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Molecular survey of canine parvovirus type 2: the emergence of subtype 2c in New Zealand

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ABSTRACT

Aims: To determine the genetic makeup of carnivore parvoviruses currently circulating in New Zealand; to investigate their evolutionary patterns; and to compare these viruses with those detected during the previous New Zealand-based survey (2009–2010).

Methods: Faecal samples from dogs (n = 40) with a clinical diagnosis of parvovirus enteritis were voluntarily submitted by veterinarians from throughout New Zealand. In addition, one sample was collected from a cat with comparable clinical presentation. The samples were used for DNA extraction and PCR amplification of viral protein 2 (VP2) of canine parvovirus type 2 (CPV-2). All samples produced amplicons of the expected sizes, which were then sequenced. The viruses were subtyped based on the presence of specific amino acids at defined locations. In addition, VP2 sequences were analysed using phylogeny and molecular network analysis.

Results: The majority (30/40; 75%) of CPV-2-infected dogs were younger than 6 months and 8/40 (20%) were aged between 9 months and 1 year. Most (39/41; 95%) parvoviruses were subtyped as CPV-2c, and one as the original CPV-2. The faecal sample from a cat was positive for feline panleukopenia virus. The majority (37/39; 95%) of New Zealand CPV-2c viruses were monophyletic. The remaining two New Zealand CPV-2c viruses clustered with Chinese and Sri Lankan CPV-2c viruses, separately from the main New Zealand clade.

Conclusions: There has been an apparent replacement of the CPV-2a viruses with CPV-2c viruses in New Zealand between 2011 and 2019. The source of the current CPV-2c viruses remains undetermined. The monophyletic nature of the majority of viruses detected most likely reflects a country-wide spread of the most successful genotype. However, an occasional introduction of CPV-2 from overseas cannot be excluded.

Clinical relevance: Current vaccines appear to be protective against disease caused by the CPV-2c viruses currently circulating in New Zealand. Vaccination and protection from environmental sources of CPV-2 until the development of vaccine-induced immunity remains the cornerstone of protection in young dogs against parvovirus enteritis. Ongoing monitoring of the genetic changes in CPV-2 is important, as it would allow early detection of variants that may be more likely to escape vaccine-induced immunity.

Abbreviations: AMOVA: Analysis of molecular variance; CPV-2: Canine parvovirus type 2; FPV: Feline panleukopenia virus; VP2: Viral protein 2

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Introduction

Canine parvovirus type 2 (CPV-2) is a non-enveloped, single-stranded DNA virus from the family *Parvoviridae* (Penzes *et al*. 2020). It emerged as a novel pathogen in the late 1970s, and was associated with a worldwide outbreak of acute haemorrhagic enteritis in dogs of all ages and myocarditis in neonates (Wilson 1980; Parrish *et al*. 2008). Despite the availability of effective vaccines, the virus continues to be an important cause of severe enteritis and death of young dogs worldwide, including New Zealand (Ohneiser *et al*. 2015; Kelman *et al*. 2020).

The original emergence of CPV-2 was likely due to cross-species transmission of feline panleukopenia virus (FPV) or a similar virus from wild carnivores (Figure 1). The virus continued to adapt to the new canine host, with a high rate of nucleotide substitution more typical of RNA viruses than more stable DNA viruses (Shackelton *et al*. 2005). The predominant new genotypes were designated as subtypes CPV-2a, CPV-2b and CPV-2c (Parrish *et al*. 1991; Truyen *et al*. 1996; Tucciarone *et al*. 2021). These genotypes were antigenically different from the original CPV-2 due to amino-acid substitutions in at least five loci in the viral capsid protein 2 (VP2) (Desario *et al*. 2005). The changes also affected the host range and some biological properties of the virus (Llamas-Saiz *et al*. 1996; Carmichael 2005; Moon *et al*. 2008). The original CPV-2 was replaced by CPV-2a soon after its emergence, followed by CPV-2b (Asp-426 variant; Figure 1), which by

