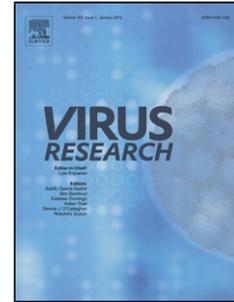


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Molecular Characterization of Canine Parvovirus and Canine Enteric Coronavirus in Diarrheic Dogs on the Island of St. Kitts: First Report from the Caribbean Region

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Highlights

- Detection of CPV (24%) and CCoV (4.8%) in dogs on Caribbean island of St. Kitts
- New CPV-2a (CPV-2a-297A) was the predominant CPV variant in St. Kitts
- The CCoV strains from St. Kitts were assigned to CCoV-I genotype
- First report on genetic diversity of CPV and CCoV from the Caribbean region

Abstract

Although canine parvovirus (CPV) and canine enteric coronavirus (CCoV) are important enteric pathogens of dogs and have been studied extensively in different parts of the world, there are no reports on these viruses from the Caribbean region. During 2015-2016, a total of 104 diarrheic fecal samples were collected from puppies and adult dogs, with or without hemorrhagic gastroenteritis, on the Caribbean island of St. Kitts (KNA). By PCR, 25 (24%, n=104) samples tested positive for CPV. Based on analysis of the complete deduced VP2 amino acid sequences, 20 of the KNA CPV strains were assigned to new CPV-2a (also designated as CPV-2a-297A). On the other hand, the VP2 genes of the remaining 5 strains were partially characterized, or could not be sequenced. New CPV-2a was the predominant CPV variant in St. Kitts, contrasting the molecular epidemiology of CPV variants reported in most studies from nearby North and South American countries. By RT-PCR, CCoVs were detected in 5 samples (4.8%, n=104). Based on analysis of partial M-protein gene, the KNA CCoV strains were assigned to CCoV-I genotype, and were closely related to CCoV-I strains from Brazil. To our knowledge, this is the first report on detection and genetic diversity of CPV and CCoV in dogs from the Caribbean region, and underscores the importance of similar studies in the other Caribbean islands.

Key words: Canine parvovirus; Canine enteric coronavirus; Genetic diversity; Caribbean region; new CPV-2a; CCoV-I

1. Introduction

Viruses are important etiological agents of diarrhea in domestic and wild canids. Among them, canine parvovirus (CPV), a member of the family *Parvoviridae*, is a major cause of hemorrhagic gastroenteritis in dogs (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016). CPV are small, nonenveloped viruses consisting of a single-stranded, negative sense DNA (~5.2 kb) molecule (Berns and Parrish, 2013; Parrish, 1999; Reed et al., 1988). The CPV genome contains two large open reading frames (ORF). The right ORF encodes 2 structural proteins (VP1 and VP2) by alternative splicing of the same mRNAs, whilst the left ORF codes for 2 nonstructural proteins (NS1 and NS2). The CPV VP2 capsid protein is antigenically significant, and has been implicated in governing host range restriction, tropism, and viral-host interactions (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016; Parrish, 1999).

Most studies on molecular epidemiology of CPV are based on the VP2-encoding gene (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016; Truyen, 2006). CPV emerged as a new enteric pathogen of domestic dogs in the late 1970s, possibly through host switching events involving a feline panleukopenia parvovirus, or a closely related virus (Berns and Parrish, 2013; Parrish, 1999). As a result of accumulation of mutations in the VP2- encoding gene, the original CPV strain (strain CPV-2) eventually got replaced with antigenic variants CPV-2a, CPV-2b, CPV-2c, new CPV-2a, and new CPV-2b that are variously distributed in dog populations worldwide (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016).

Although the current CPV vaccines, derived from the original CPV-2 strains, or CPV-2b strains, have been shown to confer protective immunity against CPV disease, and post-vaccination reactions have rarely been encountered in immunized dogs, the emergence of new genetic and antigenic variants underscores the importance of constant monitoring of evolution patterns of CPV strains circulating in dogs throughout the world (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016).

