

Review

Comparative genomics of canine parvovirus in South America: Diversification patterns in local populations

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ABSTRACT

Canine parvovirus (CPV) is a significant pathogen in domestic dogs worldwide, causing a severe and often fatal disease. CPV comprises three antigenic variants (2a, 2b, and 2c) distributed unevenly among several phylogenetic groups. The present study compared genetic variability and evolutionary patterns in South American CPV populations. We collected samples from puppies suspected of CPV infection in the neighboring Argentina and Uruguay. Antigenic variants were preliminarily characterized using PCR-RFLP and partial vp2 sequencing. Samples collected in Argentina during 2008–2018 were mainly of the 2c variant. In the Uruguayan strains (2012–2019), the 2a variant wholly replaced the 2c from 2014. Full-length coding genome and vp2 sequences were compared with global strains. The 2c and 2a strains fell by phylogenetic analysis into two phylogroups (Europe I and Asia I). The 2c strains from Argentina and Uruguay clustered in the Europe I group, with strains from America, Europe, Asia, and Oceania. Europe I is widely distributed in South America in the dog population and is also being detected in the wildlife population. The 2a strains from Uruguay formed the distinct Asia I group with strains from Asia, Africa, America, and Oceania. This Asia I group is increasing its distribution in South America and worldwide. Our research reveals high genetic variability in adjacent synchronic samples and different evolutionary patterns in South American CPV. We also highlight the importance of ancestral migrations and local diversification in the evolution of global CPV strains.

1. Introduction

Canine parvovirus (CPV) is one of the dogs' most critical pathogenic viruses, causing acute hemorrhagic enteritis and myocarditis in puppies (Appel et al., 1979). CPV belongs to the species *Protoparvovirus carnivoran1* (genus *Protoparvovirus*, subfamily *Parvovirinae*, family *Parvoviridae*). The closely phylogenetically related protoparvoviruses infect several carnivore hosts, including raccoons, minks, and felines (Cotmore et al., 2014; Péntzes et al., 2022; Péntzes et al., 2020; Zerbinì et al., 2023).

CPV has a linear single-stranded DNA genome (5.2 kb) with two open reading frames (ORFs) (Reed et al., 1988). The 3' ORF encodes nonstructural proteins 1 and 2 (NS1 and NS2) involved in DNA replication and packaging (Wang et al., 1998). The 5' ORF encodes the viral capsid proteins 1 and 2 (VP1 and VP2), the main antigens that induce neutralizing antibodies (Agbandje et al., 1995; Nelson et al., 2007; Tsao

et al., 1991).

The original type-2 CPV strain (CPV-2) emerged around the 1970s from a protoparvovirus infecting wild or domestic carnivores (Parrish, 1999). Soon after its first detection, CPV-2 reached pandemic proportions and was rapidly replaced by a new antigenic and genetic variant designated 2a (Hoelzer and Parrish, 2010; Parrish et al., 1988; Voorhees et al., 2019). This new 2a variant differed at five residues in VP2 and regained the ability to infect cats and other carnivores (Parrish, 1991; Truyen et al., 1996). CPV-2a became the new dominant lineage and is the ancestor of the variants circulating worldwide. In addition to the original 2a, two variants (2b and 2c) differ in the VP2-426 residue; 2a has Asn426, while 2b and 2c have Asp and Glu, respectively. CPV-2b was first detected in 1984 in the United States (Parrish et al., 1991), and CPV-2c was identified in 2000 in Italy (Buonavoglia et al., 2001).

The residue 426 used for typing the 2a, 2b, and 2c variants occurred

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independently in different monophyletic groups and is not appropriate to infer CPV evolution (Chung et al., 2020; Grecco et al., 2018; Voorhees et al., 2019). The discrepancy between phylogenetic and antigenic classification has led to various attempts to classify the virus using sequence data, mainly from the complete VP2 gene (Chung et al., 2020; Li et al., 2017; Tucciarone et al., 2018).

South America has a CPV viral population with a strong temporal and spatial component. The close phylogenetic relationship with foreign strains suggests that long migration and local differentiation have influenced the CPV evolution in the continent (Grecco et al., 2018). Four monophyletic groups or clades (Europe I, Asia I, Europe II, and South America I) circulate in South America (Grecco et al., 2018). These groups include strains from other continents and were denoted according to the most likely origin of emergence.

The Europe I clade comprises 2c strains from Argentina, Brazil, Ecuador, Paraguay, and Uruguay and likely emerged in Southern Europe. In the past decade, there have been periods in South America where Europe I was the most commonly circulating variant. For example, the 2c variant has been prevalent in Argentina since 2003 (Calderon et al., 2009; Calderón et al., 2011; Gallo Calderón et al., 2012) and in Uruguay until 2010 (Pérez et al., 2007, 2012).

