Check for updates

Received: 18 June 2020 | Revised: 18 November 2020 | Accepted: 21 November 2020

DOI: 10.1111/tbed.13937

ORIGINAL ARTICLE

Epidemiology and molecular characterization of *Carnivore protoparvovirus-1* infection in the wild felid *Leopardus guigna* in Chile

Irene Sacristán[1] | Fernando Esperón[2] | Rubén Pérez[3] | Francisca Acuña[4] | Emilio Aguilar[4] | Sebastián García[4] | María José López[4] | Elena Neves[2] | Javier Cabello[5] | Ezequiel Hidalgo-Hermoso[6] | Karen A. Terio[7] | Javier Millán[8,9,10] | Elie Poulin[11,12] | Constanza Napolitano[12,13]

[1]PhD Program in Conservation Medicine, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

[2]Grupo de Epidemiología y Sanidad Ambiental, Centro de Investigación en Sanidad Animal (INIA-CISA), Madrid, Spain

[3]Sección Genética Evolutiva, Departamento de Biología Animal, Facultad de Ciencias, Instituto de Biología, Universidad de la República de Montevideo, Montevideo, Uruguay

[4]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile

[5]Facultad de Medicina Veterinaria, Universidad San Sebastián, Puerto Montt, Chile

[6]Departamento de Conservación e Investigación, Parque Zoológico Buin Zoo, Buin, Chile

[7]Zoological Pathology Program, University of Illinois, Brookfield, IL, USA

[8]Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

[9]Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA), Zaragoza, Spain

[10]Fundación ARAID, Zaragoza, Spain

[11]Laboratorio de Ecología Molecular, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

[12]Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Osorno, Chile

[13]Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile

Correspondence

Irene Sacristán, PhD Program in Conservation Medicine, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile.
Email: isacristan.vet@gmail.com

Constanza Napolitano, Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Osorno, Chile.
Email: constanza.napolitano@ulagos.cl

Funding information

Comisión Nacional de Investigación Científica y Tecnológica, Grant/Award Number: Fondecyt Iniciación 11150934 and PAI 77190064; National Geographic Society, Grant/Award Number: C309-15; Morris Animal Foundation, Grant/Award Number: D15ZO-413

Abstract

Landscape anthropization has been identified as one of the main drivers of pathogen emergence worldwide, facilitating pathogen spillover between domestic species and wildlife. The present study investigated *Carnivore protoparvovirus-1* infection using molecular methods in 98 free-ranging wild guignas (*Leopardus guigna*) and 262 co-occurring owned, free-roaming rural domestic cats. We also assessed landscape anthropization variables as potential drivers of infection. Protoparvovirus DNA was detected in guignas across their entire distribution range, with observed prevalence of 13.3% (real-time PCR) and 9% (conventional PCR) in guignas, and 6.1% (conventional PCR) in cats. Prevalence in guigna did not vary depending on age, sex, study area or landscape variables. Prevalence was higher in juvenile cats (16.7%) than in adults (4.4%). Molecular characterization of the virus by amplification and sequencing of almost the entire *vp2* gene (1,746 bp) from one guigna and five domestic cats was achieved, showing genetic similarities to canine parvovirus 2c (CPV-2c) (one guigna and one cat), feline panleukopenia virus (FPV) (one cat), CPV-2 (no subtype identified) (two cats), CPV-2a (one cat). The CVP-2c-like sequence found in a guigna clustered

 © 2020 Wiley-VCH GmbH

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

together with domestic cat and dog CPV-2c sequences from South America, suggesting possible spillover from a domestic to a wild species as the origin of infection in guigna. No clinical signs of disease were found in PCR-positive animals except for a CPV-2c-infected guigna, which had haemorrhagic diarrhoea and died a few days after arrival at a wildlife rescue centre. Our findings reveal widespread presence of *Carnivore protoparvovirus-1* across the guigna distribution in Chile and suggest that virus transmission potentially occurs from domestic to wild carnivores, causing severe disease and death in susceptible wild guignas.

KEYWORDS

canine parvovirus, domestic cats, feline panleukopenia virus, infectious diseases, landscape drivers, *Leopardus guigna*

1 | INTRODUCTION

Inhabiting human-dominated landscapes has been considered a risk factor for higher pathogen prevalence in wildlife (Carver et al., 2015; Foley et al., 2013; Millán et al., 2016; Riley et al., 2004). Anthropogenic factors including habitat loss and fragmentation, conversion of natural habitats and close human presence facilitate pathogen spillover at the wildlife–domestic interface (Foley et al., 2013) and potentially impact the survival of wildlife populations. Examples of how pathogens threaten wild carnivore populations of conservation concern include canine distemper virus in African wild dogs, *Lycaon pictus* (Alexander & Appel, 1994; Laurenson et al., 1998), lions, *Panthera leo* (Harder et al., 1995; Roelke-Parker et al., 1996) and black-footed ferrets, *Mustela nigripes* (Thorne & Williams, 1988); rabies in Ethiopian wolf, *Canis sinensis* (Sillero-Zubiri et al., 1996); and feline leukaemia virus (FeLV) in Iberian lynx, *Lynx pardinus* (Meli et al., 2009) and Florida panther, *Puma concolor coryi* (Chiu et al., 2019).

Rapid native forest habitat conversion has taken place in Chile over the past two to three decades, especially affecting those animal species that rely on vegetation cover (Echeverría et al., 2006; Echeverría et al., 2008; Heilmayr et al., 2016; Schulz et al., 2010; Wilson et al., 2005). Pathogen spillover at the wildlife–domestic interface may occur in these human-dominated landscapes. The forest-dwelling wild felid guigna (*Leopardus guigna*) is endemic to Chile and a small strip of southwestern Argentina and classified as Vulnerable by the IUCN (Napolitano et al., 2015). Guigna populations have experienced a rapid decline, mainly due to habitat loss and fragmentation (Napolitano, Gálvez, et al., 2015). A previous study (Mora et al., 2015) found that guignas inhabiting human-dominated landscapes are infected by feline leukaemia virus and feline immunodeficiency virus, possibly transmitted by domestic cats, supporting the hypothesis of infectious diseases as potential threats for this species. Further information on other pathogens infecting guignas in human-dominated landscapes is scarce and includes the report of haemoplasmas (Sacristán et al., 2019).

Another group of multi-host pathogens that infect mammals is the *Carnivore protoparvovirus-1* protoparvovirus hereafter), which belongs to the family *Parvoviridae*, subfamily *Parvovirinae*. According to the most recent taxonomy, subfamily *Parvovirinae* is composed of 10 different genera: *Amdoparvovirus*, *Aveparvovirus*, *Bocaparvovirus*, *Copiparvovirus*, *Dependoparvovirus*, *Erythroparvovirus*, *Protoparvovirus*, *Artiparvovirus*, *Loriparvovirus* and *Tetraparvovirus* (Cotmore et al., 2019; Pénzes et al., 2020). The carnivore protoparvovirus-1 of the genus *Protoparvovirus* infects a broad range of domestic and wild species and is present in almost all wild and domestic carnivore populations tested (Acosta-Jamett et al., 2015; Allison et al., 2013; Calatayud, Esperón, Cleveland, et al., 2019; Calatayud, Esperón, Velarde, et al., 2019; Cotmore et al., 2014; Duarte et al., 2013; Rubio et al., 2013; Steinel et al., 2001). There are two recognized protoparvovirus variants: feline panleukopenia (FPV-like) and canine parvovirus type 2 (CPV-2 like) (Mira et al., 2019)). Studies have suggested that CPV-2 in dogs originated from an FPV-like virus circulating in wildlife, following cross-species transmission from felids or other carnivore hosts (i.e. minks, foxes or raccoons) (Allison et al., 2013; Parrish et al., 2008; Shackelton et al., 2005; Truyen et al., 1996). The appearance of CPV-2 in a novel host—domestic dogs—in Europe and other parts of the world in the mid-1970s is a clear example of an emerging disease causing a global pandemic (Parrish et al., 2008; Parrish & Kawaoka, 2005). The original CPV-2 only infected dogs but was soon completely replaced by a new lineage that initially included two different antigenic variants, CPV type-2a (CPV-2a) and CPV type-2b (CPV-2b) (Parrish et al., 1985, 1988, 1991). These variants recovered the ability to infect felids, lost by the original variant (CPV-2), and have been associated with increasing pathogenicity (Allison et al., 2013; Decaro & Buonavoglia, 2012). In contrast to FPV, the emerging CPV-2 showed rapid evolution, with substitution rates similar to RNA viruses (Shackelton et al., 2005). The newest viral variant (CPV-2c) was discovered in Italy in 2000 (Buonavoglia et al., 2001), and rapidly spread to canine populations worldwide (Calderon et al., 2009; Decaro et al., 2006, 2007; Hong et al., 2007; Kapil et al., 2007; Nakamura et al., 2004; Pérez et al., 2007; Touihri et al., 2009) and to wildlife (Calatayud, Esperón, Cleveland, et al., 2019; Calatayud, Esperón, Velarde, et al., 2019).

