




Domestic dog origin of *Carnivore Protoparvovirus 1* infection in a rescued free-ranging guiña (*Leopardus guigna*) in Chile

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Abstract

Carnivore protoparvovirus 1 is one of the most important pathogens affecting both wild and domestic carnivores. Here, we reported the genetic characterization of canine parvovirus (CPV-2) strains from a rescued guiña (*Leopardus guigna*) and domestic dogs from Chile. Guiña strain was classified as CPV-2c, and phylogenetic analysis of the complete coding genome showed that the guiña CPV-2c strain shares a recent common ancestor with Chilean domestic dogs' strains. These viruses showed >99% identity and exhibited three changes in the NS1 protein (V596A, E661K and L582F). This is the first detection and genetic characterization of CPV-2c infection in guiña worldwide, and one of the few comparative studies that show the source of infection was domestic dogs. The current findings highlight the fact that guiña is a susceptible species to protoparvovirus infection and that domestic dogs represent an important threat to its conservation. The CPV-2 cross-species transmission between domestic dogs and guiña should be taken into account for protection programmes of this endangered species.

KEYWORDS

Canine parvovirus, *Carnivore protoparvovirus 1*, genetic characterization, *Leopardus guigna*, phylogeny

1 | INTRODUCTION

Carnivore protoparvovirus 1 is a member of the genus *Protoparvovirus* of the family *Parvoviridae*. Like other parvoviruses, these are

non-enveloped, small icosahedral viruses having a linear, single-stranded DNA genome of approximately 5.2 kb containing two major open reading frames (ORFs; Cotmore et al., 2014). The first ORF encodes two non-structural proteins (NS1 and NS2), while the

second ORF encodes the structural proteins VP1 and VP2 (Reed, Jones, & Miller, 1988). *Carnivore protoparvovirus 1* comprises several closely related viruses previously considered different species, including feline parvovirus (FPV), canine parvovirus (CPV-2), mink enteritis virus and raccoon parvovirus (Cotmore et al., 2014). These viruses are significant pathogens of veterinary relevance and affect both wild and domestic animals in the order Carnivora (Behdenna et al., 2019; Chen, Chang, Wada, Chen, & Tu, 2019; Cotmore et al., 2014, 2019). A fatal CPV-2 infection was recently described in a rescued Taiwanese pangolin, providing the first evidence of CPV-2 infection in a non-carnivorous species (Wang et al., 2020).

FPV and CPV-2 share a common recent ancestor that likely infected felids or a related wild carnivorous species (Truyen & Parrish, 1992). When CPV-2 emerged, it only infected canids. This first CPV-2 type was soon replaced by a new lineage CPV-2a that regained the ability to infect felines and spread worldwide in few years. The high mutation capacity of CPV-2 promoted the emergence of new genetic variants, including the antigenic/genetic variants known as 2a, 2b and 2c (Buonavoglia et al., 2001; Shackelton, Parrish, Truyen, & Holmes, 2005). These variants regained the ability to infect felines and started to pose a threat for non-dog species (Ikeda et al., 2000; Truyen, Evermann, Vieler, & Parrish, 1996).

In South America, all CPV-2 genetic and antigenic variants were described. The analysis of complete genome from several countries evidenced two migration events from Europe, an introduction from Asia and a lineage that likely diverged in South America (Grecco et al., 2018). Chile, Uruguay and Argentina have a similar scenario with the predominance of 2c strains of European origin (Castillo et al., 2020) and the existence of 2a strains belonging to two different lineages (Gallo-Calderón et al., 2015; Pérez et al., 2014).

In wild species from South America, there is evidence of circulation of *Carnivore protoparvovirus 1* in wild animals by serologic tests, including culpeo, grey and crab-eating foxes (*Lycalopex culpaeus*; *Lycalopex griseus*; *Cerdocyon thous*; Acosta-Jamett, Cunningham, Bronsvort, & Cleaveland, 2014; Martino et al., 2004), maned wolves (*Chrysocyon brachyurus*; de Almeida Curi et al., 2012) and Geoffrey's cats (*Leopardus geoffroyi*; Uhart, Rago, Marull, del Ferreyra, & Pereira, 2012). Recently, a CPV-2c strain has been isolated and genetically characterized from a dead coati (*Nasua nasua*) in Argentina (Bucafusco et al., 2019).

