Genetic Variability and Evolutionary Patterns of Canine Parvovirus in South America

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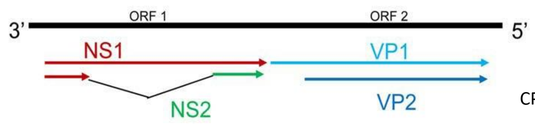
INTRODUCTION

- Canine parvovirus (CPV) is a well-recognized cause of acute and often fatal hemorrhagic gastroenteritis in puppies. The single-stranded DNA genome encodes capsid (VP1/VP2) and non-structural proteins (NS1/NS2) (1).



CPV genome has 5,2 kb

- CPV emerged in the late 1970s through an interspecies jump from other carnivores to dogs (3). The newly emerged CPV (CPV-2) rapidly spread worldwide, causing a pandemic in the new canine host (4). In 1979, a new genetic lineage (CPV-2a) replaced the original CPV-2 strains in only two years and became the ancestor of all the variants currently circulating worldwide: 2a, 2b, 2c (5). The CPV-2a lineage underwent further evolution showing a high substitution rate that resembled fast-evolving RNA viruses (2).

The present study maps the more recent viral genomic variability and evolutionary patterns in South America.

MATERIALS & METHODS

267 samples collected (2008- 2019) from suspected CPV-infected puppies from Argentina and Uruguay were analyzed using PCR-RFLP, Sanger, and NGS-Illumina sequencing (5). A full-length coding genomes (4269 nt) dataset was created (duplicated and vaccinal sequences were removed). Nucleotide and amino acid sequence alignments were performed using MAFFT (6). Recombination, NGS, and phylogenetic analysis were performed using RDP4 (7), SplitsTree4 (8), and Geneious software (9).

RESULTS & DISCUSSION

- PCR-RFLP and partial sequencing identified CPV-2c and CPV-2a strains in Uruguay (Fig 1A), while in Argentina CPV-2a/b/c were detected (Fig. 1B). One sample from Argentina had a CPV-2a/2b RFLP-pattern but has a GAG at 426 codon that codes for the CPV-2c characteristic amino acid (glutamate) (Fig. 2).
- The recombination analysis revealed sporadic events: one Uruguayan and one Argentine recombinant strain. In both cases, the recombination was between 2c (ns) and 2a (vp) strains (Fig. 3).
- NGS analysis identified two coinfections in Uruguay between field strains (2a and 2c) that differed by 18 nucleotides (Table 1).

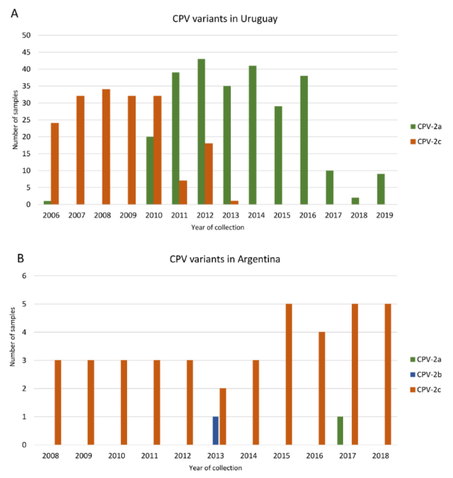


Figure 1 A. Evolution of CPV variants in Uruguay from 2012 – 2019. B. Evolution of CPV variants in Argentina from 2008 – 2018.

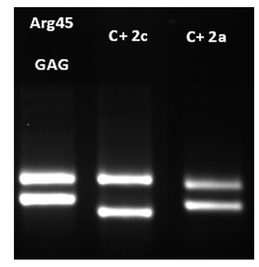


Figure 2. RFLP pattern of CPV Argentine sample with alternative CPV-2c codon (GAG) controls CPV-2a and CPV-2c are included.

Table 1. Variant frequency (CPV-2a minority strain) of the coinfected samples in Uruguay.

