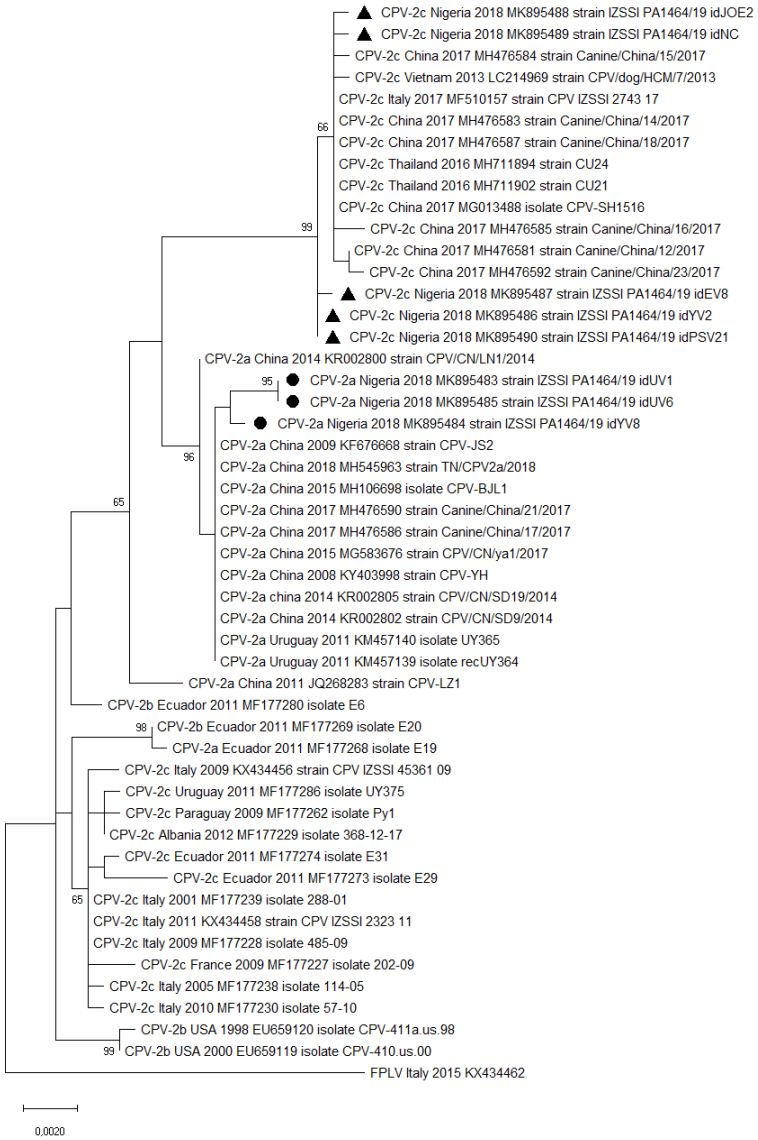

Figure 1. Maximum-likelihood tree based on 49 full-length VP2 gene sequences of canine parvovirus type 2 strains (bootstrap 1000 replicates; bootstrap values greater than 65 are shown). Black dots (●) and black triangles (▵) markings indicate CPV-2a and CPV-2c strains from Nigeria, respectively, analysed in this study. Each sequence is indicated with virus type (FPLV: feline panleukopenia virus - CPV: canine parvovirus) or variant (CPV-2, CPV-2a, CPV-2b, CPV-2c), country and year of collection, accession number and strain/isolate name. The scale bar indicates the estimated numbers of nucleotide substitutions per site.