The Europe II clade includes strains from Brazil and Ecuador that emerged in Europe and were introduced to South America via Ecuador in the middle 1980s. Some Ecuadorian strains of this clade acquired *de novo* the residue 426E of the 2c variant. Therefore, these are 2c strains that do not share a recent ancestor with the Europe I clade.

The South American I clade comprises strains from Argentina, Brazil, and Uruguay (2a and 2b), which emerged and differentiated in Argentina in the 1990s (Grecco et al., 2018).

The Asia I clade includes 2a strains from Uruguay and most likely emerged in Asia during the late 1980s. This clade was introduced in Uruguay in 2010 and reached a frequency of 85% within two years (Grecco et al., 2018; Maya et al., 2013; Pérez et al., 2012). Although the spreading was fast, this strain has yet to be described in other South American countries (Gallo Calderón et al., 2015).

The study aimed to analyze phylogenetically South American CPV strains to provide insights into the population genetic diversity and differentiation, particularly in neighboring geographic populations of Argentina and Uruguay.

2. Material and methods

2.1. Argentine and Uruguayan samples

Two hundred sixty-seven fecal samples were collected from puppies suspected of having parvovirus infection from Argentina ($n = 41$) and Uruguay ($n = 226$). The samples were obtained from 2012 to 2019 (Uruguay) and 2008–2018 (Buenos Aires, Argentina) from veterinary clinics in different geographic regions that are depicted in a map (<http://microreact.org/>) (Supplementary Table 1 and Supplementary Fig. 1).

2.2. DNA extraction, polymerase chain reaction (PCR), and restriction fragment length polymorphism (RFLP)

Viral DNA was extracted from fecal samples using the QIAmp DNA Mini kit (Qiagen). All strains were characterized by amplifying a partial VP2 coding region (1315 nt), including codon 426 (Castillo et al., 2020; Ikeda et al., 2000). The CPV-positive samples were further processed for restriction fragment length polymorphism (RFLP) analysis and Sanger sequencing to confirm CPV variant classification. The RFLP was performed with the restriction enzyme *MboII* for 2 h at 37 °C. Digested products (15 µl) were assayed on 1.5% agarose gel to analyze the RFLP amplicon pattern. The enzyme recognizes the target sequence GAAGA, which is present only once at positions 4006–4010 in the CPV-2, 2a, and 2b amplicons. The restriction site appears twice in the 2c variant, at

positions 4006–4010 and 4062–4066; the latter site includes the GAA codon that encodes the characteristic amino acid of CPV-2c (Glu426).

2.3. Sequencing

Full-length coding genome amplification of five selected samples from Argentina and 31 from Uruguay was performed as previously described (Pérez et al., 2014). Samples for sequencing were chosen to increase the likelihood of detecting divergent strains. For Argentina, we selected three 2c samples (2c strains used to be relatively homogenous) and all non-2c samples. For Uruguay, we selected samples from different variants, locations, and years.

Sequence assembly and consensus sequences were obtained using Geneious Prime (Kearse et al., 2012). The nucleotide sequences were submitted to the GenBank database (<http://www.ncbi.nlm.nih.gov>) (Accession numbers: OK888554–OK888589) (Table 1).

2.4. Dataset creation and curation

Full-length coding genomes (4269 nt, $n = 339$) and vp2 gene sequences (1755 nt, $n = 1568$) of five selected samples from Argentina and thirty-one from Uruguay (Table 1) were combined with sequences retrieved from the GenBank database (Supplementary Tables 2 and 3) to create two datasets. Duplicated and vaccinal sequences were removed, and nucleotide and amino acid sequence alignments were performed using MAFFT (Katoh et al., 2002).

Table 1
Argentina and Uruguayan samples obtained in the present study according to GenBank accession number, year and country of collection, CPV variant, and phylogenetic group.