Name	Position	Frequency	Change	Amino Acid	Quality	CDS
G	81	7.5%	A -> G		35	NS1
T	342	6.5%	C -> T		37	NS1
G	516	7.8%	A -> G		36	NS1
G	1062	7.5%	A -> G		35	NS1
G	1098	7.7%	A -> G		35	NS1
T	1173	8.2%	C -> T		34	NS1
G	1875	7.5%	A -> G		36	NS1
C	1975	8.0%	T -> C		36	NS1
AG	2085	11.7%	GA -> AG		33	
A	2432	8.4%	G -> A	R -> K	34	VP1
A	2550	8.3%	G -> A		37	VP2
A	2574	8.2%	T -> A		35	VP2
T	2817	8.6%	C -> T		36	VP2
T	3314	5.8%	A -> T	Y -> F	35	VP2
TA	3484	3.4% -> 5.3%	AT -> TA	I -> Y	34	VP2
GAA	3790	6.7% -> 6.8%	AAT -> GAA	N -> E	35	VP2

Name	Position	Frequency	Change	Amino Acid	Quality	CDS
G	81	3,4%	A -> G		35	NS1
T	342	4,1%	C -> T		36	NS1
G	516	4,1%	A -> G		37	NS1
G	1062	4,3%	A -> G		35	NS1
G	1098	4,4%	A -> G		36	NS1
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A	2574	3,2%	T -> A		36	VP2
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T	3314	3,0%	A -> T	Y -> F	32	VP2
A	3485	3,0%	T -> A	I -> N	36	VP2
GAA	3790	3,9%	AAT -> GAA	N -> E	36	VP2