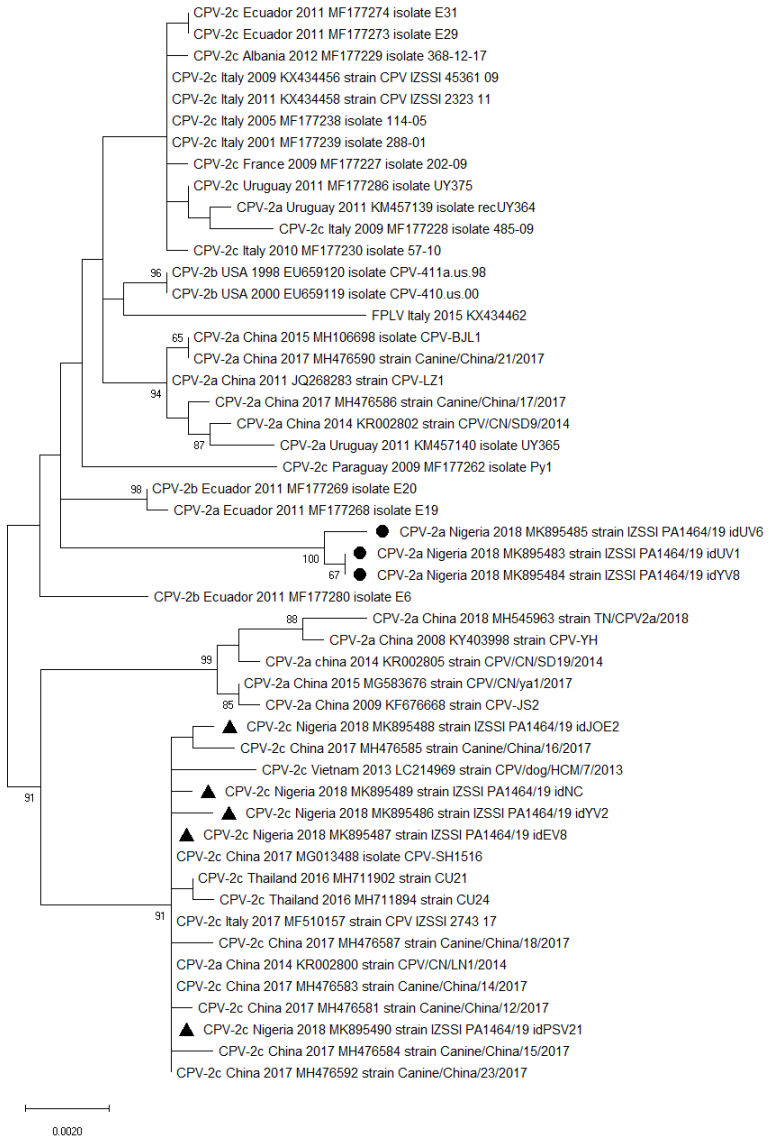

Figure 2. Maximum-likelihood tree based on 49 full-length NS1 gene sequences of canine parvovirus type 2 strains (bootstrap 1000 replicates; bootstrap values greater than 65 are shown). Black dots (●) and black triangles (▲) markings indicate CPV-2a and CPV-2c strains from Nigeria, respectively, analysed in this study. Each sequence is indicated with virus type (FPLV: feline panleukopenia virus - CPV: canine parvovirus) or variant (CPV-2, CPV-2a, CPV-2b, CPV-2c), country and year of collection, accession number and strain/isolate name. The scale bar indicates the estimated numbers of nucleotide substitutions per site.