Accession number	Year	Country	Diagnostic	Phylogroup
OK888559	2012	Uruguay	CPV-2c	Europe I
OK888560	2012	Uruguay	CPV-2a	Asia I
OK888561	2012	Uruguay	CPV-2c	Europe I
OK888562	2012	Uruguay	CPV-2a	Asia I
OK888563	2012	Uruguay	CPV-2a	Asia I
OK888564	2012	Uruguay	CPV-2c	Europe I
OK888565	2012	Uruguay	CPV-2c	Europe I
OK888566	2012	Uruguay	CPV-2c	Europe I
OK888567	2012	Uruguay	CPV-2c	Europe I
OK888568	2012	Uruguay	CPV-2a	Asia I
OK888569	2012	Uruguay	CPV-2c	Europe I
OK888570	2013	Uruguay	CPV-2a Rec	No group - Recombinant
OK888571	2013	Uruguay	CPV-2a	Asia I
OK888572	2013	Uruguay	CPV-2a	Asia I
OK888573	2013	Uruguay	CPV-2a	Asia I
OK888574	2013	Uruguay	CPV-2a	Asia I
OK888575	2013	Uruguay	CPV-2c	Europe I
OK888576	2013	Uruguay	CPV-2a	Asia I
OK888577	2013	Uruguay	CPV-2a	Asia I
OK888578	2014	Uruguay	CPV-2a	Asia I
OK888579	2014	Uruguay	CPV-2a	Asia I
OK888580	2014	Uruguay	CPV-2a	Asia I
OK888581	2014	Uruguay	CPV-2a	Asia I
OK888582	2015	Uruguay	CPV-2a	Asia I
OK888583	2015	Uruguay	CPV-2a	Asia I
OK888584	2015	Uruguay	CPV-2a	Asia I
OK888585	2016	Uruguay	CPV-2a	Asia I
OK888586	2016	Uruguay	CPV-2a	Asia I
OK888587	2016	Uruguay	CPV-2a	Asia I
OK888588	2017	Uruguay	CPV-2a	Asia I
OK888589	2017	Uruguay	CPV-2a	Asia I
OK888554	2013	Argentina	CPV-2b	Europe I basal
OK888555	2015	Argentina	CPV-2c	Europe I
OK888556	2017	Argentina	CPV-2a Rec	No group - Recombinant
OK888557	2017	Argentina	CPV-2c	Europe I
OK888558	2017	Argentina	CPV-2c	Europe I

2.5. Recombination analysis

The full-length genome dataset was analyzed using a recombination detection program (RDP4) to identify recombinant and parental sequences and locate breakpoints (Martin et al., 2015). Only potential recombination events detected by two or more algorithms, coupled with phylogenetic evidence of recombination, were considered significant using the highest acceptable *P*-value cutoff of 0.05. SplitsTree4 (Huson and Bryant, 2006) was used to infer a recombination network from the dataset. A Phi test value of *p* 0.05 was considered statistical evidence of recombination in the dataset.

2.6. Sequence analysis and phylogenetic inferences

After removing duplicates and recombinant strains detected from the dataset, the best-fit model of nucleotide substitution (GTR + G + I) was selected under the Akaike and the Bayesian information criteria in jModelTest (Posada, 2008) to perform phylogenetic analysis. Maximum-likelihood trees were inferred in Geneious Prime (Kearse et al., 2012) using the PhyML plugin (Guindon et al., 2010), node support was assessed using SH-like branch support (Anisimova et al., 2011), and the tree was visualized using iTOL (Letunic and Bork, 2021). Determining variable residues in the protein alignments was performed using the DIVEIN web server (Deng et al., 2010).

3. Results

3.1. CPV identification and classification

3.1.1. Argentine samples

PCR and RFLP were used to discriminate between 2c and non-2c (2a and 2b) CPV strains (Supplementary Fig. 2). We identified 38 strains as 2c and three strains as non-2c. Characterization is straightforward when the restriction pattern corresponds to a 2c strain; if the pattern is different, it is necessary to determine whether it is a 2a or 2b variant by sequencing the 426 codon.

One strain was 2a (collected in 2017), and one was 2b (collected in 2013) (Fig. 1A and Supplementary Table 1). In the 2017 strain, there was a GAG codon instead of the typical GAA codon found in 2c strains. Both codons encode for the 426Glu of 2c strains, but the GAG codon is not recognized by the *MboII* enzyme used in RFLP (Supplementary Fig. 2).

3.1.2. Uruguayan samples

In Uruguay, we identified 18 2c strains in 2012 and one in 2013, along with 207 2a/2b strains between 2012 and 2019. The 2a/2b strains were later classified as 2a by sequencing the genomic region containing codon 426 (Fig. 1B and Supplementary Table 1).

