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

Nicholas J. Clarka,⁎, Jennifer M. Seddona, Myat Kyaw-Tannera, John Al-Alawneha, Gavin Harperb, Phillip McDonaghb, Joanne Meersa

a School of Veterinary Science, University of Queensland, Gatton, Queensland 4343, Australia
b Boehringer Ingelheim Pty Limited, North Ryde, NSW 2113, Australia

ARTICLE INFO

Keywords:
Bayesian Skygrid
Canine parvovirus
CPV-2
Disease emergence
Molecular epidemiology
Surveillance

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; Cleveland 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 hemorrhagic 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...
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).

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. Extracts 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 1755 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 X551151), 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 (CP12) and allowed independent rates for the third position CP3. We specified a GTR + I + Γ model (following Shackleton 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

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 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.)
molecular clock rates were assessed using TRACER v1.4 (Rambaut and Drummond, 2007).

### 2.3. Population dynamics of canine parvovirus strains

To explore possible variation in the rates of population demographic change between CPV-2a and CPV-2b subtypes, Bayesian Skygrid population dynamic models were constructed separately for each subtype. As in the previous model, phylogenetic trees were inferred using the GTR + I + Γ substitution model with codon partitioning in BEAST v1.1.8. MCMC chain lengths and sampling frequencies of runs were identical to those in the above analysis.

#### 2.4. Temporal variation in CPV-2b infection probability

The phylogenetic demographic models above use rates of sequence evolution to estimate changes in effective population sizes of the two subtypes. We supplemented this analysis by examining possible temporal variation in the proportion of clinical infections that were attributed to CPV-2b in our dataset. We tested whether sample collection date influenced the probability that infections were classified as either CPV-2a (coded as 0) or 2b (coded as 1) using a logistic regression with a binomial error distribution and logit link function. Some studies have reported geographic variation in the relative frequencies of canine parvovirus antigenic variants (Miranda et al., 2016; Miranda and Thompson, 2016). We accounted for possible influences of climatic variation on CPV-2b infection probability by including minimum temperature of the coldest month, maximum temperature of the warmest month, minimum precipitation of the driest month and maximum precipitation of the wettest month as covariates. To account for underlying geographical variation, we included postal code as a random grouping term, allowing regression intercepts to vary among groups. All covariates were centred and scaled by one standard deviation to allow direct comparisons of coefficient sizes. Regressions were performed using the ‘lme4’ package (Bates et al., 2015) in the R programming language (Subtype occurrence data provided in Appendix A; R code to reproduce the logistic regression provided in Appendix B).

### 3. Results

From a total of 396 parvovirus submissions, CPV-2 infections were detected by PCR in 312 individual dogs. We obtained sequences of CPV-2 in 284 of these positive samples, with 167 confirmed as subtype CPV-2a and 145 confirmed as CPV-2b. Sequences from both subtypes were recovered from dogs throughout continental Australia and Tasmania (Fig. 1). From the remaining 85 samples, results from 61 were PCR negative to CPV-2, while 23 did not yield adequate DNA for PCR testing.

Phylogenetic reconstruction of time-stamped VP sequences found strong support for the monophyly of Australian CPV-2b sequences, while CPV-2a sequences were found to be paraphyletic, represented by two well-supported clades (Fig. 2). Highest posterior credible intervals of divergence times indicated that CPV-2a likely emerged in Australia some time between 1973 and 1988, while CPV-2b likely emerged between 1985 and 1998 (Fig. 2). For both subtypes, emergence was characterised by a classical pattern of relatively high rates of evolution during early emergence, followed by subsequent decreases in evolutionary rates over time (Fig. 2).

Bayesian Skygrid reconstructions revealed that both parvovirus subtypes showed higher relative rates of 3rd codon substitutions compared to substitutions in the 1st and 2nd codons (Fig. 3a), indicating high rates of synonymous mutations in the viral VP gene. The frequency of 3rd versus 1st and 2nd codon substitutions was lower for CPV-2b strains (suggesting a greater rate of non-synonymous mutations), possibly reflecting higher selective pressures for the CPV-2b subtype (Fig. 3a). CPV-2b sequences showed comparatively faster rates of evolution than CPV-2a, though 95% credible intervals for these estimates overlapped (Fig. 3a). Temporal reconstructions of effective population size indicated that populations of both subtypes expanded over time, though the trajectories of these expansions showed considerable variation between subtypes (Fig. 3b). The CPV-2a subtype emerged earlier and expanded rapidly, followed by intermittent periods of expansion and contraction. In contrast, the CPV-2b subtype has continued to expand since initial emergence, and likely surpassed CPV-2a as the most common subtype in dogs in Australia sometime in the last four to six years (Fig. 3b).