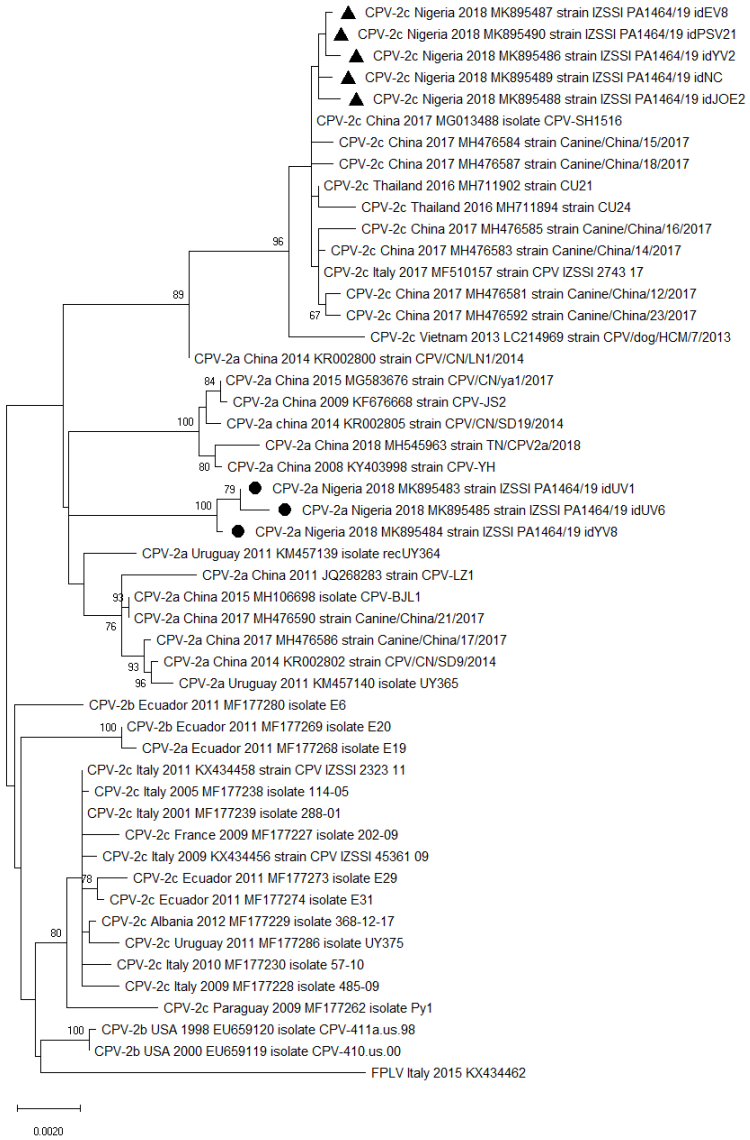

Figure 3. Maximum-likelihood tree based on 49 complete genome sequences of canine parvovirus type 2 strains (bootstrap 1000 replicates; bootstrap values greater than 65 are shown). Black dots (●) and black triangles (▲) markings indicate CPV-2a and CPV-2c strains from Nigeria, respectively, analysed in this study. Each sequence is indicated with virus type (FPLV: feline panleukopenia virus - CPV: canine parvovirus) or variant (CPV-2, CPV-2a, CPV-2b, CPV-2c), country and year of collection, accession number and strain/isolate name. The scale bar indicates the estimated numbers of nucleotide substitutions per site.

Supplementary Table 1. Identification code, origin, breed, gender, age, vaccination and clinical status of tested dogs.

ID code	City	Date of collection	Breed	Gender	Age	Vaccination status	Clinical signs	Remarks
YV 2	Makurdi	18/02/18	Caucasian	Male	6 weeks	Vaccinated	Diarrhoea, fever, anorexia, vomiting and weakness	Clinical
YV 7	Makurdi	27/02/18	Caucasian	Male	7 weeks	Vaccinated	Diarrhoea, vomiting, dehydrated and anorexia	Clinical
YV 8	Makurdi	05/03/18	Caucasian	Female	12 weeks	Vaccinated	Diarrhoea, vomiting, dehydrated emaciation and anorexia	Clinical
OBA 1	Makurdi	20/02/18	Rottweiler	Female	8 weeks	Vaccinated	Bloody diarrhoea, vomiting, emaciation, dehydrated and anorexia	Clinical
PB 3	Makurdi	10/03/18	Neapolitan mastiff	Male	6 weeks	Vaccinated	Lethargy, fever, dehydrated and vomiting	Clinical
DV 1	Makurdi	30/01/18	German shepherd	Male	12 weeks	Vaccinated	No sign	Apparently healthy
JOE 2	Makurdi	30/01/18	Chihuahua	Male	7 weeks	Vaccinated	No sign	Apparently healthy
P 1	Makurdi	08/03/18	Rottweiler	Male	6 months	Vaccinated	No sign	Apparently healthy
ZK 3	Makurdi	30/01/18	Caucasian	Male	7 weeks	Vaccinated	No sign	Apparently healthy
5	Jos	05/04/18	Caucasian	Male	4 months	Vaccinated	Diarrhoea, vomiting, dehydrated and anorexia	Clinical
NC	Jos	23/03/18	Caucasian	Male	10 weeks	Vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
CVC 6	Jos	20/01/18	Mixed breed	Male	6 weeks	Vaccinated	Bloody diarrhoea, fever, anorexia, vomiting and weakness	Clinical
CVC 11	Jos	28/01/18	Caucasian	Female	14 weeks	Vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
YA 3	Jos	14/05/18	Caucasian	Male	6 weeks	Vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical

YA 4	Jos	14/05/18	Caucasian	Female	10 weeks	Vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
NV 8	Jos	07/02/18	Boerboel	Male	10 weeks	Vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
NV 19	Jos	17/03/18	Rottweiler	Male	5 months	Vaccinated	Bloody diarrhoea, vomiting and anorexia	Clinical
PSV 14	Jos	15/01/18	Local breed	Male	8 weeks	Vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
2	Jos	20/04/18	Caucasian	Male	16 weeks	Vaccinated	No sign	Apparently healthy
NV 4	Jos	27/01/18	Rottweiler	Male	10 weeks	Vaccinated	No sign	Apparently healthy
NV 10	Jos	19/02/18	Caucasian	Female	10 weeks	Vaccinated	No sign	Apparently healthy
NV 15	Jos	28/02/18	German shepherd	Male	9 weeks	Vaccinated	No sign	Apparently healthy
CVC 1	Jos	10/01/18	Local breed	Female	2 years	Not vaccinated	Bloody diarrhoea, fever, anorexia, vomiting and weakness	Clinical
CVC 3	Jos	15/01/18	Mixed breed	Female	2 years	Not vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
CVC 10	Jos	25/01/18	Local breed	Female	9 weeks	Not vaccinated	Bloody diarrhoea, vomiting, emaciation, dehydrated and anorexia	Clinical
CVC 14	Jos	10/02/18	German shepherd	Male	6 months	Not vaccinated	Bloody diarrhoea, fever, anorexia, vomiting and weakness	Clinical
CVC 21	Jos	21/02/18	Local breed	Female	12 weeks	Not vaccinated	Bloody diarrhoea, fever, anorexia, vomiting and weakness	Clinical
PSV 17	Jos	24/01/18	Local	Male	8 weeks	Not vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical

PSV 21	Jos	01/02/18	Local breed	Male	11 weeks	Not vaccinated	Bloody diarrhoea, fever, anorexia, vomiting and weakness	Clinical
PSV 24	Jos	07/03/18	Local breed	Female	6 months	Not vaccinated	Bloody diarrhoea, vomiting, emaciation, dehydrated and anorexia	Clinical
PSV 32	Jos	22/03/18	Bull mastiff	Male	6 weeks	Not vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
V 1	Jos	10/04/18	Caucasian	Female	12 weeks	Not vaccinated	Bloody diarrhoea, fever, anorexia, vomiting and weakness	Clinical
PSV 5	Jos	09/01/18	Mixed breed	Male	8 weeks	Not vaccinated	No sign	Apparently healthy
PSV 15	Jos	19/01/18	Mixed breed	Male	8 weeks	Not vaccinated	No sign	Apparently healthy
EV 8	Abuja	24/02/18	Caucasian	Female	5 months	Vaccinated	Diarrhoea, and anorexia	Clinical
K 6	Abuja	01/03/18	Caucasian	Male	8 months	Vaccinated	Bloody diarrhoea, vomiting and anorexia	Clinical
VC 5	Abuja	04/04/18	Caucasian	Female	8 weeks	Vaccinated	Diarrhoea and weakness	Clinical
EV 1	Abuja	10/01/18	Neapolitan mastiff	Male	7 weeks	Vaccinated	No sign	Apparently healthy
EV 3	Abuja	17/01/18	Caucasian	Male	7 weeks	Vaccinated	No sign	Apparently healthy
GS 3	Abuja	21/02/18	Caucasian	Female	10 weeks	Vaccinated	No sign	Apparently healthy
K 1	Abuja	20/02/18	Bull mastiff	Female	4 months	Vaccinated	No sign	Apparently healthy
MV 1	Abuja	13/01/18	Caucasian	Male	8 weeks	Vaccinated	No sign	Apparently healthy
OY 1	Abuja	26/01/18	Bull mastiff	Female	16 weeks	Vaccinated	No sign	Apparently healthy
SHA 1	Abuja	30/01/18	Neapolitan mastiff	Female	11 weeks	Vaccinated	No sign	Apparently healthy
EV 5	Abuja	10/02/18	Bull mastiff	Male	9 weeks	Not vaccinated	Bloody diarrhoea, vomiting and anorexia	Clinical

RKB 2	Abuja	09/01/18	Lhasa apso	Male	5 weeks	Not vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
UV 1	Abuja	06/02/18	Local breed	Male	6 months	Not vaccinated	Diarrhoea and anorexia	Subclinical
UV 6	Abuja	10/03/18	Local breed	Male	10 weeks	Not vaccinated	Dullness and Diarrhoea	Subclinical
VW 1	Abuja	13/01/18	Local breed	Female	10 weeks	Not vaccinated	Diarrhoea and dullness	Subclinical
N 1	Lafia	01/03/18	Caucasian	Male	9 weeks	Vaccinated	Bloody diarrhoea, vomiting, emaciation, dehydrated and anorexia	Clinical
RF 3	Lafia	15/01/18	German shepherd	Male	7 weeks	Vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
JA	Lafia	15/03/18	Caucasian	Male	8 weeks	Vaccinated	No sign	Apparently healthy
LO 11	Lafia	20/03/18	Mixed breed	Female	2 years	Vaccinated	No sign	Apparently healthy
V 4	Lafia	15/03/18	German shepherd	Female	18 weeks	Not vaccinated	Bloody diarrhoea, vomiting, dehydrated and anorexia	Clinical
V 6	Lafia	29/03/18	Mixed breed	Female	5 months	Not vaccinated	Bloody diarrhoea, vomiting and anorexia	Clinical
V 7	Lafia	09/04/18	Caucasian	Male	5 months	Not vaccinated	Diarrhoea, vomiting, dehydrated and anorexia	Clinical
LO 4	Lafia	25/02/18	Mixed breed	Male	18 months	Not vaccinated	No sign	Apparently healthy
LO 5	Lafia	12/03/18	Mixed breed	Female	18 months	Not vaccinated	No sign	Apparently healthy
LTG 3	Lafia	20/04/18	Local breed	Male	12 weeks	Not vaccinated	No sign	Apparently healthy

