**Canine parvovirus: the worldwide occurrence of antigenic variants**

Carla Miranda¹,² and Gertrude Thompson¹,²

¹Department of Veterinary Clinics, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, 4050-313 Porto, Portugal
²Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), InBio, Laboratório Associado, Universidade do Porto, 4485-661 Vairão, Portugal

The most important enteric virus infecting canids is canine parvovirus type 2 (CPV-2). CPV is the aetiologic agent of a contagious disease, mainly characterized by clinical gastroenteritis signs in younger dogs. CPV-2 emerged as a new virus in the late 1970s, which could infect domestic dogs, and became distributed in the global dog population within 2 years. A few years later, the virus’s original type was replaced by a new genetic and antigenic variant, called CPV-2a. Around 1984 and 2000, virus variants with the single change to Asp or Glu in the VP2 residue 426 were detected (sometimes termed CPV-2b and -2c). The genetic and antigenic changes in the variants have also been correlated with changes in their host range; in particular, in the ability to replicate in cats and also host range differences in canine and other tissue culture cells. CPV-2 variants have been circulating among wild carnivores and have been well-documented in several countries around the world. Here, we have reviewed and summarized the current information about the worldwide distribution and evolution of CPV-2 variants since they emerged, as well as the host ranges they are associated with.

**Introduction**

Canine parvovirus type 2 (CPV-2) is one of the most important enteric pathogens of dogs. This virus is extremely contagious, causing high morbidity with increased incidence in shelters, pet stores and breeding kennels. The disease is characterized by a rapid clinical course with death that can often occur 2–3 days after onset of signs in non-protected hosts (Carman & Povey, 1985; Parrish, 1995). It can affect dogs at any age, but severe infection is most common in puppies between 6 weeks and 6 months of age (Houston et al., 1996). All breeds are susceptible to the disease, although the mixed breeds are described to be less susceptible than many pure-breds. Rottweilers, Doberman Pinschers, English Springer Spaniels, American Pit Bull Terriers and German Shepherd are the pure-breds that have been reported with higher risk for CPV enteritis (Glickman et al., 1985; Houston et al., 1996).

The infection is generally acquired by the faecal-oral route through the contact with faeces from infected dogs or contaminated surfaces. Upon entering the body, the virus affects mainly mitotically active tissues, such as the lymphoid tissues, intestinal epithelium and bone marrow, as well as the heart in neonatal pups. Following an incubation period of 3–7 days, the disease can be characterized by two clinical forms, the enteric form that comprises vomiting, haemorrhagic diarrhoea, depression, loss of appetite, fever and dehydration in younger dogs (Nelson et al., 1979; Carman & Povey, 1985; Parrish, 1995; Hoelzer et al., 2008a). Myocarditis may be seen after infection of neonatal puppies, where the clinical signs are seen a number of weeks after infection (Meunier et al., 1984; Sime et al., 2015). Canine parvoviral infection is also characterized by a drop in the white blood cell counts as a result of the infection of the bone marrow infection and other lymphoid tissues (Kelty, 1978; Appel et al., 1979).

The main method for controlling the disease in domestic animals is by vaccination. After the emergence of the disease, modified live virus vaccines were soon developed, being the first CPV vaccine available in 1979. The vaccines appear to be safe and to confer protective immunity allowing much of the disease to be controlled. However, the virus is still widely distributed in nature, and if pups are not vaccinated, and or when maternal antibodies interfere with their vaccination, they generally become naturally infected (Parrish, 1999). The evolution of the virus raises questions about the efficacy of some vaccines, so that an understanding of the variation is required (Truyen, 2006).

**Aetiology of CPV-2**

**Taxonomy**

The CPV-2 belongs to the genus *Protoparvovirus*, member of the *Parvoviridae* family, that has been included within the
species *Carnivore protoparvovirus 1*, together with Feline panleukopenia virus (FPV), Mink enteritis virus (MEV) and Raccoon parvovirus (RPV), according to the International Committee on Taxonomy of Viruses (Tijssen *et al.*, 2011).

**Virus structure**

Parvoviruses have small (−25 nm diameter), non-enveloped icosahedral capsids. The three-dimensional structure of CPV-2, FPV and CPV-2a particles has been determined at atomic resolution using X-ray crystallography (Tsao *et al.*, 1991; Agbandje *et al.*, 1993; Xie & Chapman, 1996). The virus has a linear, single-stranded and negative-sense DNA genome of ~5200 nucleotides, containing two major open reading frames (ORFs). One of which encodes the non-structural proteins NS1 and NS2, and the other two structural proteins VP1 and VP2. At either end of the genome, palindromic hairpins of about 150 bases are used in the replication of the viral DNA (Reed *et al.*, 1988; Parrish, 1999).