Canine coronavirus (CCoV) (family *Coronaviridae*, genus *Alphacoronavirus*, species *Alphacoronavirus-1*) usually cause mild, self-limiting enteritis in dogs, although fatal disease has been observed with a pantropic variant of CCoV (Decaro and Buonavoglia, 2008, 2011; Decaro et al., 2013; Pinto et al., 2014). CCoV are enveloped viruses with a single-stranded, positive sense RNA (27-31 kb) genome (Decaro and Buonavoglia, 2008). The CCoV membrane (M) protein is the most abundant structural protein and has been shown to elicit antibodies, whilst the spike (S) glycoprotein is the main inducer of virus-neutralizing antibodies.

Based on analysis of the M- and/or S- protein encoding genes, CCoV strains have been classified into at least two genotypes, CCoV-I and CCoV-II (Decaro and Buonavoglia, 2008, 2011). Recently, CCoV-II strains were further classified into two subtypes, CCoV-IIa (classical strains) and CCoV-IIb (strains arising from putative recombination events between CCoV-II and transmissible gastroenteritis virus of swine) (Decaro and Buonavoglia, 2008, 2011; Le Poder, 2011). CCoVs have been detected in canine populations worldwide (Decaro and Buonavoglia, 2008, 2011).

The Caribbean region has a sizeable dog population, and dogs with diarrhea, including those with hemorrhagic gastroenteritis are routinely presented at veterinary clinics on these islands. Although the prevalence and genetic diversity of CPV and CCoV in dogs have been

extensively studied in different parts of the world including nearby Latin American countries, there are no reports on these important canine viruses from the Caribbean region so far. We report here the detection and molecular characterization of CPV and CCoV strains in dogs with diarrhea on the Caribbean island of St. Kitts (KNA).

2. Materials and methods

2.1. Sampling

During 2015-2016, a total of 104 diarrheic fecal samples were collected from puppies and adult dogs, with or without hemorrhagic gastroenteritis, at two veterinary clinics (the Ross University School of Veterinary Medicine Clinic, and the Ponds Veterinary Clinic) on the island of St. Kitts, Caribbean region. The samples were stored at -20°C until further analysis. The present study was conducted in compliance with good laboratory practice (GLP).

2.2. Amplification of VP2 gene of CPV

For PCR, viral DNA was extracted from the fecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen Sciences, MD, USA). Samples were screened for the presence of CPV using a PCR-based detection assay targeting a 583-bp stretch of the 3'- portion of the VP2-encoding gene, as described previously (Buonavoglia et al., 2001). In order to determine the CPV variant, a 1799 bp fragment of CPV genome containing the complete ORF of VP2 gene was amplified using a newly designed primer VP2F (5'-ATG AGT GAT GGA GCA GTT CAA CC-3', corresponding to nucleotide [nt] 2787-nt 2809 of reference strain CPV-b), and primer 555rev (Buonavoglia et al., 2001). PCRs were performed using Platinum™ Taq DNA Polymerase (Invitrogen, CA, USA) following manufacturer's instructions. PCR-grade water was used as the negative control.

2.3. Amplification of M-protein encoding gene of CCoV

Viral RNA was extracted from fecal samples using the QIAamp Viral RNA Mini Kit (Qiagen Sciences, MD, USA). For detection of CCoV in fecal samples, RT-PCR based on a partial stretch (409 bp) of M protein-encoding gene was performed as reported previously (Pratelli et al., 1999). RT-PCRs were carried out using SuperScript® III RT (Invitrogen, CA, USA) and Platinum™ Taq DNA Polymerase (Invitrogen, CA, USA) following manufacturers' instructions. We used PCR-grade water as the negative control.

2.4. Nucleotide sequencing

For nt sequencing, PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Sciences, MD, USA) according to manufacturer's protocol. Nucleotide sequences were obtained using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA, USA) on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, CA, USA.). The PCR products were sequenced in both directions.

2.5. Sequence analysis

Homology search for related cognate sequences was performed using standard nt BLAST program (Basic Local Alignment Search Tool, www.ncbi.nlm.nih.gov/blastn). Multiple alignments of deduced amino acid (aa) sequences were performed using the CLUSTALW program (version ddbj, <http://clustalw.ddbj.nig.ac.jp/>) with default parameters. Phylogenetic trees were constructed using the MEGA (v5.2.2) software.