Chile has a little information on the circulation of CPV-2 in both domestic dogs and wild animals. Two serological studies evidenced the presence of antibodies against CPV-2 in domestic dogs and wild canids (Acosta-Jamett et al., 2014, 2015). A more recent study characterized the strains using VP2 sequence analysis and evidenced the existence of a predominant 2c variant of European origin (Castillo et al., 2020).

The guiña (*Leopardus guigna*) is a small felid inhabiting central and southern Chile and some areas in south-western Argentina (Napolitano et al., 2014). According to the red list of the International Union for Conservation of Nature (IUCN), its conservation status is vulnerable, and the main causes are the reduction and fragmentation of its habitat (Napolitano, Gálvez, Bennett, Acosta-Jamett, & Sanderson, 2015). In recent years, feline viral immunodeficiency (FIV) and feline viral leukaemia (FeLV) infections have been reported in guiña populations and in both cases, phylogenetic analyses suggested a high association between wild and domestic animals viruses (Mora, Napolitano, Ortega, Poulin, & Pizarro-Lucero, 2015).

The main objective of this study was to genetically characterize a CPV-2s strain collected from a rescued guiña and to establish the phylogenetic relationship with CPV-2 strains obtained from domestic dogs inhabiting the near geographic area in Chile.

2 | MATERIALS AND METHODS

In July of 2015, a six-month-old sick guiña was recovered in an urban area from Curicó (Chile) by the Agricultural and Livestock Service (SAG) of Chile. The animal was submitted to the Center for Rehabilitation of Wild Fauna of the Universidad de Concepción (Chillán, Chile). The individual presented an intermittent haemorrhagic diarrhoea, depression and anorexia during the clinical exam. A blood sample was collected to identify a possible infectious cause. Diagnostic tests against FeLV, FIV by nested PCR following the protocol suggested by Mora et al. (2015) and against CPV-2 by PCR were performed. A few weeks later, the animal died, and necropsy was carried out. Stool, stomach, small intestine and spleen tissues were collected.

DNA was extracted from blood sample using Thermo Fisher Scientific DNA® Extraction from Blood (processed at the Virology Laboratory, Faculty of Veterinary Sciences, University of

TABLE 1 Sample identification used in this study. Accession number, strain name, date collection, city and virus type were included

Accession number	Strain name	Date collection	City	Virus type
MT458223	CHL-guigna	2015	Curico	CPV-2c
MT458224	CHL-17	2015	Chillán	CPV-2c
MT458225	CHL-32	2016	Chillán	CPV-2c
MT458226	CHL-64	2017	Santiago	CPV-2c
MT458227	CHL-69	2017	Los Andes	CPV-2c
MT458228	CHL-70	2017	Santiago	CPV-2c
MT458229	CHL-71	2017	Santiago	CPV-2c
MT458230	CHL-73	2017	Santiago	CPV-2c

Concepción, Chillán, Chile). DNA was extracted from tissue samples using DNeasy Blood and Tissue Kit and the QIAampDNA Stool Mini Kit (Qiagen; processed at the Molecular Biology Laboratory, Faculty of Sciences, University of Chile, Santiago, Chile). The extracted genomic DNAs were stored at -20°C .

For CPV-2 diagnosis, a small viral fragment of VP2 gene (583 bp) was amplified following the protocol suggested by Buonavoglia et al., (2001). Furthermore, seven samples from Chilean domestic dogs were selected for complete coding genome sequencing. These samples were previously characterized by Castillo et al., (2020) using sequencing of the VP2 gene. All samples belongs to Central Chile, which is one of the main habitats to the guiña. The complete coding genome (4,269 pb) from dogs and guiña were amplified following the protocol suggested by Pérez et al., (2014).