The parvoviral capsid contains 60 protein subunits of VP1 (5–6 copies) and VP2 (54–55 copies), and those share a common structure. The coding regions for the VP1 (727 residues) and VP2 (584 residues) proteins overlap, apart from a 143 amino acid N-terminal region unique to VP1 (Tsao *et al.*, 1991; Agbandje *et al.*, 1993). The two structural proteins are produced by alternative splicing of viral mRNAs (Reed *et al.*, 1988; Wang *et al.*, 1998; Parrish & Kawaoka, 2005). The VP2 protein can be cleaved near its N-terminus by host proteases to produce another structural protein, VP3. The capsid proteins have a highly conserved central core composed of an eight-stranded, anti-parallel β-barrel with flexible loops between the β-strands that interact to form most of the capsid surface. The surface features of the capsid includes a 22 Å long raised region (spike) on the threefold axes, a 15 Å deep depression (canyon) surrounding cylindrical structures at the fivofold axes, and a 15 Å deep depression (dimple) at the twofold axes. In addition, the threefold axes are the most antigenic region of the capsid and serve as a target for neutralizing antibodies (Tsao *et al.*, 1991; Agbandje *et al.*, 1993).

**Virus emergence**

In the 1970s, CPV-2 emerged as a new virus in domestic dogs. It caused a pandemic disease and spread through Asia, Australia, New Zealand, the Americas and Europe in early 1978 (Parrish *et al.*, 1988, 1999). This virus was identified as CPV-2 to distinguish it from the distinctly related minute virus of canine (MVC) or also known as CPV type 1 (CPV-1) (Carmichael *et al.*, 1994). Molecular clock estimates and phylogenetic studies indicated that CPV-2 likely emerged a number of years before spreading globally in dogs in 1978 and 1979 (Parrish *et al.*, 1988; Shackelton *et al.*, 2005; Hoelzer *et al.*, 2008b). Testing for antibodies in dog sera showed that the first positive titres in dogs in Greece and Belgium were reported between 1974 and 1976 (Schwers *et al.*, 1979; Koptopoulo *et al.*, 1986), while the first positive sera in the USA, Japan and Australia were reported early in 1978 (Parrish, 1999). Since that year, the virus has been ubiquitous in dogs throughout the world (Parrish, 1999). During 1979, wild coyotes in the USA also became widely infected (Thomas *et al.*, 1984).

**Origin**

The phylogenetic relationships between CPV-2 isolates from dogs and the viruses from cats (FPV), mink (MEV), raccoon (RPV), raccoon dog (Raccoon dog parvovirus, RDPV) and blue fox (Blue fox parvovirus, BFPV) showed that all CPVs derived from a single common ancestor, and that the strains were mostly similar to viruses from different wildlife animals including raccoons and foxes (Allison *et al.*, 2012, 2013). CPV was shown to be related to a virus similar to the long recognized FPV, but likely not from cats (Truyen *et al.*, 1995). CPV-2 likely arose when it acquired mutations that allowed binding to the canine transferrin receptor (TfR) type-1 (Truyen *et al.*, 1996a; Shackelton *et al.*, 2005; Allison *et al.*, 2012). Several studies have demonstrated that the TfR plays a key role in the susceptibility of cells to infection by these viruses (Hueffer *et al.*, 2003; Palermo *et al.*, 2006).

CPV and FPV are over 98% identical in DNA sequence, but have specific host ranges, antigenic and haemagglutination (HA) properties which are controlled by the capsid protein gene (Chang *et al.*, 1992; Truyen *et al.*, 1995; Shackelton *et al.*, 2005). The successful cross-species viral transfer and adaptation to the new canine host involved few amino acid changes in and around the threefold spike (Truyen *et al.*, 1995). These six genomic changes were sufficient for CPV-2 to acquire the canine host range, but lost the ability to replicate in feline host (Chang *et al.*, 1992; Truyen & Parrish, 1992). Three differences at VP2 residues 93 (Lys to Asn), 103 (Val to Ala) and 323 (Asp to Asn) between FPV and CPV-2 could introduce the canine host range (Chang *et al.*, 1992; Truyen *et al.*, 1995). The changes of VP2 residues 80 (Lys to Arg), 564 (Asn to Ser) and 568 (Ala to Gly) were associated to the loss of ability to replicate in cats (Truyen *et al.*, 1994) and are shown in Figs 1 and 2. However, residues 232 (Val to Ile) and 375 (Asp to Asn) also changed between FPV and CPV-2 sequences. The residue 375 variation was found only in some isolates of the original strain of CPV-2, and in later CPV variants that residue reverted to an Asp, suggesting that 375Asn is not critical to the success of CPV in nature. However, VP2 residue 375 that is located on the side of the threefold spike, this amino acid together with 323 determines the pH dependence of HA (Parrish, 1991a, b; Chang *et al.*, 1992).