2.6. Nucleotide sequence accession numbers

The GenBank accession numbers for nt sequences of complete, or partial ORF of VP2 genes of the KNA CPV strains, and partial M-protein encoding genes of the KNA CCoV strains are shown in Table 1.

3. Results and Discussion

The federation of St. Kitts and Nevis is a twin island nation in the Lesser Antilles of the Caribbean region with a total human population of ~55,000 (Map is shown in Supplementary fig. S1). Although there are no official estimates on the canine population of St. Kitts, different breeds of domestic dogs, including a local island breed are kept as pets in many households on the island. Diarrhea, including hemorrhagic gastroenteritis is prevalent in domestic dogs on St. Kitts, as evident from clinical cases that are presented now and then at the two major veterinary clinics (the Ross University School of Veterinary Medicine Clinic [RVC], and the Ponds Veterinary Clinic [PVC]) on the island. In the present study, CPV, or CCoV were detected in 30 (28.8%) of the 104 fecal samples obtained from diarrheic dogs at the two veterinary clinics on St. Kitts (Table 1).

3.1. Canine parvovirus

By PCR of the partial VP2 gene, a total of 25 (24%, n= 104) dogs tested positive for CPV. Among them, 15 dogs were presented with hemorrhagic gastroenteritis, whilst the remaining 10 dogs had severe diarrhea. All the CPV positive dogs were sporadic cases from different households across the island of St. Kitts. The age of the dogs that tested positive for CPV ranged from 3 days up to 3 years of age (Table 1). Eighteen of the 25 CPV positive dogs were aged < 6 months, corroborating previous observations that dogs up to 6 months may exhibit a greater risk of infection (Decaro and Buonavoglia, 2012; Miranda et al., 2015). Most of the CPV positive samples were from mixed breeds (Table 1), contradicting a previous observation that purebreds were more susceptible to CPV disease than mixed breeds (Kalli et al., 2010). However, a few other studies have shown that breed may not be a risk factor (Miranda et al., 2015).

Vaccination is crucial to control and prevent CPV disease (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016). Lack of vaccination, incomplete vaccination schedules, or vaccine failures, primarily due to interference with maternal antibodies may predispose dogs to natural infection. Moreover, some studies have raised questions on the complete efficacy of the current CPV vaccines against the CPV antigenic variants, as these vaccines were derived from old CPV-2 strains (Decaro et al., 2008, 2009; Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016; Truyen, 2006). In the present study, nearly half (11/25) of the CPV positive dogs did not receive any vaccine, whilst 2 puppies did not attain the recommended minimum age of vaccination (6 weeks) at the time of sampling (Table 1). Among the vaccinated dogs that tested positive for CPV (4/25), only one animal had received two doses of the vaccine (Table 1). Therefore, lack of, or inadequate protective immunity might have predisposed these dogs to CPV disease, although the role/s of other risk factors, such as endoparasitism and unsanitary environments cannot be ruled out (Miranda et al., 2015). On the other hand, no information was available on the immunization status of the remaining CPV positive dogs (Table 1). Information on vaccination status was available for only 26 of the 79 dogs that tested negative for CPV. Eleven of these dogs received one, or more doses of CPV vaccine before sampling, whilst 15 were not immunized against CPV.

To study the genotype nature and evolution of CPV strains circulating in St. Kitts, we determined the complete ORF nt sequences of VP2 genes of 20 KNA strains (Table 1). The deduced VP2 aa sequences of all the 20 KNA CPV strains exhibited 297-A, 426-N and 555-V, and therefore, were classified as new CPV-2a (CPV-2a-297A) strains (Table 2; Supplementary fig. S2). On the other hand, the VP2 genes of the remaining 5 KNA strains were partially characterized, or could not be sequenced due to insufficient volumes of fecal samples (Table 1).

Other relevant mutations that may influence viral antigenicity and/or host range have also been reported in many of the recent CPV-2a/2b strains, such as G300D, Y324I and T440A (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016). However, these mutations were not observed in the putative VP2 proteins of the KNA new CPV-2a strains (Table 2, Supplementary fig. S2).