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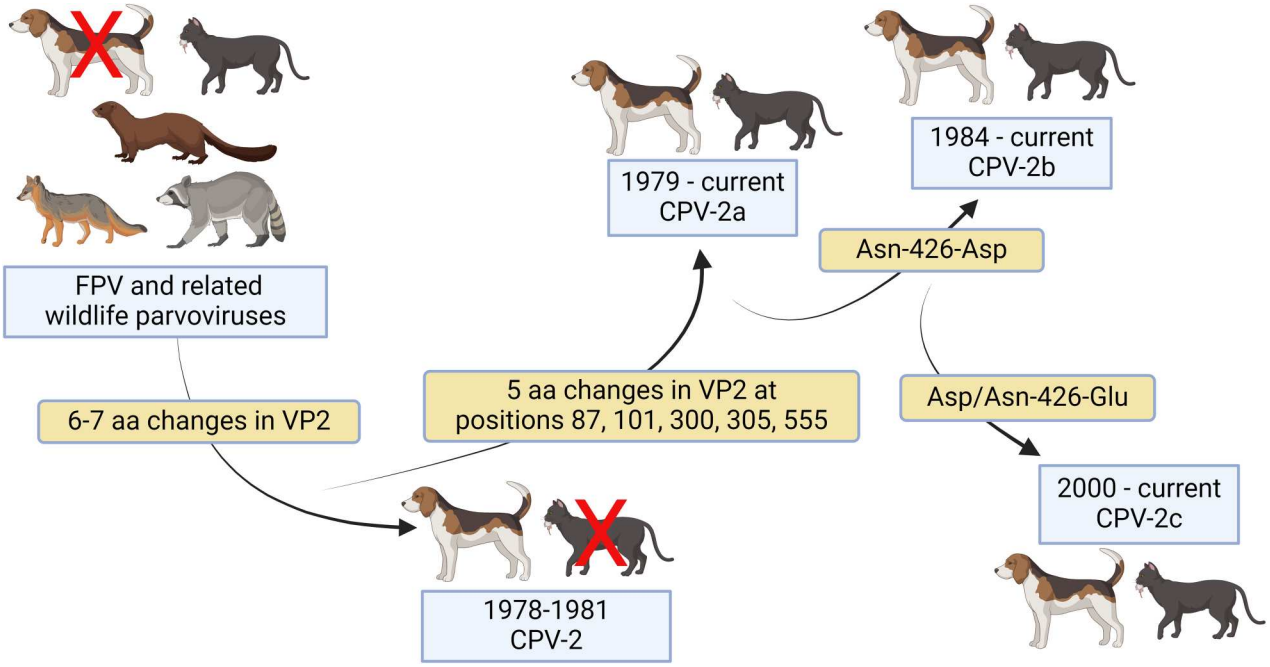

Figure 1. Canine parvovirus type 2 (CPV-2) emerged in canine populations worldwide in the late 1970s from feline panleukopenia virus (FPV) or related carnivore parvoviruses. The virus continued to adapt to its canine host through specific mutations in viral protein 2 (VP2), which affected the biological properties of the virus including its host range (non-supportive hosts indicated by crosses). The key difference between currently circulating subtypes is a change from asparagine (Asn) to aspartic acid (Asp) or glutamic acid (Glu) at amino acid (aa) position 426. The figure was made in BioRender (www.biorender.com).

the mid-to-late 1980s was circulating concurrently with CPV-2a among dogs in several countries (Parrish *et al*. 1991). A further change to CPV-2c (Glu-426 variant; Figure 1) was first described in Italy in 2001 (Buonavoglia *et al*. 2001), and later detected in Asia (Nakamura *et al*. 2004), the USA (Hong *et al*. 2007), South America (Pérez *et al*. 2007), Africa (Touihri *et al*. 2009), Australia (Woolford *et al*. 2017) and several European countries (Decaro *et al*. 2007). Co-infections with multiple CPV-2 subtypes have been reported by several authors (Miranda *et al*. 2015; Miranda and Thompson 2016; Jinadasa *et al*. 2021).

Although the change from the original CPV-2 to CPV-2a in the early 1980s was accompanied by an apparent increase in the virulence of the virus (Carmichael 2005), the clinical implications of infection with various subtypes of CPV-2 are poorly understood, as it is difficult to separate the influence of the virus itself from the influence of host-related factors such as age or the levels of virus-specific immunity (Miranda *et al*. 2015).

There is limited information available on the subtypes of CPV-2 that are present in New Zealand. The only molecular survey was conducted in 2009–2010 (Ohneiser *et al*. 2015). The results of that survey suggested that New Zealand CPV-2 sequences were monophyletic and belonged predominantly to the subtype CPV-2a, with no sequences from newer subtypes (CPV-2b and CPV-2c) detected. Hence, the aim of the current study was to determine the genetic make-up of canine parvoviruses currently circulating in New Zealand, to investigate their evolutionary patterns, and to compare these viruses with those detected during the previous survey.