**Emergence of antigenic CPV-2 variants**

**Antigenic variant CPV-2a**

After the detection of CPV-2, in many countries that virus was replaced around 1980 in the USA by an antigenically and genetically variant virus, designated CPV-2a. While the CPV-2 strain was present in the USA, Japan, Belgium and
Australia prior to 1980, it was replaced by the CPV-2a variant in all of those countries between 1979 and 1982, as well as, in France and Denmark (Parrish et al., 1988). The examination of sera collected from wild coyotes (Canis latrans) between 1979 and 1984 in the USA also indicated that these were originally infected by CPV-2 (Thomas et al., 1984), but that after 1980 the juvenile coyotes were being infected with CPV-2a, as well as by the 426Asp variant (also known as CPV-2b) (Parrish et al., 1988).

The natural global replacement of CPV-2 by CPV-2a over a period of 2–3 years indicates that CPV-2a has a strong epidemiological advantage over CPV-2 (Parrish et al., 1988). It was also seen that the CPV-2a and its derivatives had regained the ability to infect cats, and it also became the most common virus in many other carnivores (Truyen et al., 1996a). CPV-2a became the new dominant lineage and underwent further evolution, gaining several common point mutations in various lineages. Some of these mutations changed the antigenic properties of the capsid and reached high frequencies in viral populations (Maya et al., 2013). The CPV-2a strain that emerged in 1979 differs in only five or six amino acids from CPV-2 isolates (Parrish et al., 1991b). The changes of the residues 87 (Met to Leu), 300 (Ala to Gly) and 305 (Asp to Tyr) allowed the replication in cats (Figs. 1 and 2). Other changes also occurred in the capsid protein gene, residues 101 (Ile to Thr), 297 (Ser to Ala) and 555 (Val to Ile), between originally CPV-2 and -2a (Tsao et al., 1991; Agbandje et al., 1993; Truyen et al., 1996a).

**Antigenic variants CPV-2b and -2c**

In addition to the original CPV-2a antigenic type, there are two antigenic variants called CPV-2b (or VP2 426Asp) and CPV-2c (or VP2 426Glu). Although not all scientists are in agreement with the virus nomenclature, the reference to CPV-2a, -2b and -2c is prevalent in the literature. CPV-2b was first detected in 1984 in the USA (Parrish et al., 1991b) and CPV-2c was identified in 2000 in Italy (Buonavoglia et al., 2001). However, a recent study (Decaro et al., 2007) showed that the oldest CPV-2c strain was isolated in 1996, thus providing evidence that this variant had been circulating in Germany 4 years before its first detection in Italy. Antigenic differences among the three variants are associated with changes at residue 426 (Asn in CPV-2a, Asp in CPV-2b and Glu in CPV-2c). This mutation affects the major antigenic region (epitope A), which is located at the top of the threefold spike in the VP2 protein. From the DNA sequence analysis of CPV-2a and -2b, it was shown that the second variant differ only two amino acids from the first, in the VP2 protein. The two CPV-2b specific coding changes resulted in differences in VP2 residues 426, previously referred, and 555 in the VP2 region (Fig. 2). The substitution of VP2 residue 555 (Ile to Val) represented a reversion to or retention of the sequence of CPV-2, and only the difference at residue 426 determined the altered epitope recognized and represented a replacement unique to CPV-2b (Parrish et al., 1991b). As the CPV-2b and -2c antigenic strains differ from CPV-2a at only one position (VP2 residue 426), they are now considered by some authors to be variants of CPV-2a rather than distinct subtypes (Organtini et al., 2015).