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Protoparvovirus require the nucleus of rapidly dividing cells for replication (e.g. intestinal crypts, myocardiocytes and bone marrow precursor cells); thus, it can mainly affect young animals (Decaro & Buonavoglia, 2012; Goddard & Leisewitz, 2010). However, CPV-2c has been associated with severe disease in adults, which also harbour rapidly dividing cells (e.g. intestinal epithelial cells), seen even in vaccinated animals (Decaro & Buonavoglia, 2012). The main clinical signs of CPV infection in dogs are haemorrhagic enteritis, anorexia, vomiting, fever, depression and leukopenia. The mortality rate in pups may exceed 70% (Decaro & Buonavoglia, 2012). The pathogenesis of CPV in cats is unclear, although CPV-2a and CPV-2b infection pathogenesis appears to be similar to FPV (Mochizuki et al., 1996). Domestic cats infected by CPV-2c in Italy presented mild forms of the disease without abnormal haematological findings (Decaro et al., 2011). Cheetahs and tigers infected by CVP-2a/2b-type had chronic diarrhoea, enteritis and anorexia, suggesting high pathogenic potential of these viral variants in felids (Steinel et al., 2000). FPV affects cats of all ages, but kittens are more susceptible, with mortality rates over 90% (Truyen et al., 2009). The main clinical signs are diarrhoea, lymphopenia and neutropenia, followed by thrombocytopenia and anaemia, immunosuppression (transient in adult cats), abortion and cerebellar ataxia in kittens (Truyen et al., 2009).

One of the main characteristics of protoparvovirus is its high environmental stability and survival, conferring capacity of transmission by both direct and indirect contact with infected animals, as well as by environmental contamination (Berthier et al., 2000). Intrauterine transmission has also been documented (Truyen et al., 2009). The faecal-oral route is considered as the main transmission method (Truyen et al., 2009).

Exposure to protoparvovirus in Chile has been detected only by serological methods in domestic cats and dogs (Acosta-Jamett, Cunningham, et al., 2015; Acosta-Jamett, Surot, et al., 2015; Llanos-Soto et al., 2019). FPV exposure or infection has not been reported in Chilean wild species, while antibodies against CPV have been reported in grey fox (*Lycalopex griseus*) and culpeo fox (*L. culpaeus*) (Acosta-Jamett, Cunningham, et al., 2015; Rubio et al., 2013). There is no available information on the potential pathogenic effects of protoparvovirus on wild carnivore species in Chile or information about exposure or infection in the guigna.

Here we assessed potential infection risk factors associated with protoparvovirus in guignas and their domestic counterpart, the cat. We explored possible transmission pathways through phylogenetic analysis of wild and domestic strains in Chile. We also assessed the clinical status and potentially associated lesions of protoparvovirus-infected guignas by histopathology and haematological

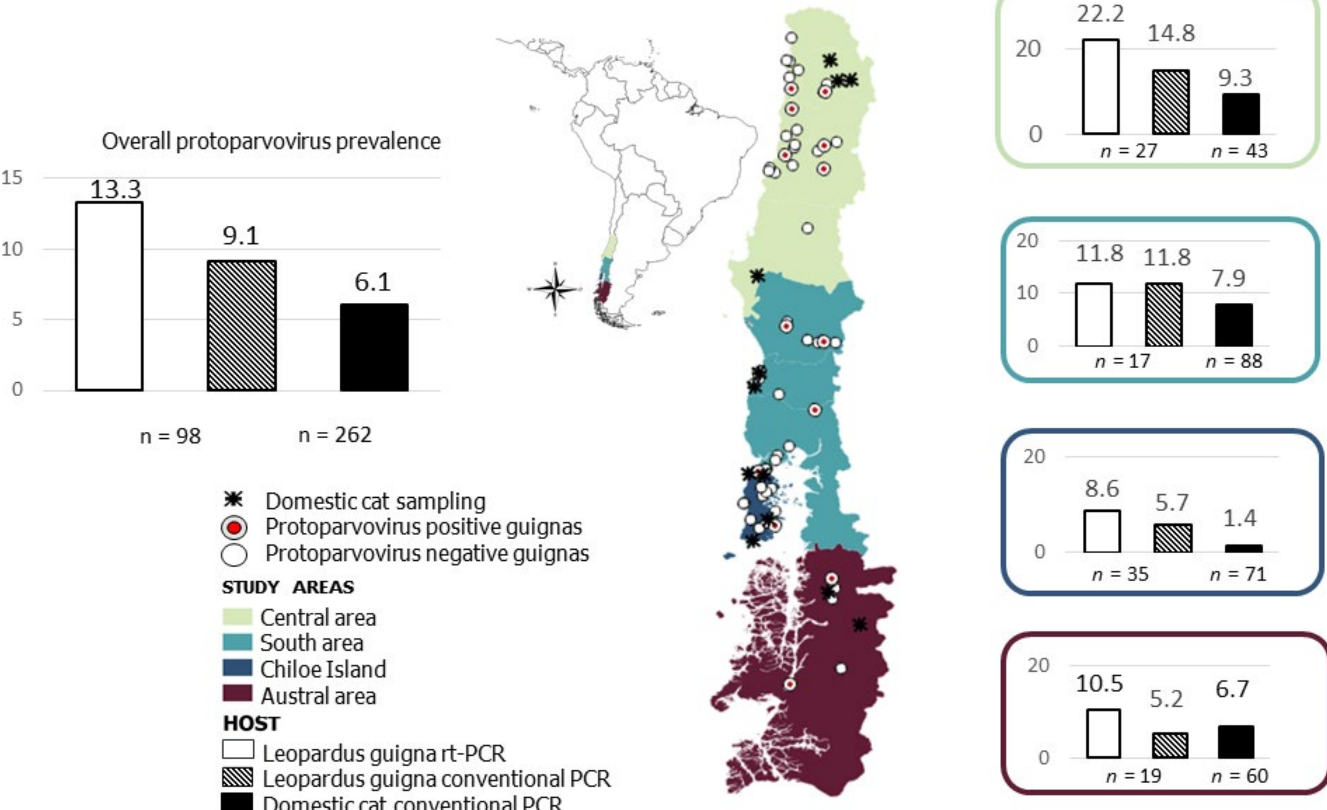

FIGURE 1 Map of study area, overall and per study area per cent prevalence of *Carnivore protoparvovirus-1* obtained by real-time PCR and conventional PCR in guignas (white colour) and by conventional PCR in domestic cats (shaded black) and the number of individuals sampled

