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Canine parvoviruses in New Zealand form a monophyletic group distinct from the viruses circulating in other parts of the world

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ABSTRACT

Canine parvovirus 2 (CPV-2) is a well-recognized cause of acute haemorrhagic enteritis in dogs worldwide. The aim of the current study was to identify which CPV-2 subtypes circulate among dogs in New Zealand, and to investigate the evolutionary patterns of contemporary CPV-2 viruses. Faecal samples were collected from 79 dogs with suspected CPV-2 infection over the period of 13 months, and tested for the presence of CPV-2 DNA by PCR. Of 70 positive samples, 69 were subtyped as CPV-2a and one as CPV-2. A majority of CPV-2 positive samples were collected from unvaccinated or not-fully vaccinated puppies ≤6 months of age. The haplotype network produced from New Zealand CPV-2 sequences showed no structure when assessed based on location, vaccination status or age of the animals sampled. International haplotype network indicated that, unlike CPV-2 from other countries, the population of CPV-2 in New Zealand appeared to be monophyletic.

1. Introduction

Canine parvovirus type 2 (CPV-2) is a non-enveloped DNA virus from the family Parvoviridae(Tijssen et al., 2012). It is a well-recognized cause of acute haemorrhagic enteritis in young dogs worldwide (Decaro and Buonavoglia, 2012), including New Zealand (Parrish et al., 1980, 1982). The virus emerged as a novel pathogen in the late 1970's, most likely as a result of cross-species transmission of feline panleukopenia virus (FPV) (Parrish et al., 2008; Wilson, 1980). Since then, it has continued to change, probably as a result of the on-going adaptation to the new canine host. The original CPV-2 was replaced with a new variant Gln-426 (CPV-2a), soon after its emergence (Parrish et al., 1988, 1985). The Gln-426 variant further evolved into Asp-426 (CPV-2b), with both viruses circulating concurrently in dog populations worldwide (Parrish et al., 1991). In 2001, another variant Glu-426 (CPV-2c) was detected in Italy (Buonavoglia et al., 2001). Initially, CPV2c was detected in adult vaccinated dogs with severe gastroenteritis, which raised concerns that it could represent a vaccine escape mutant (Decaro et al., 2008, 2009a). These concerns were later discounted, as vaccinated dogs were protected against CPV-2c challenge under experimental conditions (Larson and Schultz, 2008; Spibey et al., 2008), and the rate of CPV-2c detection from sick vaccinated dogs in the USA appeared to be similar to that of other CPV-2 subtypes (Hong et al., 2007). Finally, the increased rate of detection of CPV-2c in Italy did not seem to be accompanied by the increase in reported cases of parvovirus enteritis (Martella et al., 2005). The Glu-426 variant has also been reported from other European countries (Cavalli et al., 2014; Decaro et al., 2007, 2010; Filipov et al., 2014; Ntafis et al., 2009), South America (Perez et al., 2007), Asia (Nakamura et al., 2004), Africa (Touihri et al., 2009), and the USA (Hong et al., 2007).

The antigenic differences between CPV subtypes are a consequence of amino-acid substitutions in five loci in the viral capsid protein VP2 (Desario et al., 2005). These changes are thought to be associated with the antigenicity, host range and pathogenicity of the virus (Carmichael, 2005; Chang et al., 1992; Llamas-Saiz et al., 1996; Moon et al., 2008b). Although clinical implications of infection with various subtypes of CPV are currently poorly understood, CPV-2 is clearly an evolving virus, and monitoring of the on-going changes is important.

New Zealand comprises two main islands separated by the 24 km wide Cook Strait. It is geographically isolated from the rest of the world by Pacific and Indian oceans. This separation, coupled

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with strict quarantine measures, provides freedom from a number of canine pathogens common in other parts of the world (Hill, 1999). However, the first cases of CPV-2 gastroenteritis were reported in New Zealand soon after the worldwide emergence of the virus, suggesting that neither the geographical separation, nor strict quarantine measures prevented introduction of CPV-2 to New Zealand. The earliest suspected CPV-2 cases occurred in the South Island in 1979 (Gumbrell, 1979), and the virus was first isolated in the same year from a sick puppy in the North Island (Horner et al., 1979). There have been no investigations of CPV-2 in New Zealand since the early reports following the initial introduction of the virus (Parrish et al., 1980; Parrish et al., 1982).

Canine parvovirus type 2 is closely related to FPV and several other parvoviruses of wild carnivores, which are all classified within Feline panleukopenia virus species (Tijssen et al., 2012). These viruses are known to be able to infect a range of species within the order Carnivora, including large and small cats, raccoons, mink and foxes (Steinel et al., 2001). All of these, except for the wild domestic cats, are exotic to New Zealand. It has been suggested that passage through racoons, and possibly other intermediate hosts, played a role in the original transmission of the virus from cats to dogs, and in the subsequent evolution of the virus within its canine host (Allison et al., 2012). Hence, New Zealand provides a unique ecosystem in which epidemiology and evolution of CPV-2 might have followed a different pattern to that observed in other countries.