Materials and methods

Sample collection

"Parvo packs" consisting of a sampling container with faecal transport media, a sterile swab, a submission form, a letter explaining the purpose of the study, and an addressed pre-paid courier envelope were distributed among veterinarians throughout New Zealand between January 2021 and January 2022. The submission form included signalment of the dog, vaccination history, and a statement confirming that the owners/handlers consented to participation in the study. Additional packs were supplied to veterinarians upon request until June 2023. Participating veterinarians were asked to collect faecal samples from any dog that tested positive on a CPV-2 in-house test or was suspected to be infected with CPV-2 based on clinical presentation. Veterinarians were asked to store the samples at 4°C and to courier them to the laboratory as soon as feasible after collection. The sample collection protocol has been approved by the MSD Animal Health Animal Ethics Committee (approval number AEC 4-21).

Collection and processing of samples

Upon receipt, the samples were coded as "CPV21/number" and either processed within 1–2 days of

arrival (stored at 4°C) or placed at −80°C for later processing. The faecal samples were used for DNA extractions using the E.Z.N.A. Stool DNA kit (Omega Bio-Tek Ltd., Norcross, GA, USA) and tested for the presence of CPV-2 DNA in a PCR reaction designed to amplify an approximately 2-kb fragment of the VP2 gene using published virus-specific primers (Supplementary Table 1). Each reaction consisted of 0.4 µM of each primer (CPV.VP2.JS1 and CPV.VP2.JS2) and 2 µL DNA template in a total volume of 20 µL using HotFirePol master mix with the final concentration of 2 mM $MgCl_2$ (Solis BioDyne, Tartu, Estonia). The cycling conditions included an initial denaturation at 95°C for 15 minutes, followed by 40 cycles of denaturation (95°C for 15 seconds), annealing (52°C for 10 seconds) and extension (72°C for 2 minutes), with the final extension step at 72°C for 7 minutes followed by a 4°C hold. DNA from a contemporary CPV-2 positive sample (confirmed by sequencing) and water were included with every PCR run as positive and negative controls, respectively. The remaining faecal material from each sample was stored at −80°C for future reference. On occasions, additional primers (CPV.555.F and CPV.555.R) were used to amplify a shorter (582 bp) PCR product containing the sequence of the defining amino acid at position 426.

CPV subtyping

The CPV-2 VP2 PCR products were sent for sequencing to Massey Genome Service (Massey University, Palmerston North, NZ) using four to six individual primers (Supplementary Table 1). The obtained sequences were assembled and compared to the reference CPV-2 sequence (GenBank accession number M38245) using Geneious version 9.1.8 (Kearse *et al*. 2012). The amplicons were assigned to specific CPV-2 subtypes based on published differences at selected amino-acid residues (Parrish *et al*. 1991; Truyen *et al*. 1996; Meers *et al*. 2007).

Phylogeny and network analyses

A consensus CPV-2 sequence generated from each assembly was manually curated. Additional CPV-2 VP2 sequences (n = 478) originating from various countries were obtained from the National Center for Biotechnology Information (NCBI) database. All 519 nucleotide sequences were aligned using MUSCLE alignment within Geneious, and trimmed to the same length. The sequences that were shorter than the trimmed alignment and sequences with large internal gaps were removed from the alignment so that the final alignment comprised 492 sequences. This included 99 parvovirus sequences from New Zealand, which comprised 41 sequences obtained in the current study (39 CPV-2c, one CPV-2, one FPV) and 58 archival sequences (53 CPV-2a, four CPV-2, one FPV) from the previous study (Ohneiser *et al*. 2015). The alignments were exported to MEGA11 (Tamura *et al*. 2021) and saved as a nexus file, which was then used as an input file to generate a haplotype list in the DnaSP software.[1] Sites with gaps or missing/ambiguous data were excluded. The sequences representative of the haplotypes (n = 276) were then realigned in Geneious and exported in nexus format with trait blocks added to represent geographical region and subtype. Median-joining haplotype networks were drawn using default parameters in POPART version 1.6 (Leigh and Bryant 2015).

The New Zealand CPV-2 sequences obtained in this study have been deposited in GenBank under accession numbers PQ159853 to PQ159893.