TABLE 1 Oligonucleotide sequences targeting *vp2* gene used in rt-PCR and conventional PCR assays for biological samples from wild guignas and domestic cats sampled in central-southern Chile

Primer	Sequence (5′ to 3′)	Binding site[a]	Sense	Reference
VPF (mc)	ATGGCACCTCCGGCAAAGA	2285–2303	Forward	Mochizuki et al. (1996)
VPR (mc)	TTTCTAGGTGCTAGTTGAG	5285–5302	Reverse	
P1 (mc)	ATGAGTGATGGAGCAGTTC	2786–2804	Forward	Battilani et al. (2001)
P4 (mc)	AAGTCAGTATCAAATTCTT	4200–4218	Reverse	
Primer *F* (s)	TGGAACTAGTGGCACACCAA	3454–3473	Forward	Streck et al. (2013)
Probe (s)	6FAM-CAGGTGATGAATTTGCTACAGG-BHQ1	3555–3576	Forward	
Primer R (s)	AAATGGTGGTAAGCCCAATG	3636–3655	Reverse	
M5mod (mc)	ATAACAAACCTTCTAAATCCTATATCAAAT	4681–4709	Reverse	Steinel et al. (2000)

Note: (s) = primers and probe used for protoparvovirus screening analysis; (mc) = primers used for protoparvovirus molecular characterization analysis.

[a]Binding site calculated with respect to the reference sequence CPV-N (GenBank accession Number M19296).

analysis. Our goal was to evaluate the possible effects of landscape anthropization on the interspecific transmission of protoparvovirus between guignas and domestic cats as well as to evaluate possible pathogenicity of protoparvovirus in guignas.

2 | MATERIAL AND METHODS

2.1 | Study area

The study area included different macro-regions of central and southern Chile (33°S–46°S), encompassing the entire current distribution range of the guigna in Chile (Napolitano, Gálvez, et al., 2015) (Figure 1). We defined four study areas: Central, South, Chiloé Island and Austral areas, which correspond to the phylogeographic structure of guigna populations (Napolitano et al., 2014). The study area has different degrees of human-dominated landscapes, including continuous near pristine forest areas and areas with high human population density (INE, 2017).

2.2 | Sample collection

Between 2008 and 2018, 98 free-ranging guignas were sampled, through active capture with tomahawk-like live traps (n = 48) or opportunistically immediately following admission into wildlife rescue and rehabilitation centres (WRRC; n = 8), euthanized at WRRC (n = 4), or found road-killed (n = 38).

Captured animals were immobilized with a combination of dexmedetomidine (0.05 mg/kg) and ketamine (5 mg/kg) injected intramuscularly. When the guigna started to regain consciousness, an intramuscular injection of atipamezole (five times the dose of dexmedetomidine previously applied) was given to antagonize the dexmedetomidine. The anaesthetic protocol was adapted from protocols described in other species of South American wild felids (Beltrán et al., 2009).

Whole blood samples were collected from live animals by jugular venipuncture from 55 guignas (0.5 ml tube with EDTA anticoagulant for genetic analysis, 0.5 ml EDTA anticoagulant tube for haematological analysis, 1 ml tube without anticoagulant for biochemical analysis). Faecal samples were collected directly from the rectum of 20 guignas and preserved frozen (−20°C). Complete necropsies of road-killed and euthanized animals (at WRRC) were performed, and faecal (n = 31), spleen (n = 27), intestine (n = 8) and thoracic blood samples (n = 7) were collected.

Sex, age range (estimated from dentition) and GPS location of each animal sampled were recorded. All live animals were given a complete physical examination by a veterinarian. A total of 38 females and 60 males, 62 adults and 16 juveniles (no age data was available for 20 individuals) were sampled.

Whole blood (n = 258) (0.5 ml tubes with EDTA anticoagulant for genetic analysis) and/or faeces directly collected from the rectum (n = 83) were also collected from 262 owned, free-roaming domestic cats from rural communities across the guigna distribution range in Chile. Four spleen samples were collected during necropsies of road-killed domestic cats. Sex, age class and location of each cat were recorded. A total of 129 females and 133 males, 226 adults and 36 juveniles were sampled. None of the cats was vaccinated (no information available for the four road-killed cats) or neutered.

Guigna captures and tissue collection followed proven techniques (Napolitano, Díaz, et al., 2015), and handling and supervision protocols in accordance with bioethical and animal welfare frameworks, with permission from the Chilean Agriculture and Livestock Service (SAG) (capture permits 814/13 2008, 109/9 2009, 1220/22 2010, 1708/26 2010, 7624/2015, 2288/2016, 2185/2017, 4072/2018).

Guigna and domestic cat samples were stored frozen at −20°C until molecular analyses. Samples for haematological and biochemical analysis were stored refrigerated and sent to the laboratory within two days of collection.

2.3 | Genetic analysis

Total DNA extraction from guigna blood, faecal and tissue samples was performed by a pressure filtration method (QuickGene DNA Tissue Kit S, Fujifilm), following the manufacturer's instructions

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(using same amount of tissue). Domestic cat DNA extraction from blood, faecal and tissue samples was performed with a commercial kit (DNeasy Blood & Tissue kit, Qiagen®), following the manufacturer's instructions (using same amount of tissue). To monitor for cross-contamination during the extraction process, negative controls consisting of 100 μl phosphate-saline buffer were prepared concurrently with each batch of 15 samples.

Ultrapure water was used as a negative control in all PCR assays. The commercial CPV-2 vaccine (Nobivac® Puppy DP, MSD Animal Health) was used as a positive control for guigna sample analysis and DNA from a sequenced parvovirus from a domestic dog was employed as positive control for domestic cat sample analysis.

DNA amplification of protoparvovirus from guigna samples was performed by a conventional PCR adapted from primers described in Streck et al. (2013) (95°C, 5′, followed by 40 cycles: 95°C 30″; 58°C 30″; 72°C, 30″; with a final extension of 72°C for 7′), and also by real-time PCR method based on TaqMan probes for comparison purposes, amplifying 200 bp of the parvovirus *vp2* gene of both CPV-2 and FPV, as described by Streck et al. (2013).

DNA amplification of protoparvovirus from domestic cat samples was performed by same conventional PCR protocol adapted from Streck et al. (2013), amplifying 200 bp of the *vp2* gene.

Molecular characterization of positive guignas and domestic cats after the screening stage was carried out by amplifying and sequencing almost the entire *vp2* gene, a procedure regarded as the gold standard for this pathogen (Truyen et al., 1996). A nested PCR was performed to amplify 1,746 bp. The external PCR amplified a 2,401 bp fragment, and was conducted by combining primers VPF and M5mod (Mochizuki et al., 1996; Steinel et al., 2000); the internal PCR was conducted using primers P1 and VPR (Battilani et al., 2001; Mochizuki et al., 1993) (Table 1). The temperature profile for the external PCR was set at 94°C for 5′, followed by 45 cycles: 94°C for 30″, 55°C for 30″ and 72°C for 2′30″, with a final extension of 72°C for 7′. The internal PCR was set at 94°C for 5′, followed by 40 cycles: 94°C for 15″, 52°C for 15″ and 72°C for 2′, with a final extension of 72°C for 7′. Samples with the corresponding 1,746 bp amplicon were sequenced with seven different primers (Table 1). PCR products were separated by electrophoresis in 2% agarose gels and directly sequenced by Sanger methods.

Multiple sequence alignments were conducted using the CLUSTAL W algorithm (Geneious®). The best model of evolution was selected by the jModelTest2 (version 2.1.6) program (Darriba et al., 2012), under the Akaike Information Criterion (AIC) (Posada & Buckley, 2004) (GTR + I). Phylogenetic trees were constructed based on maximum likelihood methods; RaXML software version 1.5 (Stamatakis et al., 2008). The data set was resampled 1,000 times to generate bootstrap values.