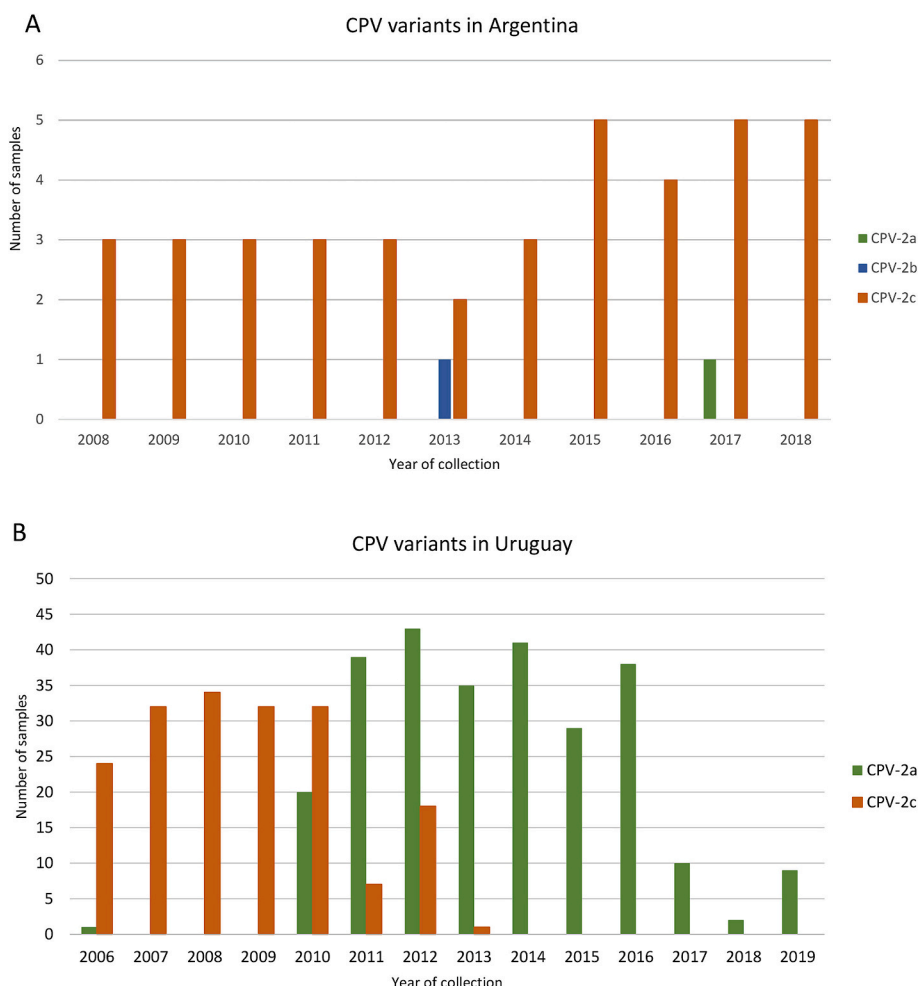


Fig. 1. A. Circulating pattern of CPV variants in Argentina from 2008 to 2018. B. Circulating pattern of CPV variants in Uruguay from 2012 to 2019.

3.2. Recombination analysis

When constructing phylogenetic trees, the signal of vertical evolution can be confounded by recombination. Therefore, the initial step was examining the strains and identifying potential recombinants. Thirty-three recombinant strains were detected and characterized (Supplementary Table 4).

Moreover, we detected one Argentine and one Uruguayan recombinant strain among the newly obtained sequences. The phi test found statistically significant evidence for recombination ($p = 5.372\text{E-}4$). In both cases, the recombination was between 2c (ns) and 2a (vp) strains, located between the 2c and 2a clusters in the network analyzed (Supplementary Fig. 3). The parental strains for the Argentine recombinant were KM457110 (2c) and JX660690 (2a), and for the Uruguayan recombinant, they were KM457110 (2c) and OK888589 (2a).

3.3. Phylogenetic analysis and sequence comparison

A maximum likelihood tree was constructed using a complete coding

genome dataset (Fig. 2). Argentine and Uruguayan 2c strains fell in the Europe I clade in the phylogenetic tree. Europe I also included previously reported 2c strains from Argentina, collected between 2008 and 2013, from Uruguay, collected between 2006 and 2011, and other related CPV-2c from America (United States, Mexico, Brazil, Chile, Ecuador, Paraguay, Peru), Europe (Italy, France, Albania), Asia (Iran) and Oceania (Australia) (Fig. 2). The sequences of this clade shared a unique amino acid in position 426 of VP2 protein (426E) and synonymous changes at positions 1975C, 2086G, and 2575A. The 2b strains from Argentina sequenced here, and the 2b strains from the United States were basal of Europe I clade.

The Uruguayan 2a strains collected during 2012–2019 clustered in well-supported Asia I clade of 2a strains from Asia (China, Viet Nam, Iran, India, South Korea, Bangladesh, Singapore), Africa (Nigeria), North America (Canada), and Oceania (Australia) (Fig. 2). In addition, most strains shared the molecular signature: 267Y, 324I, 426N (VP2).