Statistical analyses

The signalment data were analysed using descriptive statistics. Differences between the median ages of vaccinated and unvaccinated dogs that were positive for CPV-2 were tested using the Mann–Whitney test, with p-values < 0.05 considered to be significant. Analysis of molecular variance (AMOVA; within POPART) was used to test for correlation between the genetic structure of the CPV-2 sequences and selected traits. The strength of correlation was represented by a PhiPT value, with 0 indicating no correlation and 1 indicating perfect correlation. The corresponding p-values were generated by reference to 1,000 random permutations of the input data.

Results

Faecal samples were collected from 40 dogs with suspected canine parvovirus enteritis from throughout New Zealand (Figure 2). Although both islands were represented, the majority (33/40; 82%) of samples came from the North Island, with half (20/40; 50%) collected from two regions (Manawatū-Whanganui and Waikato). In addition, a faecal sample from a recently vaccinated 16-week-old kitten with clinical suspicion of parvovirus infection (CPV21/09) was also submitted from the Auckland region. All samples generated expected PCR products when tested with CPV-2 primers. The most frequent breeds included dogs from the Terrier group and crosses (n = 10), Huntaway and crosses (n = 7), Mastiff and crosses (n = 3), Labradors and crosses (n = 5; including one cross with a Terrier and one cross with a Mastiff). Several dogs were listed as "mixed breed" (n = 8) and the make-up

[1] http://www.ub.edu/dnasp/ (accessed 1 March 2024).

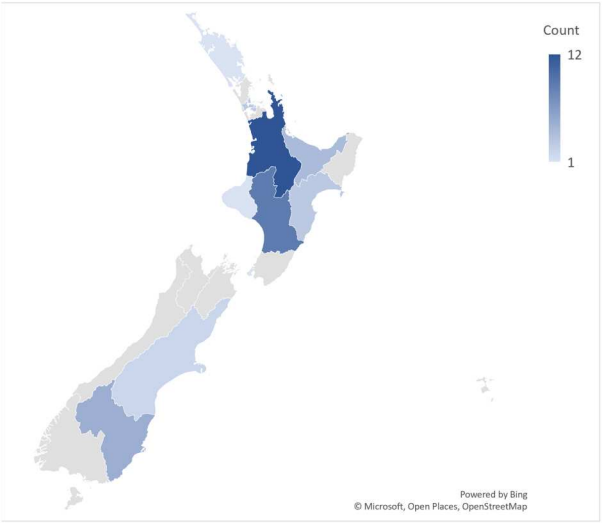

Figure 2. Geographical regions of New Zealand from which canine faecal samples were submitted for the study, shaded according to the number of samples obtained from each region.

of some crosses with the main breed listed were not specified (Supplementary Table 2).

The majority (30/40; 75%) of CPV-2 infected dogs in the current study were younger than 6 months and 8/40 (20%) were aged between 9 months and 1 year. One dog was listed as "1–2 years" of age and was considered to be 18 months for the purpose of the analyses, and one dog was of unknown age. There was no difference between the median age of vaccinated (10 weeks; n = 17) and unvaccinated (11 weeks; n = 22) dogs (p = 0.79). However, the exact vaccination details (brand of vaccine and dates of vaccination) were often not available, and there was no information on the vaccination status for one dog. Half (9/17; 47%) of the dogs reported as vaccinated were 10 weeks old or younger when they developed parvovirus enteritis.

Most (39/41; 95%) parvoviruses in the current study were subtyped as CPV-2c, one (CPV21/40) as the original CPV-2, and one (CPV21/09) as FPV. The majority (37/39; 95%) of CPV-2c viruses detected were monophyletic (Figure 3 and Supplementary Figure 1), which was supported by a strong (94%) bootstrap support value. The remaining two of the New Zealand CPV-2c viruses (CPV21/28 and CPV21/29) clustered with Chinese and Sri Lankan CPV-2c viruses, separately from the main New Zealand clade (Figure 3 and Supplementary Figure 1), although the bootstrap support for such clustering was low (26%). These two viruses also had a mutation at nucleotide position 3,586, resulting in an amino acid change from phenylalanine to tyrosine at amino acid position 267 of VP2, which has been frequently detected in CPV-2c viruses from Asia (Franzo *et al*. 2023).