**Other VP2 mutations reported in the antigenic variants**

Other non-synonymous substitutions at the VP2 region were also reported in the variants. An amino acid change at VP2

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**Fig. 1.** The locations of the mutations between FPV and CPV variants in the structure of the capsid. (a) The surface of the capsid is shown as a projection, where an icosahedral asymmetric unit of the capsid is shown as a triangle. (b) Surface exposed of the CPV capsid, where the three VP2 426 residues (1 to 3) from the three different VP2 monomers located on the top of the threefold spike are visible and are equivalent to shown in (a). VP2 residues 80, 564 and 568 in FPV, as well as residues 87, 297, 300 and 305 in the new antigenic of CPV-2a, -2b and -2c (along with residue 101, which is not surface exposed) appear to determine the ability to replicate in cats. Residues 93 and 323 differ between CPV-2 and FPV and control canine host range and influence canine TIR binding, while residue 426 differs between the new antigenic variants [Based on Steinel et al. (2000) and Allison et al. (2014)]. These locations were visualized using the PyMOL Molecular Graphics System, version 1.5.0.4 Schrödinger, LLC.
position 297 (Ser to Ala) was observed both in CPV-2a and -2b. Residue 297 is located in a minor antigenic site close to epitope B, but no changes in the antigenicity of those variants have been reported. CPV-2a/2b having a mutation at 297 residue (Ser to Ala) have sometimes been designated as New CPV-2a and -2b (Martella et al., 2005; Ohshima et al., 2008).

The mutation of VP2 residue 440 (Thr to Ala) has been observed in the same isolates which showed a mutation of VP2 residue 324 (Tyr to Ile). VP2 residue 440 may influence the antigenic structure as it is located in the GH loop of the VP2 protein on the surface of the capsid, while VP2 residue 324 is likely to have an effect on CPV host range similar to the previously characterized residue 323 (Hong et al., 2007; Mittal et al., 2014). An additional mutant, 300Asp, of CPV-2a/2b has been detected in recent years in domestic or wild felids in southern Asia, as well as in raccoons. The mutation 300Asp is probably the expression of a further adaptation of the virus to replication in the feline or raccoon hosts (Ikeda et al., 2000; Allison et al., 2012).

Nowadays, the three antigenic variants have a worldwide distribution, and are found to infect a variety of different hosts. The regaining of the feline host range and infection of other hosts is likely to be a selective advantage for the virus (Truyen et al., 1996a). While CPV-2 is considered a host range variant of a virus closely related to FPV that gained the ability to infect dogs via wild carnivores, as previously reported, the emergence of CPV-2a was previously suggested to be due to host adaptation in dogs (Allison et al., 2012). A recent study (Allison et al., 2012) showed that raccoons from the USA have harboured parvoviruses similar to CPV for over 20 years. In a phylogenetic analysis of the VP2 protein many RPV sequences, as well as a single isolate from a bobcat, fell in intermediate locations between the dog-associated CPV-2 and -2a strains. Hence, RPVs may have played a central role in the transition between CPV-2 and the later CPV-2a and related variants that not only infected dogs but also regained the ability to infect cats, a property lost by CPV-2. The CPV-2a-specific residues at 87 and 101 position were likely acquired during evolution of the virus in raccoons, while the changes at 300 and 305 were acquired when the virus transferred back to the canine host (Fig. 2). However, other wild animals may have also played a role in the evolution and spread of CPV-2, such as, wolves, foxes, jackals and coyotes that have been found to be susceptible to CPV-2 disease (Thomas et al., 1984; Steinel et al., 2001).

The significance of co-infections

Based on clinical signs, it is not possible to distinguish any of the CPV sequence variants (Markovich et al., 2012).
Although a few reports suggest that CPV-2c (426Glu) may cause more severe clinical signs and mortality particularly in adult dogs than other strains (Decaro et al., 2008), others describe less-severe disease and lower mortality rates in dogs infected with that virus (Decaro et al., 2005).

Co-infections by multiple CPV variants in domestic dogs are not commonly reported, however, two cases have been detected (Battilani et al., 2007; Vieira et al., 2008a). A recent study by Pérez et al. (2014) reported two co-infections, in which one dog was infected by CPV-2c and -2a strains, where the sequences examined differed in 29 nucleotides, and in the other dog the CPV sequence included a minor CPV-2a strain (13.3 % of the viral population) and a major recombinant strain (86.7 %). The recombinant strain arose from the inter-genotypic recombination between CPV-2c and -2a strains within the VP1/VP2 gene boundary. These data indicate that co-infections and recombination are currently occurring between circulating strains in natural populations of CPV, indicating that both may be relevant forces in the generation of viral diversity and the emergence of new genotypes. Rapid genomic evolution by genetic interchange may be regulated by restricting recombination to particular hotspots in the genome that favour the interchange of the entire VP2 gene (Pérez et al., 2014).