2.4 | Spatial variable analysis

To describe the landscape features associated with protoparvovirus infection in guigna, we generated a circular area surrounding each guigna sample location, which was defined as the buffer area. This buffer corresponded to the mean home range area described for guignas (males = 446 ha; females = 170 ha) (Dunstone et al., 2002; Sanderson et al., 2002; Schüttler et al., 2017). We described and quantified six landscape variables in each buffer area: (a) percentage of vegetation cover (Hansen et al., 2013, v.1.4), (b) presence of houses within the buffer, (c) number of houses within the buffer, (d) distance from the sample location to the nearest house (either inside or outside the buffer area), (e) land use (fragmented landscape or continuous forest) and (f) study area: Central, South, Chiloé Island and Austral area.

Per cent vegetation cover was defined based on Hansen et al. (2013, v.1.4), which included canopy closure for all vegetation >5 m height in both native and timber plantations (both native and timber plantations suppose functional connectivity for guignas) (Gálvez et al., 2013, 2018; Sanderson et al., 2002).

Presence of houses and number of houses were defined based on the presence of roofs extracted from Google Earth (Google Inc., 2013), using roofs as a proxy for houses (Villatoro et al., 2016).

For land use (variable 5), we defined continuous landscape as a buffer area composed only of continuous vegetation, which may or may not include roads (functional connectivity for guignas is not limited by roads) (Gálvez et al., 2013, 2018; Sanderson et al., 2002). We defined a fragmented landscape as a buffer area composed of human settlements, agricultural land and/or fragments of forest surrounded by a matrix of human activities.

Geographic Information System layers were obtained from the Ministerio de Bienes Nacionales website (Ministerio de Bienes Nacionales, 2019). The QGIS 2.14® software was used to extract the attribute values of landscape variables corresponding to each sampled guigna for spatial analysis. To address spatial autocorrelation in our data, we conducted a Global Moran I test (Pfeiffer et al., 2008) using ArcGIS Pro.

2.5 | Assessment of clinical signs of disease

Guigna haematological, biochemical and histological parameters were evaluated, as well as clinical signs, by direct inspection. Guigna whole blood preserved in EDTA (n = 20) and serum samples (n = 19) were submitted to haematological and biochemical analysis, respectively. The haematological parameters analysed included erythrocyte count (RBC), white blood cell count, haemoglobin concentration, mean cell volume, mean corpuscular haemoglobin concentration and haematocrit determination, using the Abacus Junior Vet Analyzer (Diatron®). The biochemical parameters evaluated were glucose, total protein, albumin, globulin, total bilirubin, total cholesterol, blood urea nitrogen, creatinine, calcium, phosphorus, alanine aminotransferase, aspartate aminotransferase and gamma glutamyltransferase, analysed by Microlab 100 of MERCK®, employing Wiener® Lab products.

Histopathological analysis was performed in tissue samples collected during the necropsies of 32 guignas. Histopathological

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 Characteristics (sex, age, study area) and spatial variables (land use of the buffer, per cent vegetation cover, number of houses within the buffer, presence of houses within the buffer and distance to the nearest house) of each *Carnivore protoparvovirus*-1-positive guigna

ID	Sex	Age	Study areas	Landscape use of the buffer	(%) vegetation cover within the buffer	Number of houses within the buffer	Presence of houses within the buffer	Distance to the nearest house (km)	Cycle threshold (C_t)	Type of tissue
LG145	Female	Juvenile	Central	Fragmented landscape	9.0	27	Yes	1.5	25.8	Faeces
LG148	Male	Adult	Chiloé Island	Fragmented landscape	91.4	39	Yes	0.1	28.6	Intestine
LG171	Male	Adult	South	Fragmented landscape	20.8	329	Yes	0.5	31.3	Faeces
LG131	Male	Adult	Central	Fragmented landscape	2.6	190	Yes	1.5	31.4	Faeces
LG137	Male	Adult	Chiloé Island	Fragmented landscape	64.9	468	Yes	0.2	31.5	Faeces
LG081	Female	Adult	Chiloé Island	Fragmented landscape	18.2	7	Yes	17.6	33.5	Intestine
LG186	Male	Adult	South	Fragmented landscape	72.9	51	Yes	0.1	35.1	Intestine
LG176	Male	Adult	Austral	Continuous forest	85.5	3	Yes	0.1	36.8	Faeces
LG165	Female	Juvenile	Central	Fragmented landscape	6.4	102	Yes	0.3	36.8	Faeces
LG166	Female	Juvenile	Central	Fragmented landscape	52.7	18	Yes	0.2	37.2	Faeces
LG098	Male	Unknown	Austral	Continuous forest	60.4	0	No	11.7	37.8	Blood
LG173	Female	Adult	Central	Fragmented landscape	5.6	56	Yes	0.0	38.1	Spleen
LG174	Male	Juvenile	Central	Continuous forest	21.6	183	Yes	0.8	39.5	Faeces

Note: The Rt-PCR cycle threshold and type of tissue used for each sample is shown.

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

evaluation was performed on formalin-fixed tissues embedded in paraffin wax, sectioned at 3–5 μm and stained with haematoxylin eosin.

2.6 | Statistical analysis

Spatial and biological variables associated with protoparvovirus infection were assessed with crude and adjusted odds ratios (ORs) calculated by a logistic regression analysis with 95% confidence intervals (CIs) The goodness of fit models were assessed using the Hosmer Lemeshow test and analysis of residuals (Hosmer et al., 2013).

Differences in infection prevalence between domestic cats and guignas, as well as between biogeographic regions, were analysed using non-parametrical tests, either Mann–Whitney *U* or Kruskal–Wallis (Zar, 1999). Haematological and biochemical parameters of infected and non-infected guignas were compared by Kruskal–Wallis tests. All statistical analyses were performed in R studio software 3.0.1 (R Core Team, 2013) with a significance level of $p < .05$.

3 | RESULTS

DNA of protoparvovirus was detected in 13/98 guignas (13.3%, 95% Confidence Interval (CI) = 6.4%–20.1%) using real-time PCR method and 9/98 guignas (9.1%, CI = 3.3%–15.0%) using conventional PCR. Comparing between real-time PCR and conventional PCR, the latter was able to detect 4/13 (30.8%) less protoparvovirus-positive guignas than real-time PCR. DNA of protoparvovirus was detected in 16/262 (6.1%, CI = 3.1%–9.0%) of domestic cats (conventional PCR) (Table S1). Differences in guignas and domestic cats by conventional PCR methods were not statistically significant ($p = .30$; $U = 12,440$).

No statistically significant differences in protoparvovirus prevalence were observed in guignas in relation to sex, age, study area or landscape variables (Tables 2 and S1, Figure 2). A significantly higher prevalence was found in juvenile domestic cats (16.7%) compared to adults (4.4%) ($U = 3,570$; $p = .004$). No statistically significant difference was found according to study area ($K = 4.04$; $p = .25$) or sex ($U = 8,301$; $p = .27$) in domestic cats (Table S1). No association between protoparvovirus prevalence and year of sampling was found in guignas or domestic cats ($K = 3.18$, $p = .21$; $K = 6.828$, $p = .07$). We obtained non-significant results in the spatial autocorrelation analysis (Moran's index = 0.38, *z*-score = 0.46, *p*-value = 0.64), suggesting that there is no pattern of data spatial clustering.

Positive guignas showed high rt-PCRct (cycle threshold) values, with 12/13 samples presenting values above 28.5 (Table 2). The lowest C_t value (25.67) corresponded to a juvenile female guigna which showed clinical signs of disease at the time of sampling. This juvenile female was admitted into a WRRC with haemorrhagic diarrhoea, anorexia and cachexia; she died four months after admission (Ortega et al., 2020). Unfortunately, haematological and biochemical parameters and *post-mortem* examination were not available from this individual. No clinical signs were observed in any of the other positive guignas or domestic cats.