Supplementary Table 2. Primers used in the conventional PCR assays and sequencing analyses.

Assay	Primer	Sequence	Position[a]	Amplicon size (bp)	Reference
Conventional PCR	VP2-850	GAGCATTGGGCTTACCA	3633-3649	700	Touhiri et al., 2009
	VP2-1550	GCAGATGCATCAGGATC	4316-4332		
VP2 gene sequencing	P1	ATGAGTGATGGAGCAGTTC	2786-2804	1745	Battilani et al., 2001
	VPR	TTTCTAGGGTGCTAGTTGAG	4512-4530		Mokizuki et al., 1996
Internal primers[b]	41-FOX2	GCCCTTGTGTAGACGC	3824-3839		Truyen et al., 1998
	353	CCTGGATTAAACCAAACTCC	3137-3156		kindly provided by Dr. A. Balboni
Whole genome sequence	NS-Fext	GACCGTTACTGACATTCGCTTC	206-227	2255	Pérez et al., 2014
	NS-Rext	GAAGGGTTAGTTGGTTCTCC	2441-2460		
	2161F	TTGGCGTTACTCACAAAGACGTGC	2160-2183	2788	
	4823R	ACCAACCACCCACACCATAACAAC	4924-4947		
ORF1 internal primers[c]	NS-Fint	GTTGAAACCACAGTGACGACAG	1055-1076		
	NS-Rint	CATCATCCAGTCTTCAGGTG	1167-1186		
ORF2 internal primers[c]	3475R	GTTGGTGTGCCACTAGTTCCAGTA	3451-3474		
	R2	TTTTGAATCCAATCTCCTTCTGGAT	4011-4035		

[a]Nucleotide positions refer to the prototype CPV strain CPV-N (U.S.A. – 1978; accession n.: M19296). [b]Primers used for Sanger sequencing of the partial VP2 gene sequence or [c]of the full-length genome.

Supplementary Table 3. VP2 synonymous changes of analysed CPV strains described in this study.

CPV variant	ID Code	VP2 nucleotide (amino acids)															
		36 (12)	**135 (45)**	**207 (69)**	**303 (101)**	**318 (106)**	**639 (213)**	**831 (277)**	**963 (321)**	**969 (323)**	**1014 (338)**	**1074 (358)**	**1083 (361)**	**1275 (425)**	**1509 (503)**	**1629 (543)**	**1659 (553)**
CPV-2a	UV1	g (Q)	c (F)	a (V)	c (T)	a (V)	a (Q)	c (H)	t (N)	t (N)	t (Y)	t (A)	g (R)	g (T)	g (A)	t (H)	t (I)
CPV-2a	UV6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPV-2a	YV8	-	-	-	-	-	g	-	-	c	-	a	-	a	-	-	-
CPV-2a	LTG3	-	-	-	-	-	-	-	-	c	-	a	-	a	-	-	-
CPV-2a	RKB2	-	-	-	-	-	-	-	-	c	-	a	-	a	-	-	-
CPV-2c	[a]	a	t	g	t	-	-	t	c	c	-	a	-	a	a	-	c
CPV-2c	JOE2	-	-	-	-	-	-	-	-	-	c	-	-	-	-	-	-
CPV-2c	NC	-	-	-	-	g	-	-	-	-	-	-	-	-	-	-	-
CPV-2c	PSV15	-	-	-	-	g	-	-	-	-	-	-	-	-	-	-	-
CPV-2c	CVC3	-	-	-	-	g	-	-	-	-	-	-	-	-	-	-	-
CPV-2c	EV3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	c	-
CPV-2c	EV8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	c	-
CPV-2c	NV4	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-	-

[a]All other CPV-2c strains (ID Code: PB3, YV2, NV19, YA3, NV10, CVC14, PSV21, CVC1, CVC21, PSV5, VC5, MV1, EV5, RF3, N1, LO11, V4). "-" same amino acid as in the first row.