The VP2 of KNA CPV strains shared 99-100% nt and deduced aa identities among themselves, and with those of other new CPV-2a strains. Nucleotide and deduced aa identities of 99% were also observed with the VP2 of the CPV-2, CPV-2a, CPV-2b, new CPV-2b, and CPV-2c strains. By phylogenetic analysis of complete ORF nt sequences of VP2 genes, the KNA strains clustered together, and were found to be more closely related to new CPV-2a strains than other CPV-2 variants (Fig. 1), corroborating the observations at deduced aa level.

Although the present study is the first report on CPV variants from the Caribbean region, the molecular epidemiology of CPV has been documented in nearby North and South American countries (Miranda and Thompson, 2016). In Ecuador and USA, CPV-2b and CPV-2c were more prevalent than CPV-2a. Only CPV-2c strains have been reported from Mexico and Paraguay. CPV-2c was the major variant in Argentina and Brazil (Calderón et al., 2012; Pinto et al., 2012). CPV-2b was the major genotype in Canada (Gagnon et al., 2016). In Uruguay, CPV-2c was the predominant variant during 2006-2010 (Miranda and Thompson, 2016). However, new CPV-2a variants emerged in 2010 (Pérez et al., 2012), and went on to become the major CPV-2 variant in Uruguay in 2011 (Maya et al., 2013). A recent study reported CPV-2a as the major variant in Columbia (Duque-García et al., 2017). In the present study, new CPV-2a was found to be the predominant CPV variant in domestic dogs on the Caribbean island of St. Kitts, contrasting the

molecular epidemiology of CPV reported in most studies from North and South American countries.

3.2. *Canine enteric coronavirus*

By RT-PCR targeting a partial fragment of the M protein encoding gene, 5 of the 104 samples tested positive for CCoV (Table 1). Among these sporadic cases, one sample was from an adult dog with mild enteritis, whilst the remaining fecals were from puppies exhibiting mild to severe diarrhea. In the present study, high quality nt sequences of the partial M-protein gene (369 bp, excluding the 5'- and 3'- end primers) could be obtained for 4 of the KNA CCoV strains (Table 1).

The partial M-protein gene of the KNA CCoV strains shared 100% nt sequence identities between themselves. With other CCoVs, the partial M protein gene sequences of the KNA CCoV strains shared maximum nt and deduced aa sequence identities of 99% and 100%, respectively, with those of several Brazilian CCoV-I strains. Absolute deduced aa sequence identities were also observed with cognate stretch of reference CCoV-I strain 23/03/ITA. On the other hand, nt and deduced aa identities of < 91% and < 94%, respectively, were observed with those of CCoV-II (CCoV-IIa and -IIb) strains. Alignment of partial deduced M protein aa sequences of the KNA CCoV strains with those of CCoV-I, and CCoV-IIa and -IIb strains is shown in supplementary fig. S3. By phylogenetic analysis of partial M protein genes, the KNA CCoV strains were found to be closely related to CCoV-I strains from Brazil within the CCoV-I cluster, and were distantly related to CCoV-II and feline coronavirus strains (Fig. 2). Based on analysis of partial M-protein gene, the KNA CCoV strains were found to belong to CCoV-I genotype.

Whilst the molecular epidemiology of CCoV has been studied in different Asian and European countries (Cavalli et al., 2014; Decaro and Buonavoglia, 2008, 2011; Decaro et al.,

2010, 2011, 2013; Erles et al., 2009; Jeoung et al., 2014; McElligott et al., 2011; Ntafis et al., 2013; Soma et al., 2011; Wang et al., 2016), there is a dearth of data on distribution of CCoV genotypes from the North and South Americas. In Brazil, CCoV-I and CCoV-II strains, including pantropic CCoV-IIa variants were found to be circulating in the domestic dog population (Moutinho et al., 2014; Pinto et al., 2014). CCoV-IIa and -IIb genotypes have been associated with fatal enteritis in puppies in USA (Licitra et al., 2014). Although CCoVs were detected at lower frequencies in St. Kitts, this is the first report on detection and genotyping of CCoV strains from the Caribbean region.