Despite the worldwide origin of the sequences included in the network, all appeared to be closely related. The average number of base substitutions per site in pair-wise alignments was 0.007 (calculated in MEGA 11). When the haplotype network was coloured by the geographical origin of CPV-2 sequences (Figure 3b), some well-structured regions (e.g. the New Zealand CPV-2c sequences or the cluster of sequences from mainland Asia, both in the right-hand area of the network) were visible. The main cluster of New Zealand CPV-2c sequences comprised 37/39 (95%) sequences that formed one monophyletic group of six haplotypes (Figure 3). However, sequences from the same geographic region were often scattered throughout the network and on occasion, sequences from one region were more closely related to sequences from a different region than to each other. This can be exemplified by the two CPV-2c sequences from New Zealand, which belonged to the same haplotype as several international CPV-2c sequences from Asian countries within a cluster of similar haplotypes in the central area of the network (Figure 3). Overall, most of the sequence variability observed was within different geographical regions, and only 23% of the variability was attributed to variations between populations, indicating a fairly barrier-free spread of canine parvoviruses throughout the world (Table 1).

Interestingly, the New Zealand CPV-2a sequences from the 2010 survey (Ohneiser *et al*. 2015) were no longer monophyletic, but mixed with newer CPV-2a sequences from Australia. The contemporary FPV and original CPV-2 sequences clustered together with older New Zealand and international FPV and CPV-2 sequences, respectively. This was supported by the AMOVA results, which indicated that 35.5% of the observed genetic variation in the sequences may be explained by the subtype designation (Table 1). However, over 60% of variation was not attributed to subtype designation. This was reflected in the network; while many sequences of the same subtype clustered together, it was also apparent that the newer subtypes (CPV-2b and CPV-2c) originated from the older subtypes (CPV-2a) independently in several different areas of the network (Figure 3a).

Discussion

The current study was initiated to address the lack of contemporary data on the genetic make-up of CPV-2 viruses circulating in New Zealand. The majority of dogs affected by parvovirus enteritis in the current study were young and unvaccinated. In addition, nearly half of the dogs reported as vaccinated were 10 weeks old or younger when they developed parvovirus enteritis. As such, they were likely infected before the development of vaccine-induced immunity or vaccinated during the incubation period. This is similar to the results of the previous New Zealand-based survey (Ohneiser *et al*. 2015) and to data reported from other

