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Research paper

Emergence of canine parvovirus subtype 2b (CPV-2b) infections in Australian dogs

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ABSTRACT

Tracing the temporal dynamics of pathogens is crucial for developing strategies to detect and limit disease emergence. Canine parvovirus (CPV-2) is an enteric virus causing morbidity and mortality in dogs around the globe. Previous work in Australia reported that the majority of cases were associated with the CPV-2a subtype, an unexpected finding since CPV-2a was rapidly replaced by another subtype (CPV-2b) in many countries. Using a nine-year dataset of CPV-2 infections from 396 dogs sampled across Australia, we assessed the population dynamics and molecular epidemiology of circulating CPV-2 subtypes. Bayesian phylogenetic Skygrid models and logistic regressions were used to trace the temporal dynamics of CPV-2 infections in dogs sampled from 2007 to 2016. Phylogenetic models indicated that CPV-2a likely emerged in Australia between 1973 and 1988, while CPV-2b likely emerged between 1985 and 1998. Sequences from both subtypes were found in dogs across continental Australia and Tasmania, with no apparent effect of climate variability on subtype occurrence. Both variant subtypes exhibited a classical disease emergence pattern of relatively high rates of evolution during early emergence followed by subsequent decreases in evolutionary rates over time. However, the CPV-2b subtype maintained higher mutation rates than CPV-2a and continued to expand, resulting in an increase in the probability that dogs will carry this subtype over time. Ongoing monitoring programs that provide molecular epidemiology surveillance will be necessary to detect emergence of new variants and make informed recommendations to develop reliable detection and vaccine methods.

1. Introduction

Identifying patterns of infectious disease emergence is key to developing effective mitigation strategies (Brooks and Ferrao, 2005; Cleaveland et al., 2001; Tompkins et al., 2015). Monitoring programs that identify temporal shifts in pathogen demographics are central to improving our understanding of disease emergence dynamics (Grogan et al., 2014; Wilson et al., 1997; Zhu et al., 2015). With increasing availability of molecular sequence data, phylogenetic tools have become essential for uncovering complex population and evolutionary histories from a diverse suite of emerging pathogens (Alkhamis et al., 2017; Biek et al., 2007; Clark and Clegg, 2017; McKee et al., 2017; Shackelton et al., 2005). Here, we use a temporal dataset to describe the emergence, population expansion and molecular epidemiology of canine parvovirus subtype 2b (CPV-2b) infections in Australian domestic dogs.

Canine parvovirus (CPV-2) is one of the most globally important enteric pathogens infecting domestic dogs (Houston et al., 1996; Parrish et al., 1991). Since first emerging in domestic dogs in the 1970s, CPV-2 has caused severe disease pandemics, with symptoms including haemorrhagic diarrhoea, gastroenteritis, vomiting and immunosuppression (Hoelzer and Parrish, 2010; Miranda et al., 2016; Miranda and Thompson, 2016). In the 1980s, circulating strains of CPV-2 around the world mutated into two widespread antigenic subtypes, CPV-2a and CPV-2b, which quickly began to replace the original CPV-2 virus (Decaro and Buonavoglia, 2012). An additional antigenic subtype, CPV-2c, was identified in 2000 in Italy and has since been reported in many regions, including a recent report from Australia (Woolford et al., 2017). These subtypes are typically distinguished by testing against a panel of monoclonal antibodies, or by PCR and DNA sequencing of specific nucleotide positions of the VP capsid protein gene (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016).

A previous study of CPV-2 infections in Australian dogs reported an overwhelming majority of cases were associated with CPV-2a through

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the year 2007 (Meers et al., 2007). This is surprising given the widespread and rapid replacement of the CPV-2a subtype by CPV-2b in a number of countries around the world (Meers et al., 2007; Miranda et al., 2016). This result raised important questions about why the 2b subtype seemingly failed to emerge in Australia, and also provided a unique monitoring opportunity to track the temporal dynamics of the two subtypes and identify environmental factors that may govern their population expansions. Identifying factors that govern the circulation of different antigenic CPV-2 variants has important implications for our understanding of selective pressures and for developing targeted vaccine programs to prevent outbreaks. For instance, CPV-2 vaccines (many of which rely on the original 1980s strain) may not be 100% effective against CPV-2a and CPV-2b, possibly resulting in vaccine failure (Pratelli et al., 2001). Here, we use a nine-year dataset of CPV-2 infections in Australian domestic dogs to describe the temporal population dynamics of the CPV-2a and 2b subtypes. Using temporal phylogenetic and epidemiological models, we report a rapid population expansion of subtype CPV-2b in Australian dogs following 2007.

2. Materials and methods

2.1. Sample collection, molecular methods and sequencing of the canine parvovirus VP gene

A total of 396 samples, collected between 2008 and 2016, were analysed in this study. Samples were from cases of possible vaccine failure or unvaccinated dogs in all states within Australia (Fig. 1). All dogs had clinical signs typical of parvovirus infection. Samples consisted mostly of faecal samples, faecal swabs and occasional rectal swabs, most of which had tested positive to various CPV antigen tests, including the WitnessTM Parvo (Zoetis, USA) or SNAP® Parvo Antigen Test (IDEXX, USA).