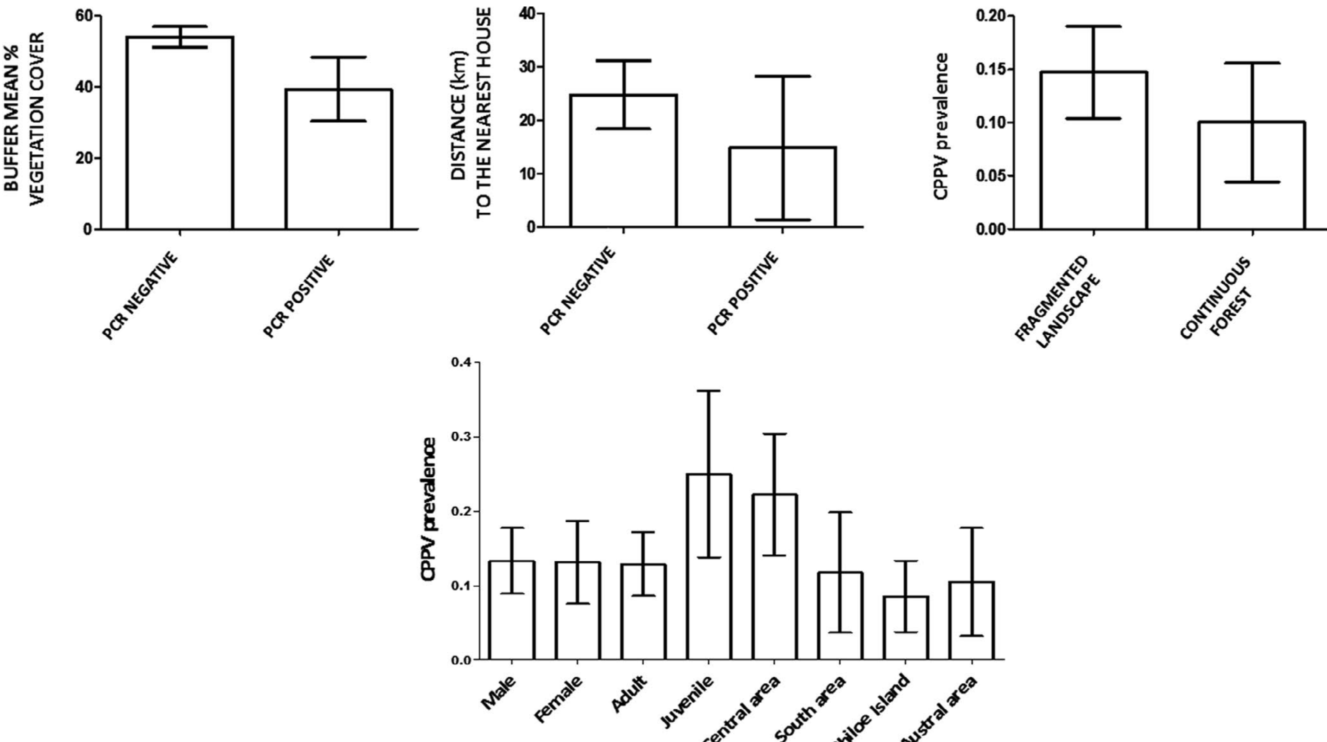

FIGURE 2 Graphical representation of protoparvovirus prevalence in relation with landscape variables (percentage of vegetation cover, distance from the sample location to the nearest house and land use), sex, age and study areas in guignas

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 Biochemical parameters (minimum, median, maximum and 95% CI) of *Carnivore protoparvovirus-1* PCR-positive and PCR-negative guignas

Carnivore Protoparvovirus 1		TP	Albumin	Globulin	TBIL	ALT	FA	GGT	AST	Calcium	Phosphorus	Creatinine	BUN	Total cholesterol	Glucose
		g/dl	g/dl	g/dl	mg/dl	IU/L	IU/L	IU/L	IU/L	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
PCR-positive guignas	*n*	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Minimum	5.9	2.1	1.6	0.2	25.8	40.0	1.6	27.6	9.1	5.2	0.2	9.6	63.0	42.0
	Median	6.4	3.9	3.3	0.2	37.4	223.6	3.4	51.0	9.6	6.4	0.9	38.8	104.0	126.5
	Maximum	8.4	4.3	4.6	0.6	47.0	389.8	4.3	162.0	9.8	10.0	1.1	61.3	158.0	367.2
	95% CI	5.0–8.6	2.0–5.2	0.7–5.7	0.01–0.7	22.0–51.8	−93.3–31.8	1.1–5.3	−23.6–69.4	9.0–10.0	3.6–10.4	0.1–1.4	3.3–70.9	45.1–169.4	−58.2–89.2
PCR-negative guignas	*n*	8	8	8	8	9	8	7	9	9	9	9	9	8	8
	Minimum	5.8	2.2	1.9	0.1	6.4	35.0	2.0	36.0	1.8	1.6	0.4	14.4	101.0	14.0
	Median	7.2	4.3	3.4	0.3	42.0	80.40	2.9	150.0	9.5	6.0	1.1	49.0	154.0	97.0
	Maximum	11.2	4.8	6.8	0.4	182.0	8,000	4.0	296.0	10.8	13.6	29.0	79.0	212.0	249.4
	95% CI	6.2–9.1	3.4–4.7	2.3–4.9	0.2–0.3	17.7–7.5	−1,251–422	2.1–3.6	69.1–211.4	6.7–10.8	4.0–9.0	−3.0–11.3	29.9–7.6	124.0–183.5	47.6–165.5

No differences in haematological or biochemical parameter values were found comparing PCR-positive to PCR-negative guignas (Tables 3 and 4, Tables S2 and S3). However, one PCR-positive guigna presented haematological alterations (anaemia, leukocytosis, lymphocytosis) (based on parameters of Geoffroy´s cat, *Leopardus geoffroyi*, the species most closely related genetically to the guigna (Teare, 2002), compatible with an infectious process (Table 4). Histopathological analysis comparing PCR-positive and PCR-negative guignas did not reveal any lesions consistent with active protoparvovirus infection in the former.

Despite repeated attempts, molecular characterization of the *vp2* gene was successful only in one PCR-positive guigna sample and five domestic cat samples, possibly due to low pathogen loads in samples or suboptimal quality of field samples.

Phylogenetic analysis showed well-supported clades; sequences of one guigna and four domestic cats from this study (GDAY17, GDRM19, 15,028, GDNH21), along with three domestic dogs from Chile clustered in the CPV clade. One domestic cat from this study clustered in the FPV clade (GDNH15) (Figure 3, Table S4).

4 | DISCUSSION

Protoparvovirus is known to infect a wide range of wild carnivores, including wild felids (Acosta-Jamett, Cunningham, et al., 2015; Calatayud, Esperón, Velarde, et al., 2019; Cotmore et al., 2014; Duarte et al., 2013; Rubio et al., 2013; Steinel et al., 2001). However, most studies on wildlife have been conducted on animals in captive settings; studies on free-ranging felids are scarce (Calatayud, Esperón, Cleveland, et al., 2019; Filoni et al., 2006; Hofmann-Lehmann et al., 1996; Santos et al., 2009; Steinel et al., 2001).

The present study showed relatively high protoparvovirus DNA observed prevalence (13.3%) in guigna, with widespread occurrence across the species' distribution range in Chile. A study based on molecular analysis in a wild felid found 13.7% prevalence of FPV in lions (*P. leo*) from Tanzania; FPV is considered an endemic pathogen in this population (Calatayud, Esperón, Cleveland, et al., 2019). The only infection with CPV-2c was detected in a wildcat *(Felis silvestris silvestris)* from the Iberian Peninsula (Calatayud, Esperón, Velarde, et al., 2019).

