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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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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.

## 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

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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-I*) 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 CCoVs 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