Estimates of the probability that infections would be attributed to CPV-2b corroborated the above findings. Samples collected more recently had a higher probability of being CPV-2b (logistic regression coefficient for time: mean = 1.085, range = [0.692, 1.478]), equating to a 52.67% increase in CPV-2b infection likelihood per year since the earliest sampling date in our database (December 19, 2007; Fig. 4; Supplementary data). Coefficient standard errors for all other covariates (minimum temperature of the coldest month, maximum temperature of the warmest month, minimum precipitation of the driest month and maximum precipitation of the wettest month) overlapped zero, indicating no apparent effect of climatic variation on the probability that an infection would be attributed to CPV-2b (Appendix B).

### 4. Discussion

Our study presents multiple lines of evidence to suggest that CPV-2b is becoming the dominant parvovirus subtype in dogs in Australia. We confirm findings from other countries that CPV-2b has progressively evolved to supplant the CPV-2a subtype (Decaro et al., 2009; Shackelton et al., 2005). We also provide crucial evidence that local climate variables do not appear to influence the subtype that dogs are likely to carry, indicating that the continued spread of this virus is unlikely to be limited by environmental conditions. Continuous surveillance of parvovirus in dogs will be key to our understanding of CPV-2 epidemiology and whether current vaccines and diagnostic tests need to be refined.

As one of the largest temporal analyses of CPV-2 sequences to date, this study provides meaningful new insights into the evolutionary origins CPV-2a and 2b. Both subtypes exhibited classical patterns of rapid evolutionary rates during early emergence, a pattern that has been repeatedly observed for other CPV-2 populations (Pérez et al., 2012; Shackelton et al., 2005). Our molecular population models estimate that CPV-2b infections have likely been circulating in Australia for nearly two decades, yet have only recently begun to surpass CPV-2a as the most common subtype in dogs. Considering that this replacement happened swiftly in many other countries (including Ireland, the UK and many African countries; Hong et al., 2007; Miranda and Thompson, 2016; Touihri et al., 2009), why this has taken so long to occur in Australia remains unclear. Previous authors have concluded that the widespread co-existence of multiple CPV-2 subtypes may indicate that neither has a particularly strong evolutionary advantage over the other (Steinel et al., 1998). However, experimental studies that assess the pathogenic potential of different CPV-2 subtypes are limited. Importantly, our results highlight that the CPV-2b subtype has continued to evolve at a relatively rapid rate in recent years. It therefore remains possible that, until recently, the CPV-2b subtype has caused milder symptoms and was less frequently encountered by practicing clinicians in Australia than CPV-2a (Meers et al., 2007). If this were the case, then our findings of more rapid evolution and a more steady population expansion rate for CPV-2b (compared to CPV-2a) may indicate an increase in virulence over the last decade in Australia. Some evidence supports the idea that subtypes 2a and 2b reach higher shedding rates and cause more severe disease than the ancestral virus CPV-2 (Decaro and Buonavoglia, 2012; Hoelzer and Parrish, 2010; Miranda et al., 2016), which may explain the rapid spread of these two subtypes. Although we do not have clinical evidence to assess whether CPV-2a and CPV-2b corroborated the above findings. Samples collected more recently had a higher probability of being CPV-2b (logistic regression coefficient for time: mean = 1.085, range = [0.692, 1.478]), equating to a 52.67% increase in CPV-2b infection likelihood per year since the earliest sampling date in our database (December 19, 2007; Fig. 4; Supplementary data). Coefficient standard errors for all other covariates (minimum temperature of the coldest month, maximum temperature of the warmest month, minimum precipitation of the driest month and maximum precipitation of the wettest month) overlapped zero, indicating no apparent effect of climatic variation on the probability that an infection would be attributed to CPV-2b (Appendix B).

### 4. Discussion

Our study presents multiple lines of evidence to suggest that CPV-2b is becoming the dominant parvovirus subtype in dogs in Australia. We confirm findings from other countries that CPV-2b has progressively evolved to supplant the CPV-2a subtype (Decaro et al., 2009; Shackelton et al., 2005). We also provide crucial evidence that local climate variables do not appear to influence the subtype that dogs are likely to carry, indicating that the continued spread of this virus is unlikely to be limited by environmental conditions. Continuous surveillance of parvovirus in dogs will be key to our understanding of CPV-2 epidemiology and whether current vaccines and diagnostic tests need to be refined.