4. Conclusions

We reported here the detection and molecular characterization of CPV and CCoV on the Caribbean island of St. Kitts. Although PCR/RT-PCR have been used extensively for detection of CPV/CCoV in many studies including recent reports, these screening assays have been shown to be relatively less sensitive than qPCR/RT-qPCR (Decaro et al., 2005a, b; Duque-García et al., 2017; Kumar and Nandi, 2010; Miranda and Thompson, 2016; Ntafis et al., 2013; Wang et al., 2016). One of the limitation of this study was the use of PCR/RT-PCR as screening assays. However, considering the lack of data from the Caribbean region, the present study primarily focused on detection and molecular characterization of circulating strains rather than strictly monitoring prevalence. New CPV-2a (CPV-2a-297A) was found to be the predominant CPV variant on St. Kitts. CPV-2a has been shown to be the major variant in Asia, whilst CPV-2c, or CPV-2b were predominant in most studies from the North and South Americas (Miranda and Thompson, 2016; Zhou et al., 2017). However, the predominance of CPV-2a variants in recent studies from Columbia, Uruguay, and St. Kitts pointed towards the changing epidemiology of CPV in this part of the world. The KNA CCoV strains were genetically closely related to

Brazilian CCoV-I strains, which may be attributed to the geographical proximity of the Caribbean region to Latin American countries. Although this is the first report on detection and genetic diversity of CPV and CCoVs from the Caribbean region, the present study was based on a single island. In order to gain vital insights into the molecular epidemiology of these important canine viruses across the entire Caribbean region, similar studies are required in the other islands.

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Figure legends:

Fig 1. Phylogenetic analysis of the complete ORF nucleotide sequences of VP2 genes of KNA CPV strains with those of CPV-2, CPV-2a, CPV-2b, CPV-2c, new CPV -2a, new CPV-2b, and vaccine strains. Feline parvovirus (FPV) strain CU-4 clustered as the outgroup. The phylogenetic tree was created by the Maximum Likelihood (ML) method, and statistically supported by bootstrapping with 1000 replicates. Phylogenetic distances were measured by the Tamura-3-parameter model. The clustering patterns of KNA CPV strains were validated by constructing ML trees with other models, such as the Jukes–Cantor model, Kimura 2-parameter model, and Hasegawa–Kishino–Yano model (data not shown). In the tree, positions of the KNA CPV strains are highlighted with dark circles. GenBank accession numbers are shown in parentheses. Bootstrap values < 70% are not shown. Scale bar, 0.005 substitutions per nucleotide.