The worldwide distribution of antigenic CPV-2 variants

Spreading of antigenic variants in the global dog population

The early evolutionary history of CPV was characterized by global dissemination and strain replacement. CPV-2a replaced the CPV-2 worldwide within 2 or 3 years, indicative of an increased fitness in dogs (Parrish et al., 1988). The end of 1983, Houston et al. (1996) showed that CPV infection had been reported in 50 countries around the world. The dynamics of the spread and evolution of CPV may have changed since it emerged. In contrast to the early period, the most recent endemic phase of the disease appears to be characterized by geographical genetic differentiation (Hoelzer et al., 2008b; Clegg et al., 2011).

Between 1979 and 2005, nearly 600 articles, papers, numerous text chapters and monographs have been published on the subject of CPV-2, according to Carmichael (2005), with over 1000 listed in PubMed. CPV infection has been reported from Africa, Asia, Australia, the Americas and Europe (Steinell et al., 1998; Decaro et al., 2007; Meers et al., 2007; Kumar & Nandi, 2010; Markovich et al., 2012; Pérez et al., 2012; Castanheira et al., 2014), and there are large numbers of publications which have reported the frequencies of different CPV variants in various countries and regions. Table 1 summarizes the presence of the CPV variants around the world in the domestic dog population, based on the analysed references. Moreover, the CPV-2 variants have been reported in 42 countries distributed among five continents. The CPV-2a (VP2 426Asn) has been reported in 37 countries, the CPV-2b (VP2 426Asp) in 31 countries and the CPV-2c (VP2 426Glu) reported in 21 countries. Those three strains have been reported to co-circulate in 15 countries, mainly in European and South American countries (Fig. 3). However, these numbers may change as there are several other countries where current studies are based on CPV positive serology testing, namely Cape Verde (Castanheira et al., 2014), Pakistan (Muzzaffar et al., 2006) or Zimbabwe (McRee et al., 2014).

Recent epidemiological reports indicate that CPV-2a with VP2 426Asn is the predominant variant in Australia (Meers et al., 2007), most of Asian (Yoon et al., 2009, Phromnoi et al., 2010; Chou et al., 2013; Yi et al., 2014; Timurkan et al., 2015) and European countries (Ntasis et al., 2010; Decaro et al., 2011, 2013; Cavalli et al., 2014; Filippov et al., 2014), and this is the only variant reported in New Zealand (Ohneiser et al., 2015), Nigeria (Dogonyaro et al., 2013), Hungary (Demeter et al., 2010), Czech Republic (Decaro et al., 2007), Slovenia and Romania (Decaro et al., 2012), while it was not detected in Vietnam and Mexico, as well as, from an outbreak in a litter in Sweden (Table 1, Fig. 4).

The prevalence of CPV-2b (VP2 426Asp) has been reported in several countries within the five continents (Table 1), and that was found to be the predominant antigenic variant in Ireland (McElligott et al., 2011), the UK (Decaro et al., 2007), the USA (Hong et al., 2007), African countries (Steinell et al., 1998; Touihri et al., 2009; Dogonyaro et al., 2013) and in four of nine Asian countries (Nakamura et al., 2004; Kumar & Nandi, 2010; Ahmed et al., 2012; Soma et al., 2013). Both CPV-2a and -2b were distributed in equal proportion in Belgium (Decaro et al., 2013), Switzerland and Austria where these antigenic types were exclusively isolated (Truyen et al., 2000). Additionally, a sequence with VP2 426Asp was isolated from a dog in Russia (GenBank) (Chausov, E.V., Ternovoi, V.A., Protopopova, E.V., Durymanov, A.G., Shestopalov, A.M., Loktev, V.B. and Netesov, S.V., unpublished data, accession number JN033694).

Approximately 20 years after the emergence of the CPV-2c, this has been found mainly in South American (Calderón et al., 2011; Pérez et al., 2012; Pinto et al., 2012; Aldaz et al., 2013) and European countries (Decaro et al., 2011; Sutton et al., 2013). However, that variant has not been detected in Oceania. In Poland, all isolates were classified as CPV-2c during 1995–2009, while between 1982 and 1985 the isolates were CPV-2a and -2b (Majer-Dziedzic et al., 2011).