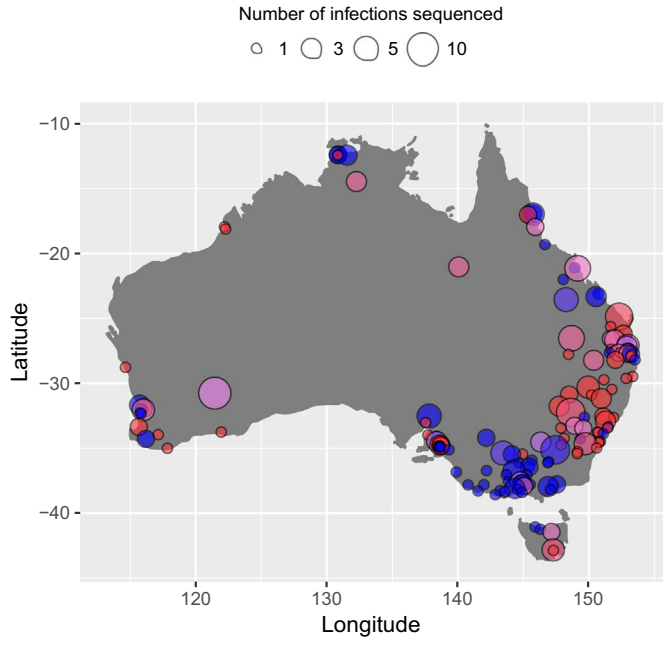

Fig. 1. Locations and number of sequenced Australian canine parvovirus samples included in the present study. Points represent the latitude and longitude of postal codes where dogs presented to a veterinary clinic with suspected parvovirus infection. Sizes of points are proportional to the number of samples submitted from each postal code across the sample collection period (December 2007 to April 2016). Colours of points reflect the proportion of samples that were confirmed as subtype CPV-2b compared to those confirmed as CPV-2a, with cooler blues indicating a higher proportion of CPV-2a subtype and warmer reds indicating a higher proportion of CPV-2b subtype. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DNA extraction, PCR amplification and DNA sequence analysis were performed as previously described (Meers et al., 2007). Briefly, DNA was extracted from all samples using the QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturer's instructions. Extractions were eluted in 200 µl elution buffer and stored at −20 °C until PCR was performed. PCR primers (JS1F, JS2R), described previously (Meers et al., 2007), were designed to amplify 1975 bp of the VP capsid protein gene encompassing all genetic variant-defining nucleotides. Products were sequenced on Applied Biosystems Hitachi 3130xl Genetic Analyzer (Applied Biosystems, Life technologies) using these primers and additional internal sequencing primers (JS3F, JS4R), described previously by Meers et al. (Meers et al., 2007). Sequences were mapped to a 1755 bp CPV-2a VP reference sequence (GenBank accession AB054213) after trimming ends with error probability of 0.02. Sequence edits and alignments were carried out in Geneious v10.0.6 (Biomatters, New Zealand; Kearse et al., 2012). We did not detect subtype CPV-2c, though a recent study reported evidence that this subtype does occur in Australian dogs (Woolford et al., 2017). Because the study by Woolford et al. only reports three CPV-2c sequences from a single timepoint, and because our central goal was to characterise the temporal evolution and molecular epidemiology of CPV-2 viruses, we focused only on CPV-2a and CPV-2b subtypes for our analyses.

2.2. Estimating the timing of CPV subtype 2b emergence in Australia

We estimated the timing of CPV-2b emergence in Australia by constructing time-structured Bayesian phylogenetic trees using BEAST v1.8.1 (Drummond and Rambaut, 2007; run on the CIPRES portal at https://www.phylo.org/; Miller et al., 2010). To improve resolution of divergence time estimates, we included timestamped CPV-2 sequences from the USA and New Zealand (accessions EU659116 and KP881645) as well as from multiple ancestral virus sequences detected in wild felids and canids as outgroups. These outgroups included feline parvovirus (FPV; accessions KP769859, KX685354 and X55115), raccoon-dog parvovirus (RDPV; accessions GU392240, KJ194463, U22192 and U22193) and mink enteritis virus (MINK; accessions M23999 and KT899745). We used the date of sample collection as a timestamp in analyses. For sequences that did not have collection date information (N = 28), we allowed uncertainty in the timing of infection by sampling dates within a 1-month timeframe prior to sample receipt (i.e. Uniform[date received, date received - 1 month]). For all outgroup taxa, only the year of sequencing was recorded, and so we sampled within a 12-month timeframe prior the recorded date to incorporate infection date uncertainty (i.e. Uniform[date recorded, date recorded - 1 year]). A conservative time interval of Uniform[1950, 1973] was specified for the most recent common ancestor of all canine parvovirus lineages.

Phylogenetic reconstructions were carried out using nucleotide sequences. To estimate variation in evolutionary rates across codon positions, we linked substitution rates and rate heterogeneities for first and second codon positions (CP_{12}) and allowed independent rates for the third position CP_3. We specified a GTR + I + Γ model (following Shackelton et al., 2005) and a Bayesian Skygrid demographic prior (with nine estimated time window parameters) to allow for variation in effective population size across time. Substitution rates associated with each branch were drawn from a single underlying distribution by specifying an uncorrelated lognormal relaxed clock with a truncated normal distribution [lower bound = 0; upper bound = 0.01; mean = 0.0001; sd = 0.001] for the substitution rate mean and an exponential distribution [mean = 0.001] for the standard deviation. Default priors were used for all other parameters. Three independent Markov Chain Monte Carlo (MCMC) chains were run for 50,000,000 iterations each, sampling every 25,000 and removing the first 25% as burn-in (resulting in 4500 posterior estimates) to ensure that estimated independent sample sizes for each parameter were above 200. Stationarity, convergence of MCMC chains and estimates of interior branch