Fig. 1

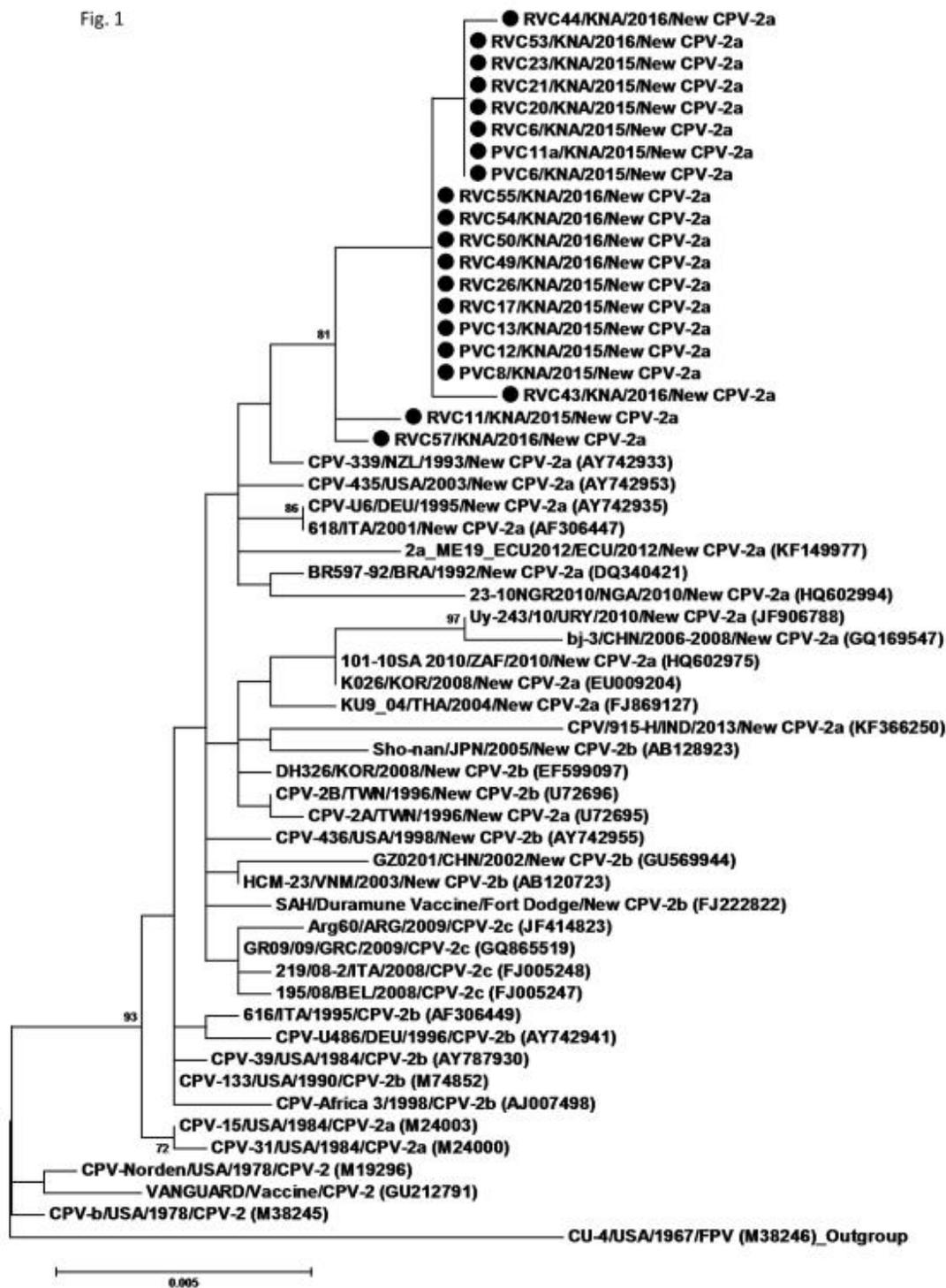


Fig 2. Phylogenetic tree constructed by the Maximum Likelihood (ML) method from nucleotide sequences of partial M-protein encoding genes (369 bp) of the KNA CCoV strains with those of CCoV-I, CCoV-II, and feline coronavirus strains. The tree was statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances were measured using the Tamura-3-parameter model of substitution. The clustering patterns of KNA CCoV strains were validated by creating ML trees with other mathematical models, such as the Jukes–Cantor model, Kimura 2-parameter model, and Hasegawa–Kishino–Yano model (data not shown). In the tree, the positions of the KNA CCoV strains are shown by dark circles. GenBank accession numbers are shown in parentheses. Bootstrap values < 70% are not shown. Scale bar, 0.05 substitutions per nucleotide.

Fig. 2

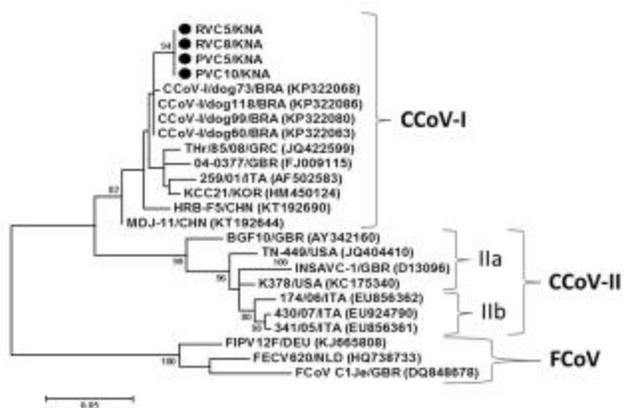


Table 1. Sampling dates, breed, age, and vaccination status of dogs that tested positive for canine parvovirus (CPV), or canine enteric coronavirus (CCoV) on the Caribbean island of St. Kitts (KNA). The GenBank accession numbers, and genotype nature of the CPV and CCoV strains are also shown.

