

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

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May 22, 2020

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 strains from a rescued guiña (*Leopardus guigna*) and domestic dogs from Chile. Guiña sequence was classified as CPV-2c and phylogenetic analysis of the complete coding genome showed that the guiña CPV-2c strain share a recent common ancestor with Chilean domestic dogs strains. These viruses presented >99% identity and showed three changes in the NS1 protein, CHL-17 V596A, CHL-71 E661K and CHL-guigna L582F. This is the first detection and genetic characterization of CPV-2c infection in guiña worldwide and one of the few comparative studies that undoubtedly determine that the source of infection were domestic dogs. The current findings highlight that guiña is a susceptible species to protoparvovirus infection and that domestic dogs represent an important threat to its conservation. The CPV cross-species transmission between domestic dogs and guiña should be taken into account for protection programs of this endangered species.

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Running Head: CPV-2c infection in guiña

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Summary

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 strains from a rescued guinea (*Leopardus guigna*) and domestic dogs from Chile. Guinea sequence was classified as CPV-2c and phylogenetic analysis of the complete coding genome showed that the guinea CPV-2c strain share a recent common ancestor with Chilean domestic dogs strains. These viruses presented >99% identity and showed three changes in the NS1 protein, CHL-17 V596A, CHL-71 E661K and CHL-guinea L582F. This is the first detection and genetic characterization of CPV-2c infection in guinea worldwide and one of the few comparative studies that undoubtedly determine that the source of infection were domestic dogs. The current findings highlight that guinea is a susceptible species to protoparvovirus infection and that domestic dogs represent an important thread to its conservation. The CPV cross-species transmission between domestic dogs and guinea should be taken into account for protection programs of this endangered species.

Keywords: Canine parvovirus, *Leopardus guigna*, protoparvovirus 1, phylogeny, genetic characterization

Main Text

Introduction

Carnivore protoparvovirus 1 (CPPV-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 *et al.*, 1988). CPPV-1 comprises several closely-related lineages previously considered different species, including feline parvovirus (FPV), canine parvovirus (CPV), 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 *Carnivore* (Behdenna *et al.*, 2019; Chen *et al.*, 2019; Cotmore *et al.*, 2014; Cotmore *et al.*, 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-carnivore species (Wang *et al.*, 2019).

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

In South-America, 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 (Pérez *et al.*, 2014; Gallo-Calderón *et al.*, 2015).

In wild species from South-America, there is antigenic evidence of the circulation of CPPV-1 in wild animals, including culpeo, grey and crab-eating foxes (*Lycalopex culpaeus* ; *Lycalopex griseus*; *Cerdocyon thous*) (Martino *et al.* , 2004; Acosta-Jammet *et al.* , 2014), maned wolves (*Chrysocyon brachyurus*) (de Almeida Curi *et al.* , 2012) and Geoffrey's cats (*Leopardus geoffroyi*) (Uhart *et al.* , 2012). Recently, a CPV-2c strain was 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 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-Jammet *et al.* , 2014; Acosta-Jammet *et al.* , 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 southwestern 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 (IUCN, 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 *et al.* , 2015).

The main objective of this study was to characterize the CPV in guiña and to establish the phylogenetic relationship with CPV strains obtained from domestic dogs inhabiting the near geographic area.

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 hemorrhagic diarrhea, depression and anorexia during the clinical exam. A blood sample was collected to identify a possible infectious cause. Diagnostic test against FeLV, FIV by Nested PCR (Mora *et al.*, 2015) and against CPV by PCR were performed, resulting positive for CPV and negative to FeLV and FIV. 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 ThermoFisher Scientific DNA[®] Extraction from Blood (processed at the Virology Laboratory, Faculty of Veterinary Sciences, University of Concepción (Chillán, Chile)). DNA was extracted from tissue samples using DNeasy Blood and Tissue Kit and the QIAamp DNA 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 diagnosis, a small viral fragment of VP2 (583 bp) was amplified following 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. Therefore, the complete coding genome (4269 pb) from dogs and guiña were amplified following Pérez *et al.* , (2014).

The dataset consisted of sequences generated in this study and CPV complete coding genome sequences retrieved from the GenBank database. 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 *et al.* , 2010). The statistical support of the nodes was evaluated using 1000 bootstrap replicates.