Based on serology, Filoni et al. (2006) found parvovirus seroprevalence of 48% in ocelot (*Leopardus pardalis*), *cougar*, (*P. concolor*) and *tigrillo* (*Leopardus tigrinas*), from Brazil. In free-ranging lions from Serengeti National Park, high seroprevalence of parvovirus (78%) related to a possible outbreak was found (Hofmann-Lehmann et al., 1996), being lower in the Ngorongoro Crater area (27%). Canine parvovirus antibodies were detected in four of 22 and one of eight studied wildcats from Spain and Portugal, respectively (Millán & Rodríguez, 2009; Santos et al., 2009).

The observed prevalence of protoparvovirus reported here in domestic cats of Chile (6.1%) was lower than that described in other South American domestic cat populations (11.8% FPV prevalence in Brazil; de Cássia et al., 2011), or in Europe (32.5% CPV

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4 Haematological parameters of *Carnivore protoparvovirus-1* PCR-positive and PCR-negative guignas and normal haematological values of Geoffroy's Cat (*Leopardus geoffroyi*), the most closely genetically related species to the guigna. In bold, guigna with haematological alterations

ID	Sex	Age	PCR status	Red blood cells (×106 μl^{-1})	Haemoglobin (g/dl)	Haematocrit (%)	Mean corpuscular volume Fl	Mean corpuscular haemoglobin concentration	White blood cells (x103 μl^{-1})	Segmented neutrophil (×103 μl^{-1})	Lymphocyte (×103 μl^{-1})	Monocyte (×103 μl^{-1})	Eosinophil (×103 μl^{-1})	Platelets (×105 μl^{-1})
Leopardus geoffroyi				**6.71–9.25**	**11.5–14.9**	**35.2–47.8**	**47–55.8**	**30.2–35.6**	**5.387–14.22**	**3.35–9.16**	**1.038–3.154**	**0.06–0.567**	**0–1.448**	**2.80–3.86**
LG029	Female	Adult	0	7.98	11.9	39.1	49	30.5	18.80	15.416	1.880	1.316	0.188	4.12
LG146	Male	Juvenile	0	8.13	12.3	46.0	57	27.0	16.10	8.211	7.406	NA	0.483	7.06
LG151	Male	Juvenile	0	6.46	11.9	38.4	59.4	31.0	3.80	1.976	1.634	0.190	0	4.15
LG158	Female	Adult	0	8.74	15.9	40.0	46	39.8	10.60	8.480	1.060	0.106	0	4.45
LG159	Male	Juvenile	0	6.67	12.0	38.0	57	32.0	10.20	6.426	2.958	0.102	0.612	5.46
LG160	Male	Adult	0	6.73	12.8	39.0	57	33.0	7.00	5.460	1.260	0.140	0.140	3.06
LG163	Female	Juvenile	0	1.11	18.9	60.0	54	35.0	5.20	4.628	0.364	0.156	NA	NA
LG164	Female	Adult	0	6.34	10.0	33.0	52	30.0	6.90	4.278	2.277	0.276	0.069	1.50
LG165	**Female**	**Adult**	**1**	**5.89**	**10.8**	**33.0**	**56**	**32.7**	**15.20**	**9.120**	**5.320**	**0.456**	**0.304**	**2.10**
LG166	Female	Adult	1	6.89	12.2	40.0	58	30.5	6.80	5.372	1.224	0.136	0.068	1.60
LG171	Male	Adult	1	7.40	12.6	40.0	54	31.5	10.80	6.588	3.672	0.432	0.108	1.48
LG172	Male	Juvenile	0	6.48	13.3	35.0	54	34.0	13.20	10.560	1.716	0.660	0	1.66
LG175	Male	Juvenile	0	6.92	13.8	45.0	65	30.6	10.24	7.070	3.120	0.060	0	7.98
LG176	Male	Adult	1	7.32	13.6	44.2	60	30.8	14.71	10.800	3.060	0.860	0	6.68
LG177	Female	Adult	0	8.89	15.9	54.8	62	28.9	21.70	10.240	11.350	0.110	0	6.64
LG185	Male	Juvenile	0	6.44	12.0	35.0	NA	NA	5.00	NA	NA	NA	NA	7.14
LG190	Male	Adult	0	9.31	15.5	48.4	52	32.0	5.20	4.056	0.832	0.260	0.052	1.96
LG191	Male	Adult	0	8.70	13.5	42.1	48	32.0	6.40	4.224	1.920	0.192	0.064	0.76
LG192	Female	Adult	0	8.34	15.5	46.9	56	33.0	12.80	10.752	1.408	0.512	0.124	1.92

Abbreviation: NA, not analysed.

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 3 Maximum likelihood phylogenetic tree of 605 bp of the *vp2* gene for guignas and domestic cats. Bootstrap values ≥ 70 at the nodes of the tree. Highlighted, guigna and domestic cat sequences from this study

prevalence in domestic cats from UK; Clegg et al., 2012), both through conventional PCR methods, supporting a limited infection rate of this virus in central-southern Chile. To the authors' knowledge, this is the first molecular report of protoparvovirus in domestic cats from Chile.

Higher observed protoparvovirus DNA prevalence in juvenile versus adult domestic cats may be explained by the fact that the virus replicates in rapidly dividing cells, thus affecting mainly, but not exclusively, young animals. Difference in prevalence between age groups may be linked to the development of a life-lasting protective immunity acquired after an infection at a young age (Decaro & Buonavoglia, 2012; Goddard & Leisewitz, 2010). In guignas, no statistically significant differences were observed between age classes. Absence of statistically significant differences may be due to low sample size and thus low statistical power; a greater sample size would be necessary to detect statistically significant differences. However, the lack of difference between age classes could indicate that that protoparvovirus infection in guignas is not endemic and spillover process are occurring. Although the pathogenic capacity of protoparvovirus in wild felids is still poorly understood (Ikeda, 2002), high mortality from both CPV and FPV in young animals has been documented in domestic dogs and cats (Decaro & Buonavoglia, 2012; Truyen et al., 2009).

High C_t values (above 28) were obtained in most rt-PCR-positive animals, suggesting that in most cases viral loads were low and the infection was probably subclinical or recovered (latent). The guigna with the lowest C_t value (25.67), and thus presumably the highest viral load, showed clinical signs consistent with an active infection and was infected by the CPV-2c subtype (Ortega et al., 2020), the most recently emerged CPV viral type, also identified as the most pathogenic one. This may imply that this viral type infection may produce severe pathogenicity in wild felids (Decaro et al., 2011; Ikeda, 2002), which should be taken into consideration in future surveillance. Notwithstanding, C_t values and viral loads may differ greatly among different kinds of tissues, depending on several factors including disease stage; thus, comparisons should be made between same tissue types.

Only one of the domestic cat sequences was identical to FPV; the other four were phylogenetically related to CPV sequences. Although FPV is the most prevalent species of parvovirus infecting cats and has been considered endemic in some populations of wild felids (Battilani et al., 2011; Calatayud, Esperón, Cleveland, et al., 2019; Truyen et al., 2009), in the present study only one

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

sequence belonged to this virus type, differing from results obtained in other countries where CPV infection in cats is rare and sporadic (Battilani et al., 2011; Truyen et al., 2009).