The obtained strains were not included in other relevant clades in the phylogenetic analysis. The 2a and 2b strains from Brazil, Argentina, and Uruguay formed a clade denoted South America I with solely South

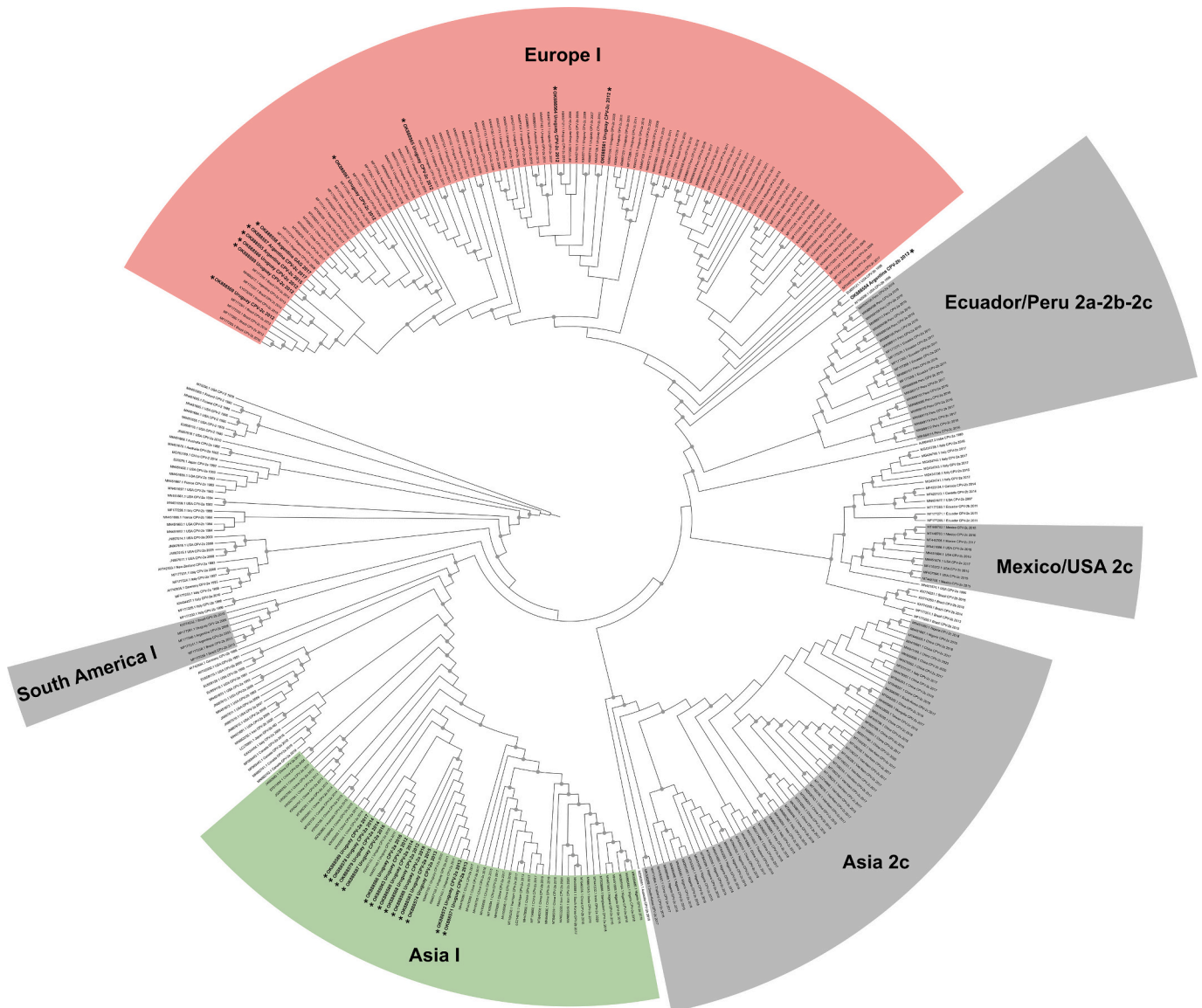


Fig. 2. CPV phylogenetic reconstruction was inferred using the maximum likelihood method with the GTR + G + I substitution model, with node support assessed using SH-like. The analysis comprised 339 complete coding genomes (4269 nt). Bootstrap values >0.7 are colored in grey. The newly generated sequences are indicated with bold, larger letters and an asterisk.

American strains. CPV strains (2a, 2b, and 2c) from Peru and Ecuador clustered together, while 2c strains from Mexico and USA comprised an exclusively North American group. One clade without strains from South America is formed with 2c strains from Asia (China, South Korea, Mongolia, Taiwan, Vietnam, Thailand), Nigeria, and Italy dated from 2013 to 2020.