Results and discussion

The blood sample of the guña was CPV positive by PCR at the arrival to the Center for Rehabilitation of Wild Fauna. *Post-mortem* stool, stomach and spleen samples were also positive, confirming the CPV diagnosis and suggesting that the virus infected and established viremia in the host efficiently. The haemorrhagic diarrhoea and characteristic of the infected tissues collected in this case were similar to those found in protoparvovirus infections in other species (Allison *et al.* , 2013; Allison *et al.* , 2014; Oosthuizen *et al.* , 2019; Bucafusco *et al.* , 2019; Wang *et al.* , 2019; Viscardi *et al.* , 2019).

We obtained eight complete sequences of the CPV coding regions, one from the guña and seven from domestic dogs. Details of these sequences are shown in Table 1. The guña and Chilean dog sequences showed high genetic identity (99.8-99.9%). The sequence analysis of the VP2 region of the guña sequence showed amino-acid changes associated with other 2c strains (Buonavoglia *et al.* , 2001; Decaro *et al.* , 2012), including 300G and 426E (Table 2). Three single nucleotide polymorphism lead to changes in the NS1 protein of three samples, CHL-17 V596A, CHL-71 E661K and CHL-guigna L582F (Table 3). Changes in the 582 NS1 position has been associated with neurological tissue tropism in FPV strains but the residue associated was different (L582S) (Garigliany *et al.* , 2016).

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 (Pérez *et al.* , 2014; Gallo-Calderón *et al.* , 2015). Phylogenetic analysis showed that the guña parvovirus clustered with CPV-2c dog strains collected between 2009-2017 from Chile, Uruguay, Argentina, and Paraguay (Figure 1). Strains from guña and coati diverged in different sub-clades, but both clustered in the Europe-I clade according to the recent classification by Grecco *et al.* . (2018). Our results, suggests that the guña virus is a recent acquisition of domestic dogs. Furthermore, apparently the 2c lineage may have a greater affinity for wildlife in South-America (Bucafusco *et al.* , 2019).

Several CPV infections in wild felids 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; Allison *et al.* , 2014) and *Leptailurus serval* (Oosthuizen *et al.* , 2019). All of them showed high identity with domestic dog strains, and similar situation was described with the coati strain (Bucafusco *et al.* , 2019). Recently, tiger parvovirus (TPV) has been described in captive tigers in China, and this has been the only variant described from a wild felid, showing greater similarity to FPV rather than to CPV (Wang *et al.* , 2019).

There are not many studies that show sequences comparison and analysis between domestic dogs and wild species (Chen *et al.* , 2019; Oosthuizen *et al.* , 2019). Most reports only compare with sequences available in GenBank (Steinel *et al.* , 2000; Nakamura *et al.* , 2001; Allison *et al.* , 2013; Allison *et al.* , 2014). For the conservation of this species, it will be important to consider the transmission of infectious agents from domestic animals. Likewise, infection by FeLV and FIV has been previously described in guñas (Mora *et al.* , 2014). So, our finding might suggest that these animals would be at permanent risk of infection by these pathogens.

Acknowledgments

We are grateful to the staff of FISENLAB for all their support in the initial processing sampling, especially to Pedro Rojas. We thank the Molecular Diagnostic Laboratory of Department of Animal Pathology, especially to Álvaro Ruiz, for all their support in providing their facilities. We thank Dr. Francisco Espina Lopez for providing clinical cases.

Funding

This study was partly funded by project VRID Inicio, UdeC number 217.152.024-1.0 to RO, the Programa Fondecyt de Iniciación No. 11170877 to VN

Conflict of interest statement

The authors declare no conflict of interest

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.

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.

Was reviewed and approved by Comité de Bioética de la Facultad de Ciencias Veterinarias, Universidad de Concepción. Dog samples was written informed consent for participation was not obtained from the owners because the animals were not identified individually. The guinea sample was taken during a clinical procedure and tissue samples were taken in the necropsy after the death.

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Figure and Table Legends

Table 1. Sample identification used in this study. Identification of accession number, strain name, date collection, city, virus type were included.

Table 2. Amino acid variations in VP2 in samples analysed in this study.

Table 3. Amino acid variations in NS1 in samples analysed in this study.

Figure 1 . Phylogenetic analysis of the CPV complete coding region. The database was constructed using the sequences obtained in this study and all CPV sequences available in GenBank. The phylogenetic tree was inferred using the maximum likelihood method in RaxML based on GTR+I+G4 nucleotide substitution model with 1000 bootstrap replicates. Bootstrap values are indicated in the main clades. Chilean domestic dog, guinea, reference domestic dog, and other species CPV strains are represented in different colors.

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