As one of the largest temporal analyses of CPV-2 sequences to date, this study provides meaningful new insights into the evolutionary origins CPV-2a and 2b. Both subtypes exhibited classical patterns of rapid evolutionary rates during early emergence, a pattern that has been repeatedly observed for other CPV-2 populations (Pérez et al., 2012; Shackelton et al., 2005). Our molecular population models estimate that CPV-2b infections have likely been circulating in Australia for nearly two decades, yet have only recently begun to surpass CPV-2a as the most common subtype in dogs. Considering that this replacement happened swiftly in many other countries (including Ireland, the UK and many African countries; Hong et al., 2007; Miranda and Thompson, 2016; Touihri et al., 2009), why this has taken so long to occur in Australia remains unclear. Previous authors have concluded that the widespread co-existence of multiple CPV-2 subtypes may indicate that neither has a particularly strong evolutionary advantage over the other (Steinel et al., 1998). However, experimental studies that assess the pathogenic potential of different CPV-2 subtypes are limited. Importantly, our results highlight that the CPV-2b subtype has continued to evolve at a relatively rapid rate in recent years. It therefore remains possible that, until recently, the CPV-2b subtype has caused milder symptoms and was less frequently encountered by practicing clinicians in Australia than CPV-2a (Meers et al., 2007). If this were the case, then our findings of more rapid evolution and a more steady population expansion rate for CPV-2b (compared to CPV-2a) may indicate an increase in virulence over the last decade in Australia. Some evidence supports the idea that subtypes 2a and 2b reach higher shedding rates and cause more severe disease than the ancestral virus CPV-2 (Decaro and Buonavoglia, 2012; Hoelzer and Parrish, 2010; Miranda et al., 2016), which may explain the rapid spread of these two subtypes. Although we do not have clinical evidence to assess whether CPV-2a and
2b show pathological differences in Australian dogs, or whether CPV-2b has become more virulent over time, the suggestions warrant further study and support calls for ongoing disease surveillance programs (Alkhamis et al., 2017; Grogan et al., 2014).

The possible presence of viral co-infections may also be important for our understanding of the epidemiology and evolutionary dynamics of CPV-2 subtypes. Infection with multiple co-circulating pathogens can lead to inaccurate results if PCR or other diagnostic tests are more sensitive to one of the occurring strains (Barbosa et al., 2017; Clark et al., 2016). While co-infections by multiple CPV-2 subtypes are not commonly reported, they are not unheard of (see for example Pérez et al., 2014), and we have insufficient evidence to speculate on whether common diagnostic tests preferentially detect one antigenic subtype over the other (Miranda and Thompson, 2016). Next-generation sequencing methods may help overcome this knowledge gap by detecting the presence of co-circulating pathogens with high precision and accuracy (Parker et al., 2017). These techniques could be especially relevant for CPV-2 studies, as recombination between co-infecting variants has been suggested as one of the possible mechanisms by which viral diversity and the emergence of new genotypes are generated (Miranda and Thompson, 2016; Pérez et al., 2014).

Although our epidemiology results suggest the continued replacement of CPV-2a by 2b, we stress that caution is necessary to interpret this finding as our samples came from suspected vaccine-failure dogs or unvaccinated dogs. This study cohort may not be an accurate representation of the domestic dog population at large in Australia, and so our ability to make inferences about infections circulating in unvaccinated animals, including feral dogs or clinically ‘well’ dogs, is limited. Nevertheless, our investigation of the potential roles of climate variables in driving subtype occurrence probabilities are worthy of consideration. Parvoviruses are considered extremely stable outside the host, with indirect environmental transmission speculated to be a key factor in ongoing maintenance of the viral population (Decaro and Buonavoglia, 2012). Yet few studies of parvovirus infection in domestic or wild canines have assessed the roles of environmental variables in driving viral prevalence and/or community composition (but see Bagshaw et al., 2014; Rika-Heke et al., 2015). The fact that we identified sequences from both CPV-2 subtypes all around Australia, together with our finding that local climate variables do not influence a dog’s likelihood of carrying one subtype over the other, raises important questions about the potential spread of these viruses. Many of the sampled regions in Australia receive little rainfall and reach very high summer temperatures, yet our findings suggest these conditions do not favour one subtype over the other. Studies over narrow spatial scales are needed, as it is possible that sufficient infected dogs (including feral dogs, which are known to spread many pathogens; Clark et al., 2017) are inhabiting harsh environments to ensure viral maintenance without the need for steady environmental transmission.

An interesting outcome of our phylogenetic analyses is the finding that the CPV-2a subtype in Australia is paraphyletic. Our study is not the first to detect such a pattern, as paraphyly of CPV-2a was also found in multiple other CPV-2 studies, including examples from Japan.
Fig. 3. Estimated evolutionary rates and population demographic trends for canine parvovirus strains in Australia. (a) Estimated relative frequencies of substitutions in 1st and 2nd codon positions versus 3rd codon substitutions (left panel) and estimated mean molecular clock rates (right panel) for canine parvovirus subtypes CPV-2a (grey shading) and CPV-2b (black shading). (b) Bayesian Skygrid estimations of changes in effective population size over time for the two subtypes. Lines represent median effective population size, while shading indicates 95% highest posterior density credible intervals. The dashed vertical line represents the median age of the CPV-2b most recent common ancestor. All parameters were gathered from a posterior distribution of 4500 trees estimated using a Bayesian Skygrid population demographic prior.