Considering that CPV infection is unusual in felids (Calatayud, Esperón, Cleveland, et al., 2019; Calatayud, Esperón, Velarde, et al., 2019, but see Allison et al., 2014), one possible origin in guignas and domestic cats of this study may be cross-species transmission from domestic dogs or other wild canids; however, we cannot conclusively conclude this with our current data. Free-ranging domestic dogs are abundant in rural Chile and are not usually subjected to any sanitary control or movement restriction, roaming freely in natural areas and therefore facilitating contact possibilities with domestic and wild species (Villatoro et al., 2016) and spreading of dog infectious agents. Another study (Ortega et al., 2020) found that dogs may be the most probable origin of infection with protoparvovirus in guignas. High environmental survival of protoparvovirus may allow the possibility of wildlife being in contact with the virus for several months, even in the absence of direct animal contact (Berthier et al., 2000). Likewise, the ability of the virus to survive in the environment may explain its wide distribution across the study area.

We acknowledge the limited length of our sequence fragments and that our viral sample size was constrained by the observed prevalence of the viruses. Future studies should aim to sequence whole viral genomes to provide more complete evidence and also include serology analysis for the determination of exposure and better identification of recovered (latent) infections.

The results of this study reveal widespread presence of protoparvovirus across the guigna distribution in Chile and suggest that interspecific transmission of the virus from domestic to wild carnivores may be a possibility, being capable of, but not always causing, severe disease and fatal infections in wild guignas. Although the impact of protoparvovirus infection in guigna populations is still unclear, elucidating the dynamics of pathogen transmission between domestic and wild species is essential to enable the implementation of integrative management measures to prevent negative effects for the long-term survival of wildlife populations.

ACKNOWLEDGEMENTS

We gratefully acknowledge local inhabitants of rural communities for kindly giving us the opportunity to sample their domestic cats. We are grateful to CONAF, especially Patricio Contreras, Patricia Barría, Andrea Bahamonde and Dennis Aldrigde; SAG, especially Diego Ramírez and Rodrigo Villalobos; the Ministry of the Environment, especially Sandra Díaz; Tantauco Park, especially Alan Bannister and Catherine Chirgwin; and the Valdivian Coastal Reserve and Camila Dünner for logistic support. Special thanks to Debora Mera, Diego Peñaloza, Gonzalo Canto, Camila Núñez, Héctor Basualto, Nicolás Gálvez, Eduardo Silva, Maximiliano Sepúlveda, José Luis Brito, Daniel González, Nicole Sallaberry, Angelo Espinoza, Jorge Valenzuela, Daniela Poo, Francisca Astorga, Violeta Barrera, Macarena Barros, Gonzalo Medina, Claudia Hernández, Nora Prehn, Camila Sepúlveda, Gerardo Morales, Daniela Ormazával, Pía Astudillo, Andrea Roa, Gaby Svensson, Ricardo Pino, Frederick Toro, Elfego Cuevas, Mario Alvarado, Brayan Zambrano, Tomás Valdés and Manuel Valdés for their valuable support in sample collection. Our work was funded by CONICYT FONDECYT Iniciación 11150934 (CN), Morris Animal Foundation D15ZO-413 (CN), National Geographic Society C309-15 (CN), Mohamed bin Zayed Species Conservation Fund 152510351 (CN), 2018 Endeavour Research Fellowship (Australian government) (CN), ANID PAI 77190064 (CN), CONICYT PIA APOYO CCTE AFB170008 (CN, EP), the Wild Felid Association (IS), Fondo Interno UNAB DI-778-15/R (JM), Morris Animal Foundation D16Z-825 (JM), and CONICYT FONDECYT Regular 1161593 (JM, CN).

ETHICAL APPROVAL

All procedures followed animal welfare and ethical protocols previously approved by the Animal Ethics Committee of the Institute of Ecology and Biodiversity of Universidad de Chile (resolution of November 20, 2015).

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The genetic data that support the findings of this study are openly available in Genebank, at https://www.ncbi.nlm.nih.gov/genbank, reference numbers: MT367584, MT367582, MT367581, MT367580, MT367579, MT367583. Other data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Irene Sacristán https://orcid.org/0000-0002-4169-4884
Rubén Pérez https://orcid.org/0000-0003-4961-4743
Ezequiel Hidalgo-Hermoso https://orcid.org/0000-0002-1291-514X
Karen A. Terio https://orcid.org/0000-0003-4924-5503
Javier Millán https://orcid.org/0000-0001-5608-781X
Elie Poulin https://orcid.org/0000-0001-7736-0969
Constanza Napolitano https://orcid.org/0000-0002-7081-6975

REFERENCES

Acosta-Jamett, G., Cunningham, A. A., Bronsvoort, B. M. D. C., & Cleaveland, S.(2015). Serosurvey of canine distemper virus and canine parvovirus in wild canids and domestic dogs at the rural interface in the Coquimbo Region, Chila. *European Journal of Wildlife Research*, *61*(2), 329–332. https://doi.org/10.1007/s10344-014-0886-0

Acosta-Jamett, G., Surot, D., Cortés, M., Marambio, V., Valenzuela, C., Vallverdu, A., & Ward, M. P. (2015). Epidemiology of canine distemper and canine parvovirus in domestic dogs in urban and rural areas of the Araucanía region in Chile. *Veterinary Microbiology*, *178*(3), 260–264. https://doi.org/10.1016/j.vetmic.2015.05.012

Alexander, K. A., & Appel, M. J. (1994). African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. *Journal of Wildlife Diseases*, *30*(4), 481–485. https://doi.org/10.7589/0090-3558-30.4.481

Allison, A. B., Kohler, D. J., Fox, K. A., Brown, J. D., Gerhold, R. W., Shearn-Bochsler, V. I., Dubovi, E. J., Parrish, C. R., & Holmes, E. C. (2013). Frequent cross-species transmission of parvoviruses among diverse

carnivore hosts. *Journal of Virology*, *87*(4), 2342–2347. https://doi.org/10.1128/JVI.02428-12

Allison, A. B., Kohler, D. J., Ortega, A., Hoover, E. A., Grove, D. M., Holmes, E. C., & Parrish, C. R. (2014). Host-specific parvovirus evolution in nature is recapitulated by in vitro adaptation to different carnivore species. *PLoS Path*, *10*(11), e1004475. https://doi.org/10.1371/journal.ppat.1004475

Battilani, M., Balboni, A., Ustulin, M., Giunti, M., Scagliarini, A., & Prosperi, S. (2011). Genetic complexity and multiple infections with more Parvovirus species in naturally infected cats. *Veterinary Research*, *42*(1), 43. https://doi.org/10.1186/1297-9716-42-43

Battilani, M., Scagliarini, A., Tisato, E., Turilli, C., Jacoboni, I., Casadio, R., & Prosperi, S. (2001). Analysis of canine parvovirus sequences from wolves and dogs isolated in Italy. *The Journal of General Virology*, *82*(Pt 7), 1555–1560. https://doi.org/10.1099/0022-1317-82-7-1555

Beltrán, S., Fabián, L., Nallar, G., Villalba, M., Lilian, M., Delgado, E., & Berna, M. (2009). Inmovilización química, evaluación haematológica y coproparasitología de *Leopardus colocolo* en Khastor, Potosí, Bolivia. *Revista de Investigaciones Veterinarias Del Perú*, *20*(2), 297–305.

Berthier, K., Langlais, M., Auger, P., & Pontier, D. (2000). Dynamics of a feline virus with two transmission modes within exponentially growing host populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1457), 2049–2056.