The VP2 residue 426 variants of CPV coexist in different ratios in dog populations around the world (Truyen et al., 1996a). Their relative frequencies and genetic characteristics vary according to the geographic region analysed and the time of sample collection. The reasons for the different ratios in various countries are unknown, but immune-selection by vaccines based on different antigenic types seem unlikely, as many vaccines used in different parts of the world are based on the original type CPV-2 (Steinell et al., 1998). Cross-protection has been demonstrated with the use of these vaccines against infection with the different
VP2 426 variants (Spibey et al., 2008; Wilson et al., 2013). The co-existence of those three variants in various populations in the world and in different ratios shows that there is likely no strong evolutionary advantage for one type or the other (Steinel et al., 1998).

**The temporal and geographic variability and the relative frequencies**

Depending on the year of collection of the canine samples, the frequency of the CPV variants has revealed interesting changes in their distribution and variability. Table 1 shows the variant types reported globally in the dog population, while Table 2 shows their temporal variation within different countries.

During 6 years, the variants’ prevalence was analysed on collected samples of the Uruguayan dog population. CPV-2c was the main variant retrieved on samples from 2006, with only one CPV-2a (Pérez et al., 2007) and CPV-2c was the only variant detected from samples collected between 2007 and 2009. However, an unexpected epidemiological change has occurred in Uruguay in 2010, where a divergent CPV-2a strain emerged in the CPV-2c homogenous population. This variant rapidly spread through the national dog population and the sequence analysis showed amino acid substitutions (267Tyr, 324Ile and 440Ala) that were not observed in the Uruguayan CPV-2c (Pérez et al., 2012). In 2011, the frequency of the CPV-2a increased to levels of 85 % in the canine population (Maya et al., 2013). In Argentina, the high prevalence of CPV-2c strains seen in the last years continues nowadays. CPV-2c appeared in the year 2003 (Calderón et al., 2009) and has been the predominant strain (>90 %) since 2008 (Calderón et al., 2015). However, unexpectedly four CPV-2a samples were found during the year 2012, while it has reappeared with a low prevalence (<10 %). No CPV2b strains have been detected among local samples since 2009 (Calderón et al., 2009, 2011, 2012, 2015). The results obtained in Argentina, together with those reported previously in Uruguay (Pérez et al., 2012) strongly suggest that, in spite of the geographical proximity, wild-type CPV strains undergo different evolutive pathways in each country, resulting in the prevalence of different strains in related dog populations (Calderón et al., 2015).

In Brazil, all the three variants were found circulating in the canine population, however in different proportions, being the predominant variant type dependent on the year of study. CPV-2a was the predominant variant during 1980–1986, which was substantially replaced by CPV-2b from 1990 to 1995 (Pereira et al., 2000). All samples from 1995 to 2003 were identified as CPV-2a, and from 2004 to 2006, both CPV-2a and -2b were observed. From 2006 to 2009, most of the samples were characterized as CPV-2b, with only one sample classified as CPV-2c in 2008 (Castro et al., 2010), and that become the most predominant variant circulating in Brazil between 2008 and 2010 (Pinto et al., 2012).

The predominance of variants may also vary according to the regions of the country where the samples are collected (Yi et al., 2014). For example in Portugal, the predominant CPV-2 variants found in the continental south regions and
Table 1. The worldwide distribution of the antigenic CPV variants in the domestic dog population

<table>
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<tr>
<th>Continent/country</th>
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<th>Reference</th>
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</table>
in the Islands differed from those detected in the samples collected in the continental north of country (Miranda et al., 2016), however, the distribution of CPV in China did not show any geographical correlation (Yi et al., 2014). The reason for the uneven world distribution of the different viral variants is still not clearly understood but could be related to the changing dynamics that the virus has experienced since its emergence in the 1970s (Hoelzer et al., 2008b). These data provide new evidence of the role of local genetic diversity and migration events during CPV evolution and, emphasize the dynamic changes in CPV variants and highlight the importance of ongoing surveillance programs to provide a better understanding of the virus epidemiology (Pérez et al., 2012; Maya et al., 2013). However, this variability did not happen within certain countries, like Hungary (Demeter et al., 2010; Decaro et al., 2013; Csagola et al., 2014), Japan (Ohshima et al., 2008; Soma et al., 2013) and China (Zhang et al., 2010; Yi et al., 2014), where different studies during several years showed the presence of the same predominant variant type. Based on these data, the three different antigenic variants of parvovirus, 2a, 2b and 2c, are currently circulating worldwide, being however difficult to state which is predominant.