A maximum likelihood tree was constructed with the vp2 dataset (Supplementary Fig. 4). This phylogenetic tree depicted many of the clades identified using the complete coding genome, but they comprised more strains and had less support.

Argentine and Uruguayan 2c strains are associated in Europe I clade with strains from Italy, France, Albania, Belgium, Croatia, India, Portugal, Spain, and South and North America. The Indian 2c strain from 2010 (KX425920) was absent from the phylogenetic analysis with complete coding sequences because it lacks the NS ORF. As an ancestral branch, this group had an Italian 2c strain collected in 2000 (FJ222821).

South American 2a strains were included in the Asia I clade, where most strains were 2a, some 2b, and one 2c from China (2016). This clade comprised strains from Asia (China, India, Singapore, South Korea, Iran, Vietnam, Japan, Bangladesh, and Thailand), South America (Uruguay and Brazil), Europe (Italy), Africa (Nigeria), and Oceania (Australia). The oldest strain in this clade was from Thailand (2008). Most sequences within the Asia I clade shared the molecular signature in VP2: 267Y, 324I, 426N.

Some clades were exclusively circulating in South America: strains from Argentina, Brazil, Chile, and Uruguay collected between 1995 and 2019 comprised the South America I clade, formed with 2a and 2b strains. In addition, one group was composed of only 2a/2b strains from Brazil (1990 to 2015). Furthermore, 2a/2b strains from Ecuador collected in 2012 clustered together with Peruvian 2a/2b strains and one Brazilian 2a strain.

4. Discussion

CPV has been causing severe disease in domestic dogs for over 45 years (Appel et al., 1979; Hoelzer and Parrish, 2010). Genetic variation has accumulated since its emergence, resulting in several clades (Chung et al., 2020; de Oliveira Santana et al., 2022; Grecco et al., 2018; Hoelzer and Parrish, 2010) that vary in frequency and genetic characteristics depending on geographic location and collection time (Miranda and Thompson, 2016).

In this study, we compare the genomes of South American strains to gain insights into the evolutionary trends of CPV in neighboring and distant countries.

Phylogenetic analysis identified groups (phylogroups) or clades containing Argentine and Uruguayan strains (Fig. 2). It is more appropriate to refer to monophyletic groups of strains as phylogroups rather than “clades,” as the latter term is typically used for samples taken simultaneously (synchronic) (Cellinese et al., 2012).

4.1. Antigenic CPV variants circulating in Argentina and Uruguay

In the Argentine population, 95% of the samples belonged to the 2c strains (Fig. 1A), indicating that this variant is still the most prevalent, particularly in the Buenos Aires Province. In contrast, the 2a variant has wholly replaced the 2c variant in the Uruguayan dog population. In 2011, the 2a variant reached a frequency of 85% (Maya et al., 2013; Pérez et al., 2012). We have found that from 2014 on, only the 2a variant was detected in the country (Fig. 1B).

Notably, one of the Argentine strains displayed a different codon for position 426: GAG instead of GAA (4062–4064). Other point mutations affecting correct genotyping have been reported previously in Hungary and Italy (Decaro et al., 2013; Decaro et al., 2009; Demeter et al., 2010) but never in codon 426.

The observed heterogeneity in the CPV populations might be associated with differences in the vaccination plans. Some reports suggest

that 2c variants cause infection in dogs vaccinated with CPV-2. Although there is no definitive evidence of the impact of the CPV variant on immunization failure, some variants might require adjustments in vaccine schedules and formulations (Decaro et al., 2020).

4.2. Phylogenetic CPV groups in South America: Europe I

Both in the complete coding genomes and the vp2 phylogenetic analysis (Fig. 2 and Supplementary Fig. 4), there is a group composed of 2c strains from Argentina, Brazil, Chile, Ecuador, Paraguay, Peru, and Uruguay. This phylogroup, denoted as Europe I, has been reported in every South American country (Aldaz et al., 2013; Castillo et al., 2020; de Oliveira et al., 2019; Grecco et al., 2018; Luna Espinoza et al., 2022) except Colombia (Galvis et al., 2022). It is also widely distributed in Europe (Albania, Belgium, Croatia, France, Germany, Italy, Portugal, and Spain) (Decaro et al., 2009; Grecco et al., 2018; Miranda et al., 2016; Novosel et al., 2019), Oceania (Australia), and Asia (India and Iran) (Nandi et al., 2010; Nookala et al., 2016; Woolford et al., 2017). Europe I has strains exclusively of the 2c variant and has only 426Glu and synonymous SNPs as genetic signatures.