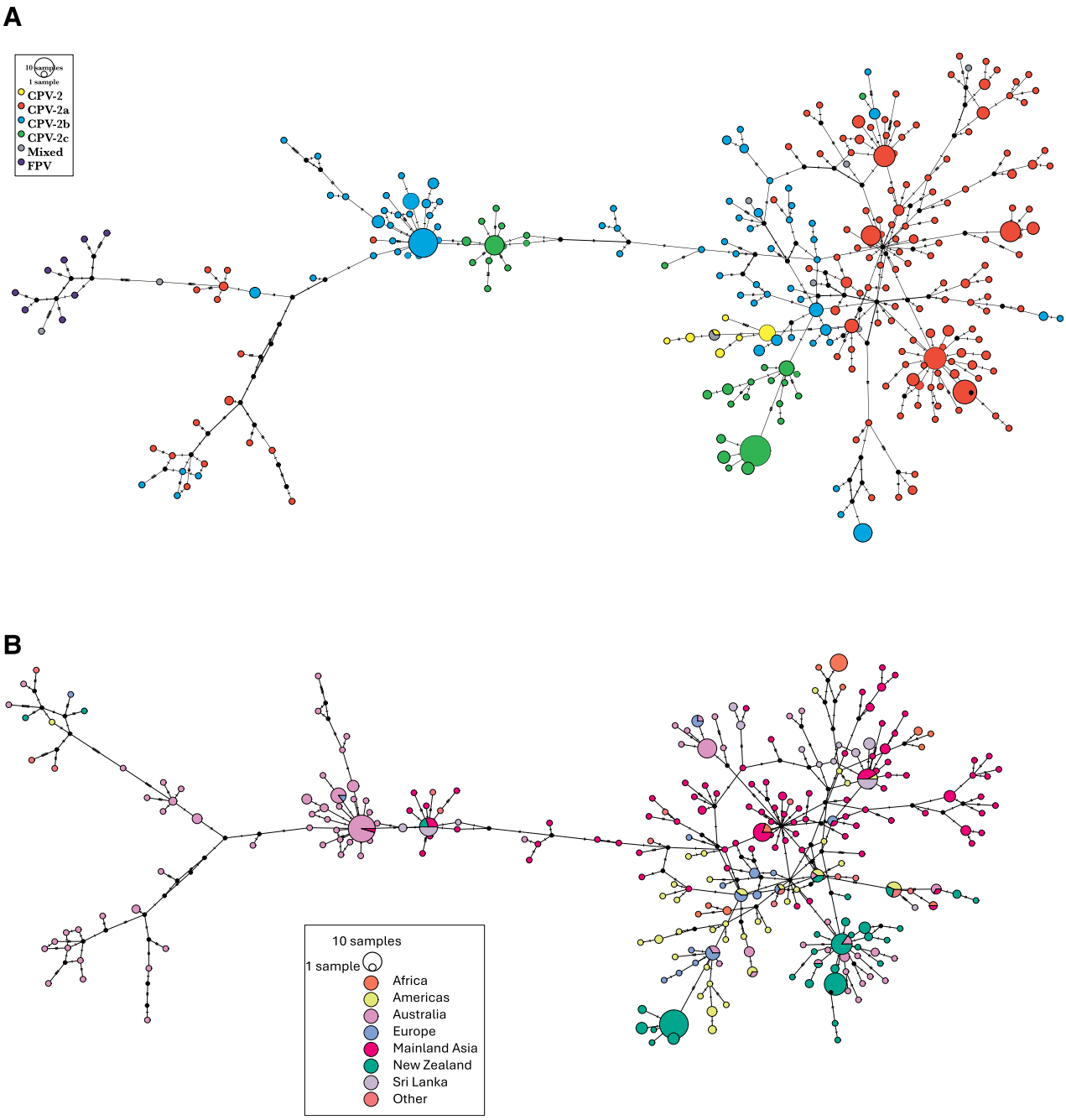

Figure 3. Haplotype network analysis based on 519 sequences of partial viral protein 2 (VP2) of canine parvovirus type 2 (CPV-2) and feline panleukopenia viruses (FPV). The sequences included 41 sequences from the current study and 58 archival New Zealand CPV-2/FPV sequences. The median-joining haplotype network of 276 haplotypes was drawn using default parameters in POPART version 1.6 (Leigh and Bryant 2015). The haplotypes are coloured by subtype (a) or geographic region of origin (b). The AMOVA results corresponding to this network are shown in Table 1.

Table 1. Analysis of molecular variance (AMOVA) results indicating the strength of correlation (PhiST) between the population genetic structure and selected traits in the molecular haplotype network (Figure 3) generated using partial sequences of viral protein 2 of canine parvovirus type 2.

Test	Variation within populations	Variation between populations	PhiST	P-value[a]
Geographical region	77.25%	22.75%	0.23	< 0.001
Subtype	64.50%	35.50%	0.35	< 0.001

[a]The likelihood that the PhiST value is true.

countries (Houston *et al*. 1996; Ohneiser *et al*. 2015; Giraldo-Ramirez *et al*. 2020). Adult dogs rarely develop canine parvovirus enteritis, most likely due to long-lasting immunity acquired either through vaccination or prior infection (Decaro and Buonavoglia 2012). Considering the high environmental stability of CPV-2 (Gordon and Angrick 1986), many dogs are likely to be regularly exposed to the virus, which may act as a booster of pre-existing immunity. The predominance of young, unvaccinated or partially vaccinated dogs among those sampled supports the importance of vaccination and appropriate

management (minimising the likelihood of contact with CPV-2 via environmental exposure) for prevention of parvoviral gastroenteritis.

Some authors suggested an association between the CPV-2 subtype and the severity of disease (Moon *et al*. 2008; Franzo *et al*. 2019). The samples for the current survey were collected from clinically affected dogs. As suggested previously (Meers *et al*. 2007; Ohneiser *et al*. 2015), this raises the possibility that the viruses detected represented the most virulent ones due to the sampling bias. However, a similar sampling strategy in the previous New Zealand-based survey (Ohneiser *et al*. 2015) resulted in detection of CPV-2a viruses only, arguing against the association between the subtype and severity of disease. Nonetheless, we cannot fully exclude the possibility that a broader range of CPV-2 genotypes may have been detected if CPV-2 positive samples from mildly or subclinically affected dogs were available for the analyses.