Virus	No.	Strain	Sampling date	Breed	Age	Variant	Vaccination status against CPV ^a	Gene analyzed	GenBank accession no.
CPV	1	RVC6/KNA	Feb 2015	Island mix ^b	1month 1 week	New CPV-2a ^c	None	VP2, complete ORF ^d	KY399033
	2	RVC11/KNA	June 2015	Mixed breed	3 months	New CPV-2a	None	VP2, complete ORF	KY399034
	3	RVC15/KNA	June 2015	Dachshund	1 month 2weeks	-	None	Not sequenced	-
	4	PVC6/KNA	June 2015	Pit bull	6 months	New CPV-2a	Not available	VP2, complete ORF	KY399035
	5	PVC8/KNA	June 2015	Rottweiler x Mastiff	5 months	New CPV-2a	Not available	VP2, complete ORF	KY399036
	6	RVC17/KNA	July 2015	Island mix	Adult ^e	New CPV-2a	None	VP2, complete ORF	KY399037
	7	RVC20/KNA	July 2015	Island mix	5 months	New CPV-2a	None	VP2, complete ORF	KY399038
	8	RVC21/KNA	July 2015	Island mix	5 months	New CPV-2a	None	VP2, complete ORF	KY399039
	9	PVC11a/KNA	July 2015	Mastiff mix	3 months	New CPV-2a	Not available	VP2, complete ORF	KY399040
	10	PVC12/KNA	July 2015	Island mix	2 months	New CPV-2a	Not available	VP2, complete ORF	KY399041
	11	PVC13/KNA	July 2015	Island mix	2 months	New CPV-2a	Not available	VP2, complete ORF	KY399042
	12	RVC23/KNA	Aug 2015	Mixed breed	2 years	New CPV-2a	None	VP2, complete ORF	KY399043
	13	RVC26/KNA	Aug 2015	Mastiff mix	6 months	New CPV-2a	None	VP2, complete ORF	KY399044
	14	RVC43/KNA	Apr 2016	Island mix	1 month 3 weeks	New CPV-2a	02-2016 ^f	VP2, complete ORF	KY399045
	15	RVC44/KNA	Apr 2016	Island mix	1 year	New CPV-2a	06-2015, 07-2015 ^f	VP2, complete ORF	KY399046
	16	PVC18/KNA	Apr 2016	Island mix	1 year	- ^g	Not available	VP2, partial ORF	KY399047
	17	PVC21/KNA	Apr 2016	Island mix	3 years	- ^g	Not available	VP2, partial ORF	KY399048
	18	PVC28/KNA	May 2016	Island mix	1 year	-	Not available	Not sequenced	-
	19	RVC49/KNA	June 2016	Mastiff mix	2 months	New CPV-2a	None	VP2, complete ORF	KY399049
	20	RVC50/KNA	June 2016	Pit bull mix	6 months	New CPV-2a	None	VP2, complete ORF	KY399050
	21	RVC53/KNA	July 2016	Rottweiler	4 months 3 weeks	New CPV-2a	05-2016 ^f	VP2, complete ORF	KY933479
	22	RVC54/KNA	July 2016	Pit bull mix	3 months	New CPV-2a	None	VP2, complete ORF	KY399051
	23	RVC55/KNA	July 2016	Island mix	3 months	New CPV-2a	None	VP2, complete ORF	KY399052
	24	RVC57/KNA	Aug 2016	Island mix	3 days	New CPV-2a	None	VP2, complete ORF	KY399053
	25	RVC60/KNA	Aug 2016	Mixed breed	1 year 8 months	-	12-2015 ^f	Not sequenced	-
CCoV	1	RVC5/KNA	Feb 2015	Malinois	1 year 5 months	CCoV-I	Not applicable	M, partial ORF	KY933480
	2	RVC 8/KNA	June 2015	Mixed breed	6 months	CCoV-I	Not applicable	M, partial ORF	KY399054
	3	PVC5/KNA	June 2015	Island mix	3 months	CCoV-I	Not applicable	M, partial ORF	KY399055
	4	PVC10/KNA	June 2015	Pit bull	1 month 1 week	CCoV-I	Not applicable	M, partial ORF	KY399056
	5	RVC41/KNA	Feb 2016	Mixed breed	2 months 1 week	-	Not applicable	Not sequenced	-

^a Vaccination status against CPV at the time of collection of fecal sample.