During 2008–2009, several field veterinarians expressed concerns about a perceived increase in the number of dogs diagnosed with parvoviral enteritis within different parts of New Zealand (Nick Cave, personal communication). This subjective view could not be substantiated due to the lack of any CPV-2 surveillance data. As such, the aim of the current study was to identify which CPV-2 subtypes currently circulate among dogs in New Zealand, and to investigate the evolutionary patterns of contemporary field CPV-2 viruses.

2. Materials and methods

2.1. Sources of samples

'Parvo' packs consisting of two sample pots, a submission form, a letter explaining the purpose of the study and an addressed prepaid courier envelope were distributed among veterinarians throughout New Zealand by the Intervet/Schering-Plough Animal Health's (currently MSD Animal Health) representatives during routine clinic visits. Similar packs were sent to Society for the Prevention of Cruelty to Animals (SPCA) shelters. The participating veterinarians were asked to collect faecal samples from any dog or puppy that tested positive for CPV-2 by an in-house test of their choice, or was strongly suspected of CPV-2 infection based on routine diagnostic criteria used by each veterinarian. Veterinarians were asked to store the samples at 4 °C and to courier them to the laboratory as soon as feasible after collection, ideally within 24 h. The submission form included the signalment, clinical history, results of in-house CPV-2 testing (if performed), and available vaccination details for the dog.

In addition to the contemporary survey samples, 12 other samples were included in the study. These comprised 3 vaccine strains of CPV-2 and 9 archival CPV-2 isolates. The latter consisted of 5 isolates in possession of Massey University from 2006 (CPV014-16), 2008 (CPV017) and 1979 (CPV018), as well as 4 isolates kindly donated by Dr. Wlodek Stanislawek (Animal Health Laboratory, Ministry of Primary Industries, Wellington, New Zealand) from 2009 (MAF.1), 1990 (MAF.2), 1986 (MAF.3), and 1980 (MAF.4).

2.2. Processing of samples

Upon receipt, the samples were either processed within 1–2 days of arrival (stored at 4 °C) or placed at −80 °C for later processing. A small amount of the faecal material (50–200 μL) was mixed with the Stool Transport and Recovery Buffer (STAR buffer, Roche) at the 1:3 ratio. The faecal samples in the STAR buffer were used for DNA extractions using the PCR Template Preparation Kit (Roche), according to the manufacturer's instructions. DNA from archival CPV-2 isolates and vaccines was extracted directly from these samples using the same DNA extraction kit.

Eluted DNA was tested for the presence of CPV-2 in a PCR reaction designed to amplify 1975 bp fragment of VP1/VP2 gene using published primers VP2.JS1.F and VP2.JS2.R (Meers et al., 2007). Each reaction consisted of 0.4 μM of each primer, 10 μL of 2× FastStart Master Mix (Roche) and 1 μL DNA template in a total volume of 20 μL. The cycling conditions included initial denaturation at 95 °C 10 min, followed by 40 cycles of denaturation (95 °C for 10 s), annealing (52 °C for 10 s), and extension (72 °C for 2 min), with the final extension step at 72 °C for 7 min, and 4 °C hold. Positive (a contemporary CPV-2 positive sample confirmed by sequencing, later included in the project as CPV058) and negative (water) controls were included with every PCR run. PCR products (entire 20 μL) were subjected to electrophoresis through a 1% agarose gel (Axygen) containing 0.5 μg/mL ethidium bromide in Tris-Acetate-EDTA (TAE) buffer for 90 min at 90 V in a mini gel tank (Bio-Rad). The PCR bands were visualised using GelDoc reader (Bio-Rad). The test was considered valid if positive and negative controls produced expected results. Any bands corresponding to the expected size of the product were extracted from the gel using Quantum Prep Freeze 'N Squeeze DNA Gel Extraction Spin columns (Bio-Rad). The samples were considered positive for CPV-2 if a band of the expected size was observed and its identity confirmed by sequencing.

2.3. CPV subtyping

The CPV-2 PCR products were sequenced using four separate primes as described by Meers et al. (2007). The obtained sequences were compared to the reference CPV-2 sequence (GenBank

Table 1
Designation of canine parvovirus type 2 (CPV-2) subtypes based on nucleotide and deduced amino acid variation at selected positions within sequences encoding viral protein 2 (VP2).

Subtype	Nucleotide (amino acid) position[a]					
	3045 (87)	3087 (101)	3684 (300)	3699 (305)	4062 (426)	4449 (555)
CPV-2	ATG (Met)	ATT (Ile)	GCT (Ala)	GAT (Asp)	AAT (Asn)	GTA (Val)
Prototype CPV2a	TTG (Leu)	ACT (Thr)	GGT (Gly)	TAT (Tyr)	AAT (Asn)	ATA (Ile)
New Zealand CPV2a	TTG (Leu)	ACT (Thr)	GGT (Gly)	TAT (Tyr)	AAT (Asn)	GTA (Val)
CPV-2b	TTG (Leu)	ACT (Thr)	GGT (Gly)	TAT (Tyr)	GAT (Asp)	GTA (Val)
CPV-2c	TTG (Leu)	ACT (Thr)	GGT (Gly)	TAT (Tyr)	GAA (Glu)	GTA (Val)

[a] Nucleotide (first nucleotide in a codon) and deduced amino acid positions are referred to the sequences of CPV-2 strain CPV-b (accession no. M38245).