A dataset consisting of the sequences generated in this study and the CPV-2 complete coding genome sequences retrieved from the GenBank database was generated. The filter criterion was to select the sequences according to its subtype, country and year

of collection and host species other than dog. The sequences were aligned using MUSCLE v3.8.3 (Edgar, 2004) incorporated in AliView v1.26 (Larsson, 2014). The best-fit evolutionary model of nucleotide substitution was selected according to the Akaike information criterion (AIC) in jModelTest v2.1.6 software (Posada, 2008). Phylogenetic tree was inferred using the maximum likelihood method with RaxML-HPC2 v8.2.12 (Stamatakis, 2014) available in CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010). The statistical support of the nodes was evaluated using 1,000 bootstrap replicates.

3 | RESULTS AND DISCUSSION

The blood sample of the guiña tested positive to CPV-2 by PCR assay and negative to FeLV and FIV by nested PCR at the arrival to the Center for Rehabilitation of Wild Fauna. *Post-mortem* stool, stomach and spleen samples were also tested positive to CPV-2, confirming

TABLE 2 Amino acid key positions in VP2 in samples analysed in this study

	80	139	267	297	300	305	323	324	426	514
CHL-guigna (MT458223)	R	V	F	A	G	Y	N	Y	E	A
CPV CHL-(MT458224-30)	R	V	F	A	G	Y	N	Y	E	A
CPV-2a (EU659118) ^a	R	V	F	S	G	Y	N	Y	N	A
CPV-2b (EU659121) ^a	R	V	F	A	G	Y	N	Y	D	A
CPV-2c (MF177239) ^a	R	V	F	A	G	Y	N	Y	E	A
CPV-2 (M38245) ^a	R	V	F	S	A	D	N	Y	N	A
FPV (EU659115) ^a	K	V	F	S	A	D	D	Y	N	A

^aReference strain.

	351	358	582	583	596	597	626	661	668
CHL-guigna (MT458223)	K	N	F	E	V	L	Q	E	D
CHL-17 (MT458224)	K	N	L	E	A	L	Q	E	D
CHL-32 (MT458225)	K	N	L	E	V	L	Q	E	D
CHL-64 (MT458226)	K	N	L	E	V	L	Q	E	D
CHL-69 (MT458227)	K	N	L	E	V	L	Q	E	D
CHL-70 (MT458228)	K	N	L	E	V	L	Q	E	D
CHL-71 (MT458229)	K	N	L	E	V	L	Q	K	D
CHL-73 (MT458230)	K	N	L	E	V	L	Q	E	D
2c Uy (KM457119/ KM457122) ^a	K/N	D/N	L	E	V	L/P	Q	E	D
2c Br (MF177251/ MF177256) ^a	K	D/N	L	E	V	L	Q/R	E	D
2c Ec(MF177264/ MF177265) ^a	N	D/N	L	E	V	L	Q	E	D
FPV (EU659115) ^a	N	D	L	E	V	L	Q	E	D
CPV-2 (M38245) ^a	N	D	L	E	V	L	Q	E	D

Red color show variations between Chilean and reference strains.

^aReference strain.

TABLE 3 Amino acid key positions in NS1 in samples analysed in this study [Colour table can be viewed at wileyonlinelibrary.com]

the diagnosis and suggesting that the virus infected and established viremia in the host efficiently.

The main findings in necropsy were multifocal gastric ulcers, petechial haemorrhages scattered throughout the intestinal mucosal surface, and petechial and ecchymotic haemorrhages on the serosal surfaces of small intestine. In addition, abnormalities were not detected in the heart. These characteristics of the infected tissues

collected in this case were similar to those found in protoparvovirus infections in other species (Allison et al., 2013, 2014; Bucafusco et al., 2019; Oosthuizen et al., 2019; Viscardi et al., 2019; Wang et al., 2020).