^b Refers to a cross between a canine breed that is native to the island of St. Kitts, and another breed.

^c In a recent study, new CPV-2a has also been designated as CPV-2a-297A (Zhou et al., 2017)

^d ORF, Open Reading Frame

^e Information on exact age not available

^f Date/s (month-year) of vaccination with VANGUARD Plus 5 L4 (Zoetis, USA).

^g As only small amounts of fecal samples were received from the clinics, it was not possible to obtain the complete ORF sequence of VP2 gene. However, the partial deduced VP2 amino acid sequences of strains PVC18 and PVC21 exhibited 426-N and 555-V, seen in CPV-2a strains.

Table 2. Comparison of evolutionary relevant amino acid (aa) residues of putative VP2 proteins of canine parvovirus (CPV) strains detected on the island of St. Kitts (KNA), Caribbean region, with those of CPV-2, CPV-2a, CPV-2b, CPV-2c, new CPV-2a, new CPV-2b, and vaccine strains. The KNA CPV strains are underlined. A dot ‘.’ indicates an identical amino acid residue at cognate position of deduced VP2 aa sequence of the concerned CPV strain with that of reference strain CPV-b/USA/1978. Alignment of complete deduced aa sequences of putative VP2 proteins of the CPV strains is shown in supplementary fig. S2.

Amino acid position ^a	80	87	93	101	103	232	267	297 ^b	300	305	321	323	324	375	426 ^c	440	555	560	564	568	570	
Strain																						Variant
CPV-b/USA/1978	R	M	N	I	A	I	F	S	A	D	N	N	Y	N	N	T	V	N	S	G	K	CPV-2
CPV-15/USA/1984	.	L	.	T	G	Y	.	.	.	D	N	.	I	CPV-2a
CPV-39/USA/1984	.	L	.	T	G	Y	.	.	.	D	D	CPV-2b
<u>219/08-13/ITA/2008</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	E	CPV-2c
CPV-435/USA/2003	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
Pome/KOR/2005	.	L	.	T	.	.	.	A	D ^d	Y	.	.	.	D	.	A	New CPV-2a ^d
Uy-243/URY/2010	.	L	.	T	.	.	Y	A	G	Y	.	.	I	D	.	A	New CPV-2a
CPV-436/USA/2003	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	D	New CPV-2b
<u>PVC6/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>PVC8/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>PVC11a/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>PVC12/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>PVC13/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC6/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC11/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC17/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC20/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC21/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC23/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC26/KNA/2015</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC43/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC44/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC49/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC50/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC53/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC54/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC55/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
<u>RVC57/KNA/2016</u>	.	L	.	T	.	.	.	A	G	Y	.	.	.	D	New CPV-2a
Vaccine VANGUARD	E	CPV-2
Vaccine (Duramune) strain SAH	.	L	.	T	.	.	.	A	G	Y	K	.	.	D	D	E	New CPV-2b

^a Positions of amino acid residues are based on those of CPV strain CPV-b/USA/1978.

- ^b The S297A mutation has been found to be fixed in VP2 of recent CPV-2a and CPV-2b strains, and these variants are sometimes designated as new CPV-2a and new CPV-2b strains (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016). In a recent study, new CPV-2a and new CPV-2b strains have also been designated as CPV-2a-297A and CPV-2b-297A, respectively (Zhou et al., 2017).
- ^c Amino Acid residue 426 of VP2 constitutes the sole basis of differentiating the new CPV-2a (426-N), new CPV-2b (426-D), and CPV-2c (426-E) strains (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016).
- ^d New CPV-2a strains exhibiting G300D are also referred to as CPV-2a-297A300D (Zhou et al., 2017).