On the other hand, this variability should include the analysis of the other unnamed mutations that are probably just as or more important than the mutations in the residue 426. In recent years, several other mutations have been reported around the world simultaneously within the VP2 region (Pinto et al., 2012; Yi et al., 2014). For example, the amino acid substitution Thr440Ala has been described in CPV-2a and -2b strains from India, Brazil, South Africa and China (Chinchkar et al., 2006; Castro et al., 2010; Dogonyaro et al., 2013; Yi et al., 2014) and in CPV-2c strains from the USA (Hong et al., 2007) and Argentina (Calderón et al., 2011). The 440 position is an important residue because it is located at the top of the threefold spike, the main antigenic site of the virus (Tsao et al., 1991). This residue is undergoing positive selection and has evolved independently in different populations, which explains its worldwide presence in unrelated CPV-2 populations (Decaro et al., 2007). The Tyr324Ile change appeared in CPV-2a, -2b and -2c. This was first reported in 2004 in CPV-2a strains from China (Zhang et al., 2010), however, it has been described in the USA (Hong et al., 2007), Brazil (Pérez et al., 2012) and Hungary (Csagola et al., 2014). Previous studies have shown that residue 324 is undergoing strong positive selection in the parvoviruses of all carnivores.

**Fig. 4.** Worldwide distribution of CPV-2 variants in domestic dogs. Red, presence of CPV-2a variant (a); pink, presence of CPV-2b variant (b); green, presence of CPV-2c variant (c).
Residue 324 is adjacent to 323, which together with residue 93 plays an important role in binding to the canine TfR and affects the canine host range (Hueffer et al., 2003), and presumably, this Tyr324Ile alteration can result in stronger receptor binding (Csagola et al., 2014). In addition, the Ala516Thr Hungarian-specific change (Csagola et al., 2014) and the Ala297Asp circulating in South African strains (Dogonyaro et al., 2013), suggest that these field viruses are under strong positive selection pressure for CPV-2 local types.

**Host ranges for antigenic CPV-2 variants**

### Domestic cats

The original CPV-2 does not replicate in cats, on the other hand, it replicates in feline cells in vitro (Parrish, 1991a;
The host ranges of the CPV variants are complex and diverse. Although CPV isolates have been recovered from domestic cats and dogs, they have naturally been detected in a variety of related carnivores. Infection with CPV has already been demonstrated in Canidae, Felidae, Procyonidae and Mustelidae family (Barker & Parrish, 2001; Steinel et al., 2001) and the families of wild carnivores where the presence of CPV were detected are summarized in Table 3. However, several studies reported the detection of CPV in other wild carnivores by serological analysis (Santos et al., 2009; Woodroffe et al., 2012), and consequently there are no sequences available on GenBank database.

The residue 300 is important in distinguishing the antigenicity and host range among parvovirus but may vary among CPV isolates (Qin et al., 2007). Alanine holds the position in CPV-2, in CPV-2a and -2b it is Gly and in CPV-2c of the Vietnamese leopard cat Asp (Ikeda et al., 2000). During 1995–1997, CPV-2a or -2b variants were isolated from three leopard cats (Felis bengalensis) in Vietnam and Taiwan. Other three leopard cats showed the substitution (Gly to Asp) at the conserved residue 300, were designated as Leopard cat parvovirus (LCPV), but currently designated by CPV-2c (Ikeda et al., 2000). In 2004, nucleotide and phylogenetic analysis of the capsid protein VP2 gene classified the Red panda parvovirus (RPPV) as a CPV-2a from red panda (Ailurus fulgens) in China. The substitution of Val for Gly at the conserved residue 300 in RPPV presents an unusual variation in the CPV-2a amino acid sequence and is a further evidence for the continuing evolution of the virus (Qin et al., 2007). Chen et al. (2011) showed that the isolates from the RDPV and Masked palm civet parvovirus (MCPV) were antigenically similar to CPV-2a, but had a change at G300S and classified as CPV-2a-like. The G300S mutation would have contributed to the adaptation of CPV-2a to raccoon dogs (Nyctereutes procyonoides) and

### Table 3. Species and taxonomic family of wild carnivores infected by CPV variants, according to the references analysed