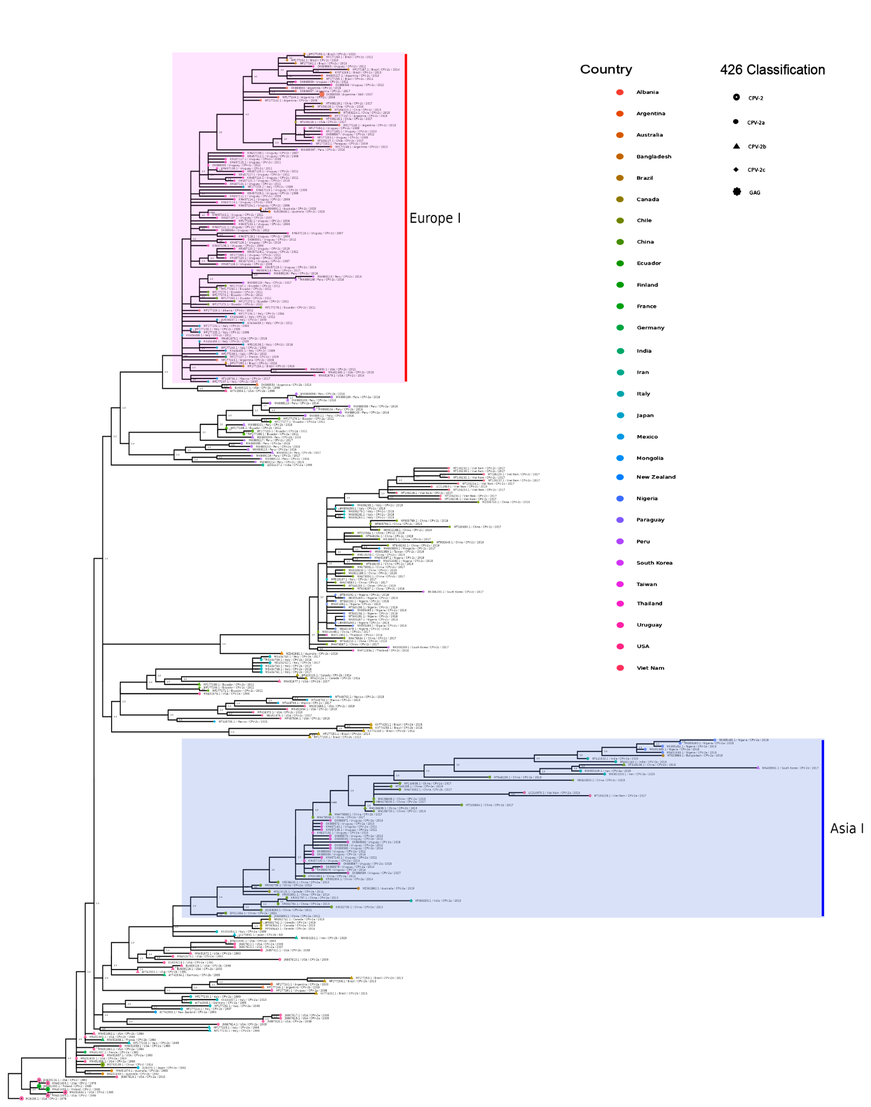

Figure 4. CPV maximum likelihood phylogenetic reconstruction (GTR +G+I) with node support assessed using SH-like. The analysis comprised 339 complete coding genomes (4269 nt). Node colored by country. Node figured by CPV variant.

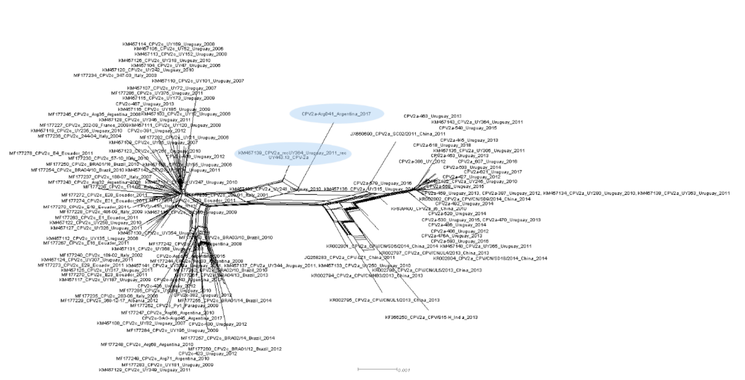

Figure 3. CPV recombination network where recombinant samples from Argentina and Uruguay are shown.

- Phylogenetic studies identified multiple CPV clades in South America. Clades of European (Europe I) and Asian (Asia I) origin (10) were widely distributed in domestic and wildlife populations, with additional clades showing regional distribution (Fig. 4). The obtained Uruguayan and Argentine CPV-2c were grouped in the Europe I clade. Europe I also included previously reported CPV-2c strains from Uruguay, Argentina, and other related CPV-2c from America (United States, Mexico, Brazil, Chile, Ecuador, Paraguay, Peru), Europe (Italy, France, Albania), Asia (Iran) and Oceania (Australia). The CPV-2b from Argentina sequenced here, and other CPV-2b from the United States were basal of Europe I clade. The Uruguayan CPV-2a strains clustered in well-supported Asia I clade of CPV-2a strains from Asia (China, Viet Nam, Iran, India, South Korea, Bangladesh, Singapore), Africa (Nigeria), North America (Canada), and Oceania (Australia) (Fig. 4).
- Since 2014, Uruguay has shown a homogenous CPV population as the Asia I clade has wholly replaced the Europe I clade. Argentina has a predominant Europe I clade (2c), except for three strains: a recombinant, a non-synonymous mutation at 426 codon, and a 2b strain. Considering the vast border and frequent movement of people and animals between these adjacent countries, the divergent pattern of CPV evolution is surprising.

CONCLUSIONS

- 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, as Asia I replaced the previous circulating strains.
- Recombination and coinfection events may be relatively frequent between field strains, but detection relies on the level of divergence among the co-infected strains.
- Our findings evidence high genetic diversity between CPV strains circulating simultaneously in neighboring countries (Uruguay and Argentina). A diverse dynamic of CPV populations in South America is becoming more complicated due to the rise in pathogens exchange between domestic dogs and cats with wild animal populations (11,12).

ACKNOWLEDGMENTS

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