Detection of the original CPV-2 from one sample most likely represented detection of a vaccine strain. The virus was obtained from a faecal sample of a 10-week-old puppy from the Royal New Zealand Society for the Prevention of Cruelty to Animals (SPCA). According to the submission form, the puppy was vaccinated on 9 May, and the CPV-2-positive faecal sample was collected nearly a month later, on 7 June (Supplementary Table 2). Hence, it is not clear if the detection of the vaccine strain was coincidental or if the vaccine strain of the virus caused clinical disease in that puppy. Unfortunately, other possible causes of the puppy's disease were not investigated. There is little published information available on the duration of detection of vaccine strain DNA in faecal samples of vaccinated dogs. In one study, a vaccine strain of CPV-2 was detected post-mortem in various tissues, including intestines, of five young dogs at the time of euthanasia, between 12 and 24 days following the last vaccination (Schiro *et al*. 2023). All were considered to have died of causes unrelated to parvovirus infection or vaccination. In another study, parvovirus DNA was detected in 62–81% of faecal samples collected 20 days after the first vaccination, and in one sample on day 28 after the second vaccination (Segev *et al*. 2022). Similarly, CPV-2 DNA was detected at low levels in faeces from 5/100 (5%) healthy adult dogs on day 28 post-vaccination (Freisl *et al*. 2017). In all of the above studies, shedding was not investigated beyond the time points mentioned above, so it may have persisted for longer than stated. Altogether, these data suggest that detection of the vaccine strain nearly a month after the last vaccination in the current study is probable. The alternative explanation is that the puppy received the second dose of vaccination closer to sample collection, but it was not recorded.

Terriers and cross-breeds were over-represented among dogs sampled, which was similar to the results from the previous New Zealand-based survey (Ohneiser *et al*. 2015). Breeds considered at increased risk of severe parvovirus enteritis in overseas studies included Rottweilers and Doberman Pinschers (Glickman *et al*. 1985; Houston *et al*. 1996). Neither was among the dogs with CPV enteritis in the current study. However, since data on the number of dogs from different breeds in New Zealand are not available, the breed composition of the sampled dogs may simply reflect the breeds most common in New Zealand.

The detection of FPV from one feline sample provides a reminder that this virus circulates among cats in New Zealand. Both FPV and CPV belong to the same species *Carnivore protoparvovirus 1*, together with mink and racoon parvoviruses (Cotmore *et al*. 2019). While FPV does not infect dogs, cats can be infected with some variants of CPV-2 (Figure 1), which is why the feline sample was processed as part of the current study.

Although tracking the emergence of new virus variants is an important component of monitoring disease outbreaks, it is difficult to estimate the required number of samples needed due to the existence of inevitable systemic and biological biases (Wohl *et al*. 2023). The initial aim was to collect samples from 100 dogs affected by parvovirus enteritis. Unfortunately, only 40 canine samples were submitted by participating veterinarians throughout the study period. This is a common problem with studies that rely on client-provided samples. Based on calculations recently reported by Wohl *et al*. (2023), 40 samples from a cross-sectional study would provide a 98% probability of detecting a genotype of CPV-2 that comprised at least 10% of the circulating genotypes, and 95% probability of detecting a genotype that was present at 7% frequency, assuming that all genotypes had the same ability to spread and cause disease, and that samples were collected in a manner that maximised geographic and temporal distribution. Hence, while we cannot exclude the possibility that genotypes other than those detected circulated at low frequencies among clinically affected dogs in New Zealand, the number of samples collected combined with the geographical/temporal distribution of the samples was sufficient to have reasonable confidence that CPV-2c was a predominant variant circulating in New Zealand during the study period.

As such, it appears that there was a change in the make-up of CPV-2 variants circulating among dogs in New Zealand between 2010 and 2021, as only CPV-2a was detected from dogs sampled in the previous survey (Ohneiser *et al*. 2015). A similar change in the make-up of genotypes within a given region over time was reported from other countries including

Australia (Woolford *et al*. 2017; Clark *et al*. 2018; Yip *et al*. 2020), Italy (Decaro and Buonavoglia 2012) and China (Fu *et al*. 2022). The reasons for such change remain elusive. The evolution of CPV-2 subtypes has likely been driven by the selection pressures exerted by the host's immune responses (Truyen *et al*. 1995; Decaro *et al*. 2008b; Yoon *et al*. 2009). Such pressures, driven in part by vaccination, have not changed in New Zealand over the past 10 years, as no new CPV-2 vaccines have been registered here since 1997, and all currently available vaccines contain the original CPV-2 virus.[2]