Fig. 4. Estimated probability of an Australian canine parvovirus infection being attributed to CPV-2b (coded as 1) compared to CPV-2a (coded as 0) over time. Points represent the 284 individual infections that were sequenced and characterised to sub-type in Australia from 2007 to 2016. The solid line represents the mean probability of CPV-2b infection estimated from a fitted logistic regression, with sampling date as the predictor and subtype as the binary response. Shading represents 95% confidence intervals of the fitted regression slope.

5. Conclusions

Despite widespread vaccination programs, CPV-2 infections remain a widespread and debilitating disease of domestic dogs (Hoelzer and Parrish, 2010; Miranda et al., 2016; Parker et al., 2017). Our understanding of the evolution and rapid emergence of CPV-2 subtypes has increased greatly with the implementation of DNA sequencing methods (Hoelzer and Parrish, 2010; Shackelton et al., 2005; Touihri et al., 2009), yet how these viruses will continue to evolve and expand should remain a key aim of ongoing research. For example, although a recent study reported several cases of CPV-2c infection in Australia (Woolford et al., 2017), our nine-year Australia-wide sample database did not detect this antigenic variant. Monitoring programs such as the one that provided data for this study should be given high priority, as continuous epidemiological surveillance will be necessary to detect new variants and make informed recommendations to develop reliable detection and vaccine methods.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2017.12.013.

Acknowledgements

Funding for the project was provided by Boehringer Ingelheim Pty Ltd. The authors thank Dr. Zahara Bensink for technical support and the many veterinarians who submitted samples.

Data statement

All new sequences used in this manuscript are stored in GenBank under accession numbers MG641444–MG641725. Infection occurrence data and R code used to run logistic regression models is provided in Appendices.

References


(Oshima et al., 2008), Europe (Decaro et al., 2009) and Brazil (Pinto et al., 2012). The taxonomy of CPV-2 subtypes has come into question multiple times in the literature (Decaro and Buonavoglia, 2012; Decaro et al., 2009; Siegl et al., 1985), and our finding of a well-supported split between two CPV-2a clades will no doubt help fuel this conversation into the future. The issue of naming DNA sequences should not be taken lightly, as this may have important consequences for our understanding of pathogen evolution. For instance, one could argue that two reciprocally-monophyletic CPV-2a clades should be treated as separate groups in population dynamics and epidemiology analyses.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2017.12.013.

Acknowledgements

Funding for the project was provided by Boehringer Ingelheim Pty Ltd. The authors thank Dr. Zahara Bensink for technical support and the many veterinarians who submitted samples.
Parish, C.R., Aquadro, C.F., Strassheim, M., Evertmann, J., Sgro, J., Mohammed, H.,
1991. Rapid antigenic-type replacement and DNA sequence evolution of canine
Pérez, R., Bianchi, P., Calleros, L., Francia, L., Hernández, M., Maya, L., Panzer, Y., Sosa,
K., Zoller, S., 2012. Recent spreading of a divergent canine parvovirus type 2a (CPV-
Pérez, R., Calleros, L., Marandonio, A., Sarne, N., Iraola, G., Grecco, S., Blanc, H.,
Vignuzzi, M., Isakov, O., Shomron, N., 2014. Phylogenetic and genome-wide deep-
sequencing analyses of canine parvovirus reveal co-infection with field variants and
Pinto, L.D., Streck, A.F., Gonçalves, R.K., Souza, C.K., Corbellini, A.O., Corbellini, L.G.,
Canal, C.W., 2012. Typing of canine parvovirus strains circulating in Brazil between
Pratelli, A., Cavalli, A., Martella, V., Tempesta, M., Decaro, N., Carmichaël, L.E.,
Buonavoglia, C., 2001. Canine parvovirus (CPV) vaccination: comparison of neu-
tralizing antibody responses in pups after inoculation with CPV2 or CPV2d modified
Oscillation Index, rainfall and the occurrence of canine tick paralysis, feline tick
102, 379-384.
Characteristics and taxonomy of Parvoviridae. Interdisciplinary 23, 61–73.
Tomkins, D.M., Carver, S., Jones, M.E., Krook, M., Skerratt, L.F., 2015. Emerging in-
Touihri, I., Bouzid, I., Daoud, R., Desario, C., El Goulli, A.F., Decaro, N., Ghorbel, A.,
variants circulating in Tunisia. Virus Genes 38, 249–258.
Wilson, M.L., Bretsky, P.M., Cooper Jr., G.H., Egbertson, S.H., Van Kuiningen, H.J.,
and temporal characteristics of animal infection and human contact. Am. J. Trop.
Zhu, H., Hughes, J., Murcia, P.R., 2015. Origins and evolutionary dynamics of H3N2