Europe I emerged in Southern Europe during 1990–98 and later spread to South America in the early 2000s (Grecco et al., 2018). The first 2c strains of this group were reported in Italy in 2001 (Buonavoglia et al., 2001) and soon spread in several European dog populations within a few years, replacing previous variants (Decaro et al., 2009; Decaro et al., 2007; Decaro and Buonavoglia, 2012; Grecco et al., 2018; Martella et al., 2005). The history of Europe I in South America started with the first report on CPV-2c in Uruguay in 2006 (Pérez et al., 2007). However, it has circulated in Argentina since 2003 (Calderon et al., 2009). According to the findings, the 2c strains of Europe I have been absent from the Uruguayan CPV population since 2014 (Fig. 1). Conversely, these 2c strains circulate frequently in Argentina. Moreover, the 2c strain found in Argentina with an alternative codon for glutamate amino acid (GAG) clustered in the Europe I phylogenetic group (Fig. 2).

The Argentine 2b strains here sequenced clustered together with other 2b strains from the United States and were basal of the Europe I clade. They were unrelated to the previous 2a/2b from Argentina from the South America I group. This could be because of previously undetected introduction from the USA. It is also possible that this unique 2b strain was generated by consecutive recombination events that were not identifiable with conventional programs.

Regarding other South American countries, Europe I has been detected in various regions of Brazil since 2008 until recent reports from 2020 (Castro et al., 2010; de Oliveira et al., 2018; Fontana et al., 2013; Gogone et al., 2020; Grecco et al., 2018; Jaune et al., 2019; Monteiro et al., 2016; Pintos et al., 2011; Streck et al., 2009; Vieira et al., 2017). In Chile, Europe I was recently reported circulating with high occurrence (Castillo et al., 2020; Ortega et al., 2021), while in Ecuador and Peru, there is the circulation of Europe I cocirculating with other phylogenetic groups (Aldaz et al., 2013; Luna Espinoza et al., 2022). Mexico and the United States have only some strains belonging to Europe I (Faz et al., 2019; Voorhees et al., 2019). In the United States, strains from this lineage ($n = 4$ in the complete genome dataset, $n = 21$ in the vp2 dataset) were reported from 2009 until 2019 (Allison et al., 2014; Allison et al., 2013; Voorhees et al., 2019), while in Mexico, two strains were detected in 2015 and 2017 (Faz et al., 2019; Pedroza-Roldán et al., 2022; Pedroza-Roldán et al., 2015).

In Asia, Europe I has been recently reported circulating in Iran (Dastmalchi Saei et al., 2017; Ghajari et al., 2021), while in Australia, it was circulating but is no longer detected (Clark et al., 2018; Kwan et al., 2021; Woolford et al., 2017). The Iranian 2c strains of Europe I are unrelated to the widely distributed phylogenetic group already reported in several Asian countries. This Asian 2c phylogroup was first detected in China (Geng et al., 2015; Wang et al., 2016) and is expanding to Europe and Africa (Mira et al., 2019; Mira et al., 2018; Ndiana et al., 2021; Ukwueze et al., 2020) but has not yet been reported in South America.

Of utmost concern is that there have been reports of the circulation of 2c strains from Europe I in wildlife animals in Argentina, Brazil, and Chile. Argentina reported the detection of these 2c strains in three coatis (*Nasua nasua*) who had anorexia and hemorrhagic diarrhea symptoms and died (Bucafusco et al., 2019), while in Chile, the same variant was reported in a wild cat (guinea: *Leopardus guigna*), which presented intermittent hemorrhagic diarrhea, depression, anorexia and died a few weeks later (Ortega et al., 2021). In Brazil, a pampas fox (*Lycalopex gymnocercus*) and a crab-eating fox (*Cerdocyon thous*) were apparently 2c strains from Europe I (Weber et al., 2020). Still, we could not confirm it because there were only partial vp2 sequences. These results confirm the ongoing transmission between domestic and wildlife animals, supporting the CPV ability for interspecies jumps (Hoelzer and Parrish, 2010).

4.3. Phylogenetic CPV groups in South America: Asia I

The first report of a 2a strain of Asia I outside Asia was in Uruguay in 2010 due to an intercontinental migration. The detection occurred in the unrelated 2c homogenous population (Pérez et al., 2012). The frequency of this 2a strain reached high frequency only one year after its detection (Maya et al., 2013). Here, we showed that the 2a Asian strain had a frequency of 71% in 2012 and 97% in 2013, reaching 100% by 2014. Since then, it has been the only variant detected in Uruguay in the last decade (Fig. 1). Notably, this drastic replacement has yet to be described in neighboring Brazil, Argentina, or any country outside the Asian continent. This behavior could be related to the small Uruguayan territory, which facilitates genetic drift and virus expansion, as may have occurred with 2c strains from Europe I (Maya et al., 2013). In addition, another recombinant strain identical to a previously reported one (Pérez et al., 2014) was detected during the co-circulation period of 2a and 2c in 2013. The recombinant strain had the ns gene from Europe I 2c strains and the vp gene of Asia I 2a strains.