We obtained eight complete sequences of the CPV-2 coding regions: one from the guinea and seven from domestic dogs. Details of these sequences are shown in Table 1. The guinea and Chilean

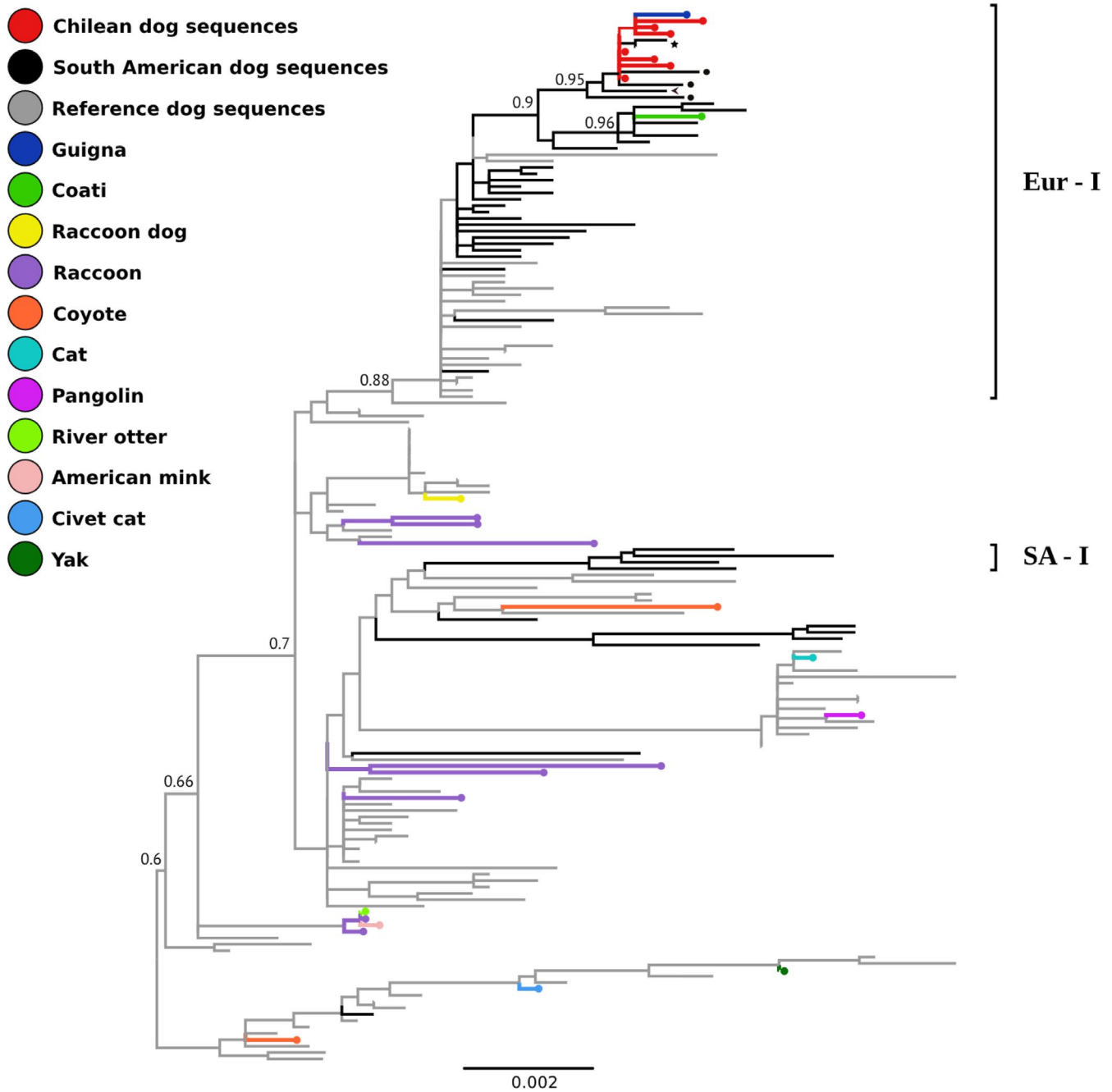


FIGURE 1 Phylogenetic analysis of the CPV-2 complete coding region. The database was constructed using the sequences obtained in this study and CPV sequences available in GenBank according to its subtype, country, year of collection and host species other than dog. The phylogenetic tree was inferred using the maximum likelihood method in RaxML based on GTR + I+G4 nucleotide substitution model with 1,000 bootstrap replicates. Bootstrap values are indicated in the main clades. Chilean domestic dog, guinea, reference domestic dog and other species CPV-2 strains are represented in different colours. Two clades were labelled according to the classification suggested by Grecco et al. (2018), and the symbols represent the sequences of the South American countries most closely related to our sequences, such as Argentina '●', Uruguay '★' and Paraguay '◀' [Colour figure can be viewed at wileyonlinelibrary.com]