Most of the New Zealand CPV-2 sequences in this study were monophyletic, which was supported by a strong (95%) bootstrap value. This suggests that they represent a country-wide spread of one successful variant of the virus. Similar results were obtained during the 2010 New Zealand survey, where all then-contemporary CPV-2a viruses were also monophyletic (Ohneiser *et al*. 2015). The source of the ancestral virus that gave rise to the main clade of CPV-2c in the current study is difficult to establish based on the available data. Although New Zealand CPV-2c viruses had common ancestors with CPV-2c viruses from several other countries including Argentina, Uruguay, Italy, Greece and Australia (Supplementary Figure 1), the bootstrap support values for such clustering were very low (< 10%), indicating low confidence in the evolutionary relationships between the main cluster of New Zealand CPV-2c viruses and CPV-2c viruses from other countries. The low bootstrap support values for the deeper branches of the phylogenetic tree likely reflect the relative similarity of all the sequences to each other, so that several different groupings are possible.

The results of the current and previously reported (Ohneiser *et al*. 2015) haplotype network analyses suggest that CPV-2c viruses evolved independently in different geographical regions from locally circulating CPV-2b variants. Although we have not detected CPV-2b viruses in New Zealand, the accuracy of any evolutionary relationships is dependent on the availability of a representative sample of viral genomes collected over a period of time. Hence, lack of New Zealand CPV-2 sequence data from between 2011 and 2019 precludes any final conclusions being drawn on the most likely origin of the current CPV-2c viruses. A considerable reticulation observed in the network is consistent with this view.

Two New Zealand CPV-2c viruses (CPV21/28 and CPV21/29) clustered with Chinese and Sri Lankan CPV-2c viruses, separately from the main New Zealand clade (Figure 3, Supplementary Figure 1). While the bootstrap support for such clustering was relatively low (26%), it was higher than the bootstrap support for many other clades at the same depths of the tree. These two New Zealand CPV-2c viruses were clearly different to the viruses from the main clade of contemporary CPV-2c viruses. Both had an additional mutation at nucleotide position 3,586 typical for CPV-2c viruses from Asia (Franzo *et al*. 2023). Based on these data, it seems likely that these two viruses were recently introduced to New Zealand from one of the Asian countries. The emergence of CPV-2c viruses in Asia and their subsequent worldwide spread, presumably linked to their increased fitness, has been described by others (Carrino *et al*. 2022; Bui *et al*. 2023; Franzo *et al*. 2023).

Altogether, the results of the current study support the conclusions from the 2010 survey (Ohneiser *et al*. 2015) that CPV-2 is not frequently introduced to New Zealand from overseas. This contrasts with the situation observed in some other countries. For example, CPV-2 sequences detected in a similar survey in Sri Lanka were not monophyletic and belonged to all three subtypes (CPV-2a, CPV-2b and CPV-2c), even though both New Zealand and Sri Lanka are island countries with no immediate neighbours (Jinadasa *et al*. 2021). Factors such as differences in the management of border biosecurity, canine vaccination coverage or the ecology of wildlife susceptible to CPV-2 infection are possible explanations for different epidemiology of CPV-2 in these two countries.

Interestingly, the New Zealand CPV-2a viruses that formed one monophyletic clade in the previous analyses (Ohneiser *et al*. 2015) clustered together with several more recent Australian CPV-2a sequences, suggesting a possible transfer of those viruses from New Zealand to Australia. However, convergent evolution of the Australian and New Zealand CPV-2a variants cannot be excluded (Voorhees *et al*. 2019).

To conclude, the results of the current study demonstrate the ongoing evolution of CPV-2, in agreement with the results of similar overseas studies (Bui *et al*. 2023; Franzo *et al*. 2023). While current vaccines appear to be protective against disease caused by all three subtypes (Greenwood *et al*. 1995; Spibey *et al*. 2008; Altman *et al*. 2017), cases of suspected vaccine inefficacy have been occasionally described overseas (Decaro *et al*. 2008a, 2020; Yip *et al*. 2020). Thus, ongoing monitoring of the genetic changes in CPV-2 is important, as it would allow early detection of variants that may be more likely to escape vaccine-induced immunity.

Acknowledgements

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[2]https://eatsafe.nzfsa.govt.nz/web/public/acvm-register

Disclosure statement

HB is an employee of MSD Animal Health, which provided funding for the project.

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