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Epidemiological evolution of canine parvovirus in the Portuguese domestic dog population

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ABSTRACT

Since its emergence, canine parvovirus type 2 (CPV-2) has caused disease pandemics with severe gastroenteritis signs, infecting especially puppies. As a consequence of CPV rapid evolution a variety of genetic and antigenic variants have been reported circulating worldwide. The detection of additional variants of CPV circulating in the dog population in Portugal suggests monitoring of the disease is useful. The objectives of this study were to further detect and characterize circulating field variants from suspected CPV diseased dogs that were admitted to veterinary clinics distributed throughout the country, during 2012–2014. Of the 260 fecal samples collected, 198 were CPV positive by PCR, and CPV antigen was detected in 61/109 samples by Immunochromatographic (IC) test. The restriction fragment length polymorphism (RFLP) analysis of 167 samples revealed that 86 were the CPV-2c. Sequence analysis of the 198 strains confirmed that CPV-2c were the dominant variant (51.5%), followed by CPV-2b (47.5%) and CPV-2a (1%). The variants were irregularly distributed throughout the country and some were detected with additional non-synonymous mutations in the VP2 gene. Phylogenetic analysis demonstrated that the isolates were similar to other European strains, and that this virus continues to evolve.

1. Introduction

Canine parvovirus type 2 (CPV-2) is an important pathogen of domestic and wild canids and has spread worldwide since its emergence in the 1970s (Parrish et al., 1985; Nandi et al., 2013). This virus has originated as a host range variant from feline panleukopenia virus (FPV) that by via of wild carnivores has adapted to the canine host (Chang et al., 1992; Agbandje et al., 1993; Parker and Parrish, 1997). The CPV-2 belongs to the genus *Protoparvovirus,* member of the *Parvoviridae* family. It has a linear single-stranded genome, about 5.2 kilobases in length, with two major open reading frames (ORFs). The left ORF encodes nonstructural proteins, NS1 and NS2, which are essential for replication and DNA packaging, while the right ORF encodes the viral capsid proteins which are the main antigens that induce protective antibodies. The VP1 and VP2 capsid proteins are splice variants and are identical in sequence, except for a 143 amino acid N-terminal region that is unique to VP1 (Tsao et al., 1991; Agbandje et al., 1993).

The CPV-2 has genetically and antigenically changed and it was rapidly replaced by a new antigenic variant, called CPV-2a, in 1978 that regained the ability to infect felids due to five or six amino acid changes located in the VP2 gene (Parrish et al., 1985). Later, in 1984 in the United States and in 2000 in Italy new antigenic variants were described resulting from the presence of Asn to Asp (CPV-2b) or Glu (CPV-2c) at VP2 residue 426 located at the top of the three-fold spike on the capsid structure (Parrish et al., 1985, 1991; Buonavoglia et al., 2001). These antigenic variants of CPV-2 are currently circulating worldwide and their relative frequencies and genetic characteristics vary geographically and temporally. Infections by CPV have been reported in Africa, Asia, Australia, the Americas and Europe (Steinel et al., 1998; Decaro et al., 2007; Meers et al., 2007; Kumar and Nandi, 2010; Markovich et al., 2012; Perez et al., 2012; Castanheira et al., 2014). Houston et al. (1996) reported that at the end of 1983, CPV infection had been reported in 50 countries around the world. The prevalence of canine parvovirus in the domestic dog population in Portugal was found with an approximately equal distribution between CPV-2b

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Table 1
Fecal samples collected from dogs with clinical signs suspected of parvovirus infection between 2012 and 2014. The veterinary clinics were located in several regions: North (N); Center (C); Lisbon (L); Alentejo (A); Algarve (Al); Açores Island (Aç); Madeira Island (M). Genotype characterization was performed by DNA sequencing.

Year	CPV-2 variant	Number of samples (%)	Origin
2012	2a	1 (1.5)	1 (C)
	2b	23 (34.8)	5 (N) + 18 (C)
	2c	42 (63.6)	6 (N) + 24 (C) + 5 (A) + 7 (Aç)
		66 (100)	
2013	2b	51 (58.6)	24 (N) + 19 (C) + 1 (L) + 4 (A) + 3 (Al)
	2c	36 (41.4)	17 (N) + 6 (C) + 3 (L) + 5 (A) + 5 (Al)
		87 (100)	
2014	2a	1 (2.2)	1 (N)
	2b	20 (44.4)	6 (N) + 12 (C) + 1 (A) + 1 (Al)
	2c	24 (53.3)	7 (N) + 3 (C) + 1 (L) + 2 (A) + 1 (Al) + 5 (Aç) + 5 (M)
		45 (100)	

and CPV-2c, from samples collected in center region of the country during 2006–2007 (Decaro et al., 2007; Vieira et al., 2008). The presence of canine parvovirus in several wild carnivores has also been reported (Santos et al., 2009; Duarte et al., 2013), such as the presence of CPV-2c in a domestic cat (Miranda et al., 2014).