dog sequences showed high genetic identity (99.8%–99.9%). The sequence analysis of the VP2 region of the guña sequence showed 426E (Table 2) amino acid change associated with 2c strains (Buonavoglia et al., 2001; Decaro & Buonavoglia, 2012). Three single-nucleotide polymorphisms lead to changes in the NS1 protein of three samples: CHL-17 V596A, CHL-71 E661K and CHL-guña L582F (Table 3). Changes in the 582NS1 position has been associated with neurological tissue tropism in FPV strains, but the residue associated was different (L582S; Garigliany et al., 2016). However, the results described by Mira et al., (2019) did not evidenced association between amino acid change and tropism by neurological tissue.

In South America, Chile and Argentina have a predominance of 2c variant (Castillo et al., 2020), but in Uruguay this variant is being replaced by a phylogenetically unrelated CPV-2a variant (Gallo-Calderón et al., 2015; Pérez et al., 2014). Phylogenetic analysis showed that the guña parvovirus clustered with CPV-2c dog strains was collected between 2009–2017 from Chile, Uruguay, Argentina and Paraguay (Figure 1). Strains from guña and coati diverged different subclades, but both clustered in the Europe-I clade according to the recent classification by Grecco et al. (2018). Our results suggest that domestic dogs are likely the viral source of guña CPV-2, but lack data from guñas population and evolutionary studies before the guña are needed to elucidate this finding.

FPV or FPV-like infections in wild felids have been reported in *Puma concolor* (Allison et al., 2014), *Panthera tigris* (Duarte et al., 2009; Wang et al., 2019) *Panthera leo*, *Acinonyx jubatus*, *Felis lybica* (Calatayud et al., 2019; Steinel, Munson, van Vuuren, & Truyen, 2000), *Genetta genetta* (Calatayud et al., 2019) and *Prionodon linsang* (Inthong et al., 2019). Regarding CPV-2 infections in wild felids, it have been reported in *Felis bengalensis* (Nakamura et al., 2001), *Acinonyx jubatus*, *Panthera tigris* (Steinel et al., 2000), *Puma concolor*, *Lynx rufus* (Allison et al., 2013, 2014) and *Leptailurus serval* (Oosthuizen et al., 2019). All of them showed high identity with circulating strains from domestic dogs. Due to the fact that 2c lineage is the most prevalent in domestic species in both Chile and Argentina, it would probably be mostly detected in sympatric wild species, which is a similar context as the one described for the coati strain (Bucafusco et al., 2019). There are not FPV studies in domestic or wild felids in Chile, and there is limited information regarding CPV. Therefore, it is really important to know the situation in domestic species.

Only a few studies compare sequences between sympatric domestic dogs and wild species (Chen et al., 2019; Oosthuizen et al., 2019; López-Pérez et al., 2019). Most reports compare them only with sequences available in GenBank (Allison et al., 2013, 2014; Nakamura et al., 2001; Steinel et al., 2000). For the conservation of this species, it will be important to consider the transmission of infectious agents from domestic to wild animals. Likewise, infection by FeLV and FIV has been previously described in guñas (Mora et al., 2015). So, our finding might suggest that these animals would be at permanent risk of infection by these pathogens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. It was approved by the Bioethical Committee from the Faculty of Veterinary Sciences, from the University of Concepción. Informed consent for the dogs' samples from the owners, was not necessary because the animals were not identified individually. The guña sample was taken during a clinical procedure, and tissue samples were taken in the necropsy after the death.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank at Accession numbers MT458223, MT458224, MT458225, MT458226, MT458227, MT458228, MT458229 and MT458230.

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