Asia I had not been described in any South American country other than Uruguay. Phylogenetic analysis of vp2 sequences indicated that Asia I strains are circulating in Brazil (Supplementary Fig. 4). These sequences share the molecular signature designated to the Asia I clade for the VP2 protein (Grecco et al., 2018). The presence of the 2a strains of Asia I clade in Uruguay and Brazil, neighboring countries with dry borders, suggests that they spread from Uruguay due to an intense exchange of people and animals (de Oliveira et al., 2019; de Oliveira Santana et al., 2022; Grecco et al., 2018). However, the replacement behavior of Asia I might differ between both countries. Brazilian 2a strains from Asia I were collected five years after the first detection in Uruguay (2010). Although it seems to increase its frequency, it is not prevalent (de Oliveira et al., 2019), maybe because it has yet to be detected or because its dynamic differs in a much larger dog population.

A recombinant strain between 2c (Europe I) and 2a (Asia I) was found in Argentina, suggesting that the parental Asia I strain may be circulating there. The restricted geographic region analyzed here (Buenos Aires Province) could explain the undetected CPV-2a in Argentine territory. Moreover, in Colombia, analysis with partial vp2 sequences showed the detection of the 2b variant with the Asia I signature (Giraldo-Ramirez et al., 2020).

Our findings showed that Asia I had expanded worldwide, being detected in countries from every continent: Italy (Europe), Nigeria (Africa), Canada (North America), Uruguay (South America), and Australia (Oceania). In addition, it is composed of all three variants, which proves this group keeps evolving, maintaining some key amino acid residues and changing the 426 position.

4.4. Phylogenetic CPV groups in South America: South America I, Europe II, and a distinct Brazilian group

Additional phylogenetic groups have been identified in South America, including South America I and Europe II (as reported by Grecco et al., 2018), and a group that is currently only found circulating

in Brazil (de Oliveira Santana et al., 2022; Pereira et al., 2007).

South America I spreads across Argentina, Brazil, Chile, and Uruguay (Castillo et al., 2020; Grecco et al., 2018). The vp2 phylogenetic analysis showed 2b strains from Chile clustered together with strains from South America I (Supplementary Fig. 4). This group circulated exclusively in South America (Argentina, Brazil, and Uruguay) and had Argentina as the likeliest ancestral location (Grecco et al., 2018). From there, it migrated to Brazil, Chile, and Uruguay (Alexis et al., 2021; Grecco et al., 2018).

The original Europe II group comprised 2a/2b/2c strains from Brazil, Ecuador, and Italy. The composition of this phylogenetic group changed after including new strains from Ecuador and Peru in our analysis. As a result, the strains from Brazil and Italy were placed elsewhere on the phylogenetic tree. With new CPV strains from Peru in the database, Ecuadorian and Peruvian strains appear closely together. Lastly, the Brazilian exclusive clade comprises only 2a and 2b strains from Brazil and seems to have circulated from the early 1990s until 2015 (de Oliveira Santana et al., 2022; Pereira et al., 2007).

5. Conclusion

Overall, the South American CPV population showed different patterns of evolution and distribution. Europe I is the most widely distributed phylogenetic group in Argentina and the rest of the continent except for Uruguay and Colombia. Asia I might increase its distribution in South America; in Uruguay, it replaced the previous circulating strains and may spread in Southern Brazil. Our findings evidence that highly genetically diverse CPV phylogroups are circulating simultaneously in neighboring countries (Uruguay and Argentina). We also identified strains from other phylogroups that require further phylogenetic analysis on new strains to clarify their origin and evolution. The 426 amino acid residue in VP2 used to classify CPV does not always correspond to the identified phylogroups; they likely arose multiple times after the emergence of the CPV-2a lineage. CPV classification still represents an entanglement for the analysis of CPV variants and for understanding its origin and evolution.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2024.105633>.

Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

CRediT authorship contribution statement

Sofia Grecco: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emma Condon:** Writing – review & editing, Methodology, Investigation. **Daniilo Bucafusco:** Writing – review & editing, Resources. **Ana Cristina Bratanich:** Resources. **Yanina Panzera:** Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition, Conceptualization. **Ruben Pérez:** Writing – original draft, Visualization, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

None.

Data availability

Sequence data from this article have been deposited with the GenBank Data Libraries under Accession No. OK888554-OK888589.

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