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Regular name</th>
<th>CPV variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canidae</td>
<td>Canis lupus</td>
<td>Grey wolf</td>
<td>2a, 2b, 2c</td>
</tr>
<tr>
<td></td>
<td>Otocyon megalotis</td>
<td>Bat-eared fox</td>
<td>2b</td>
</tr>
<tr>
<td></td>
<td>Vulpes vulpes</td>
<td>Red fox</td>
<td>2a</td>
</tr>
<tr>
<td></td>
<td>Canis latrans</td>
<td>Coyote</td>
<td>2b, 2c</td>
</tr>
<tr>
<td></td>
<td>Nyctereutes procyonoides</td>
<td>Raccoon dog</td>
<td>2a</td>
</tr>
<tr>
<td>Felidae</td>
<td>Acinonyx jubatus</td>
<td>Cheetah</td>
<td>2b</td>
</tr>
<tr>
<td></td>
<td>Panthera tigris altaica</td>
<td>Siberian tiger</td>
<td>2c</td>
</tr>
<tr>
<td></td>
<td>Felis bengalensis</td>
<td>Leopard cat</td>
<td>2a, 2b, 2c</td>
</tr>
<tr>
<td></td>
<td>Puma concolor</td>
<td>Puma</td>
<td>2b, 2c</td>
</tr>
<tr>
<td></td>
<td>Lynx rufus</td>
<td>Bobcat</td>
<td>2a, 2c</td>
</tr>
<tr>
<td>Viverridae</td>
<td>Paguma larvata</td>
<td>Masked palm civet</td>
<td>2a</td>
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<tr>
<td>Mustelidae</td>
<td>Martes foina</td>
<td>Stone marten</td>
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<td>Asian small-clawed otters</td>
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<td>Ursidae</td>
<td>Ailurus fulgens</td>
<td>Red panda</td>
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<td>Ailuropoda melanoleuca</td>
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<td>Procyonidae</td>
<td>Procyon lotor</td>
<td>Raccoon</td>
<td>2a, 2b</td>
</tr>
</tbody>
</table>
masked palm civets (*Paguma larvata*). In addition, the MCPV had a change at T301A in VP2 protein. Residue 301, following residue 300, is located in loop 3 at the extremities of the threefold spike on the viral surface. Therefore, its changes might also affect antigenicity and host range of CPV. Other evidence for the continuing evolution of the CPV was the identification of the Q370R point mutation in the VP2 gene from a giant panda (*Ailuropoda melanoleuca*) classified as CPV-2a (Guo et al., 2013). All variants have succeeded in regaining the feline/carnivores host and new genomic mutations can be expected in future (Steinel et al., 1998).

Steinel et al. (2000) reported the detection of CPV-2b viral DNA in six cheetahs (*Acinonyx jubatus*) from Namibia and USA and, in a bat-eared fox (*Otocyon megalotis*) from Namibia. CPV-2a sequence was also found in the faecal sample of the Siberian tiger (*Panthera tigris altaica*) from a German zoo. The very high prevalence of CPV-2a/2b infections in these large cats compared to domestic cats may suggest a higher susceptibility of these species for these virus types (Steinel et al., 2000). An isolate from a stone marten (*Martes foina*) collected in Portugal revealed the presence of CPV-2b (Duarte et al., 2013), such a stone marten CPV-2a strain was referred by Steinel et al. (2001). In Italy, four isolates from wolves analysed by Battilani et al. (2001) were antigenically and genetically identified as CPV-2b. The phylogenetic analysis from several non-domestic animals, such as, raccoon (*Procyon lotor*), coyote (*Canis latrans*), grey wolf (*Canis lupus baileyi/nubilus/occidentalis*), puma (*Puma concolor*), striped skunk (*Mephitis mephitis*) and bobcat (*Lynx rufus*) (Allison et al., 2012, 2013) revealed the presence of the three CPV variants. A recent study by Filipov et al. (2014) detected two wild carnivores parvovirus positive, a wolf (*Canis lupus*) and a red fox (*Vulpes vulpes*), both being infected by CPV-2a strains. A diagnosis of CPV-2c was also made in a group of Asian small-clawed otters (*Aonyx cinerea*) (Gjeltema et al., 2015).

Conclusions

In approximately 40 years after the emergence of original CPV-2, three antigenic CPV variants are disseminated in 42 countries. The different antigenic variants of CPV-2 are prevalent in varying proportions in different countries. The prevalence of variants by country was found to depend on the year and region of collection of samples, however, results can differ due to the lack of current studies and the continuous monitoring of the CPV-2 within countries. Unexpectedly, the last reported variant (CPV-2c) has been only detected in 21 countries around the world.

As a result of multiple cross-species transmission events, there was a significant diversity of nondomestic animals infected by CPV-2 variants. A better understanding on the epidemiology and the evolution of CPV in other hosts than domestic cats and dogs appears essential. The summary of the data presented here contributes to map the actual geographical spread of the antigenic CPV variants worldwide and may be used as a handy information source for veterinarians and researchers interested in CPV and its evolution.

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