The aims of this study were to detect the occurrence of CPV disease and to characterize the various mutations in the viruses circulating in dogs admitted to veterinary clinics throughout the country, between 2012 and 2014.

2. Materials and methods

2.1. Samples

Fecal samples were collected from 260 dogs (aged 1 month to 13 years) of diverse breeds that had clinical signs suspected of parvovirus infection. Samples were provided by veterinary clinics located in several regions of Portugal, as North ($n = 88$), Center ($n = 101$), Lisbon ($n = 8$), Alentejo ($n = 25$), Algarve ($n = 18$), Açores Island ($n = 15$) and Madeira Island ($n = 5$), between January 2012 and November 2014.

2.2. Rapid detection of CPV antigen by immunochromatographic (IC) test

Of the 260 fecal dog samples, 109 were screened for CPV using the IC commercial kit (Antigen Rapid CPV Ag Test Kit, BioNote, Inc., Gyeonggi-do, Korea), following the manufacturer's instructions.

2.3. Detection and characterization of CPV

Viral DNA was extracted from the 260 fecal samples as previously described by Miranda et al. (2014). For CPV detection the primers amplifying a short stretch (583 nucleotides (nt)) of VP2 region as previously described by Miranda et al. (2015) were used. The restriction fragment length polymorphism (RFLP) characterization was done in the first obtained 167 PCR products that were digested with the enzyme *Mbo*II (FastDigest *Mbo*II, Fermentas, St. Leon-Rot, Germany), as described by Buonavoglia et al. (2001), to distinguish the CPV-2c variant from others. Two positive controls were used, the CPV-2b and CPV-2c variants, and a negative control.

2.4. Sequence and phylogenetic analysis

All positive samples were subject to sequence analysis. The PCR products were purified in the NZY Gel pure kit (NZYTech Genes & Enzymes, Lisbon, Portugal) before sequencing at a commercial laboratory. Selected isolates that turned positive in the initial screen were further amplified by sequencing the entire VP2 gene (1755 nt), the primer sequences are available from the authors by request. The obtained sequences were assembled, edited and aligned with reference strains obtained from the GenBank database, using the Geneious R6 software (Biomatters Ltd., Auckland, New Zeland).

A maximum likelihood (ML) phylogenetic tree of the sequences were obtained using MEGA6 (Tamura et al., 2013). The phylogenetic tree was constructed using only the full-length sequences of VP2 region, deposited in the GenBank (Fig. 3) and estimated using bootstrap from 1000 replicates.

3. Results

3.1. Detection of CPV

A total of 198 (76.2%) of the 260 extracted DNA samples were positive by PCR assay. These positive samples, 33.3% (66/89) were collected in 2012, 43.9% (87/103) in 2013 and 22.7% (45/68) in 2014. The samples in study, from different regions in the country were: 33.3% (66/88) North, 41.9% (83/101) Center, 2.5% (5/8) Lisbon, 8.6% (17/25) Alentejo, 5.1% (10/18) Algarve, 6.1% (12/15) Açores Island and 2.5% (5/5) Madeira Island. The samples are presented according the collection year and origin in Table 1.

The RFLP genotyping with *Mbo*II of the first 167 positive samples resulted in eighty-six (51.5%) digested and identified as CPV-2c variant. The remaining eighty-one (48.5%) were not digested and identified as CPV-2, -2a or -2b. Eighty of the latter samples were classified as CPV-2b and one sample as CPV-2a after sequencing. All CPV-2c that were identified by RFLP were also confirmed by sequencing.

One hundred and nine of the total samples in the study were tested by IC test, of which 61 (56%) were positive and 48 (44%) were negative.

3.2. Sequence analysis

All sequences recovered from dogs were CPV in type. According to the sequence at codon 426, one hundred and two (51.5%) were classified as CPV-2c, ninety-four (47.5%) as CPV-2b and two (1%) as CPV-2a.

Further comparative sequence analysis was performed using 68 selected sequences, that included 62 amplified as a short VP2 region (528 nt) and 6 as full-length VP2 sequences (1755 nt). Comparative sequence alignment indicated that the majority of the sequences were similar between them, showing most of them a change on the amino acid 426. Twenty-one representatives of these sequences were deposited in GenBank database (accession numbers KR559891–KR559911).