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., & Carmichael, L. (2001). Evidence for evolution of canine parvovirus type 2 in Italy. *Journal of General Virology*, *82*(12), 3021–3025. https://doi.org/10.1099/0022-1317-82-12-3021

Calatayud, O., Esperón, F., Cleveland, S., Biek, R., Keyyu, J., Eblate, E., Neves, E., Lembo, T., & Lankester, F. (2019). Carnivore parvovirus ecology in the Serengeti ecosystem: Vaccine strains circulating and new host species identified. *Journal of Virology*, *93*(13), e02220–e2318. https://doi.org/10.1128/JVI.02220-18

Calatayud, O., Esperón, F., Velarde, R., Oleagas, A., Llanezas, L., Ribas, A., Negres, N., de la Torre, A., Rodríguez, A., & Millán, J. (2019). Genetic characterization of Carnivore Parvoviruses in Spanish wildlife reveals domestic dog and cat-related sequences. *Transboundary and Emerging Diseases*, *67*(2), 626–634. https://doi.org/10.1111/tbed.13378

Calderon, M. G., Mattion, N., Bucafusco, D., Fogel, F., Remorini, P., & La Torre, J. (2009). Molecular characterization of canine parvovirus strains in Argentina: Detection of the pathogenic variant CPV2c in vaccinated dogs. *Journal of Virological Methods*, *159*(2), 141–145. https://doi.org/10.1016/j.jviromet.2009.03.013

Carver, S., Bevins, S. N., Lappin, M. R., Boydston, E. E., Lyren, L. M., Alldredge, M. W., Logan, K. A., Sweanor, L. L., Riley, S. P. D., Serieys, L. E. K., Fisher, R. N., Vickers, T. W., Boyce, W. M., McBride, R., Cunningham, M. C., Jennings, M., Lewis, J. S., Lunn, T., Crooks, K. R., & VandeWoude, S. (2015). Pathogen exposure varies widely among sympatric populations of wild and domestic felids across the United States. *Ecological Applications*, *26*(2), 367–381. https://doi.org/10.1890/15-0445

Chiu, E. S., Kraberger, S., Cunningham, M., Cusack, L., Roelke, M., & VandeWoude, S. (2019). Multiple introductions of domestic cat feline leukemia virus in endangered Florida panthers. *Emerging Infectious Diseases*, *25*(1), 92–101.

Clegg, S. R., Coyne, K. P., Dawson, S., Spibey, N., Gaskell, R. M., & Radford, A. D. (2012). Canine parvovirus in asymptomatic feline carriers. *Veterinary Microbiology*, *157*(1–2), 78–85. https://doi.org/10.1016/j.vetmic.2011.12.024

Cotmore, S. F., Agbandje-McKenna, M., Canuti, M., Chiorini, J. A., Eis-Hubinger, A.-M., Hughes, J., Mietzsch, M., Modha, S., Ogliastro, M., Pénzes, J. J., Pintel, D. J., Qiu, J., Soderlund-Venermo, M., Tattersall, P., & Tijssen, P. (2019). ICTV virus taxonomy profile: Parvoviridae. *Journal of General Virology*, *100*(3), 367–368. https://doi.org/10.1099/jgv.0.001212

Cotmore, S. F., Agbandje-McKenna, M., Chiorini, J. A., Mukha, D. V., Pintel, D. J., Qiu, J., Soderlund-Venermo, M., Tattersall, P., Tijssen, P., Gatherer, D., & Davison, A. J. (2014). The family parvoviridae. *Archives of Virology*, *159*(5), 1239–1247. https://doi.org/10.1007/s00705-013-1914-1

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772. https://doi.org/10.1038/nmeth.2109

de Cássia Nasser Cubel Garcia, R., de Castro, T. X., de Miranda, S. C., Júnior, G. L., de Lima, M., Labarthe, N. V., & Gagliardi Leite, J. P. (2011). Characterization of parvoviruses from domestic cats in Brazil. *Journal of Veterinary Diagnostic Investigation*, *23*(5), 951–955. https://doi.org/10.1177/1040638711417140

Decaro, N., & Buonavoglia, C. (2012). Canine parvovirus—A review of epidemiological and diagnostic aspects, with emphasis on type 2c. *Veterinary Microbiology*, *155*(1), 1–12. https://doi.org/10.1016/j.vetmic.2011.09.007

Decaro, N., Desario, C., Addie, D. D., Martella, V., Vieira, M. J., Elia, G., Zicola, A., Davis, C., Thompson, G., Thiry, E., Truyen, U., & Buonavoglia, C. (2007). Molecular epidemiology of canine parvovirus. *Europe*. *Emerging Infectious Diseases*, *13*(8), 1222. https://doi.org/10.3201/eid1308.070505

Decaro, N., Desario, C., Amorisco, F., Losurdo, M., Colaianni, M. L., Greco, M. F., & Buonavoglia, C. (2011). Canine parvovirus type 2c infection in a kitten associated with intracranial abscess and convulsions. *Journal of Feline Medicine and Surgery*, *13*(4), 231–236. https://doi.org/10.1016/j.jfms.2010.11.012

Decaro, N., Martella, V., Desario, C., Bellacicco, A. L., Camero, M., Manna, L., D'Aloja, D., & Buonavoglia, C. (2006). First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. *Journal of Veterinary Medicine, Series B*, *53*(10), 468–472. https://doi.org/10.1111/j.1439-0450.2006.00974.x

Duarte, M. D., Henriques, A. M., Barros, S. C., Fagulha, T., Mendonça, P., Carvalho, P., Monteiro, M., Fevereiro, M., Basto, M. P., Rosalino, L. M., Barros, T., Bandeira, V., Fonseca, C., & Cunha, M. V. (2013). Snapshot of viral infections in wild carnivores reveals ubiquity of parvovirus and susceptibility of egyptian mongoose to feline panleukopenia virus. *PLoS One*, *8*(3), e59399. https://doi.org/10.1371/journal.pone.0059399

Dunstone, N., Durbin, L., Wyllie, I., Freer, R., Jamett, G. A., Mazzolli, M., & Rose, S. (2002). Spatial organization, ranging behaviour and habitat use of the kodkod (*Oncifelis guigna*) in southern Chile. *Journal of Zoology*, *257*(1), 1–11. https://doi.org/10.1017/S0952836902000602

Echeverria, C., Coomes, D. A., Hall, M., & Newton, A. C. (2008). Spatially explicit models to analyze forest loss and fragmentation between 1976 and 2020 in southern Chile. *Ecological Modelling*, *212*(3–4), 439–449. https://doi.org/10.1016/j.ecolmodel.2007.10.045

Echeverría, C., Coomes, D., Salas, J., Rey-Benayas, J. M., Lara, A., & Newton, A. (2006). Rapid deforestation and fragmentation of Chilean temperate forests. *Biological Conservation*, *130*(4), 481–494. https://doi.org/10.1016/j.biocon.2006.01.017

Filoni, C., Catão-Dias, J. L., Bay, G., Durigon, E. L., Jorge, R. S. P., Lutz, H., & Hofmann-Lehmann, R. (2006). First evidence of feline herpesvirus, calicivirus, parvovirus, and ehrlichia exposure in Brazilian free-ranging felids. *Journal of Wildlife Diseases*, *42*(2), 470–477. https://doi.org/10.7589/0090-3558-42.2.470

Foley, J. E., Swift, P., Fleer, K. A., Torres, S., Girard, Y. A., & Johnson, C. K. (2013). Risk factors for exposure to feline pathogens in California mountain lions (*Puma concolor*). *Journal of Wildlife Diseases*, *49*(2), 279–293. https://doi.org/10.7589/2012-08-206

Gálvez, N., Guillera-Arroita, G., John, F. A. V. S., Schüttler, E., Macdonald, D. W., & Davies, Z. G. (2018). A spatially integrated framework for assessing socioecological drivers of carnivore

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

decline. *Journal of Applied Ecology*, *55*(3), 1393–1405. https://doi.org/10.1111/1365-2664.13072

Gálvez, N., Hernández, F., Laker, J., Gilabert, H., Petitpas, R., Bonacic, C., Gimona, A., Hester, A., & Macdonald, D. W. (2013). Forest cover outside protected areas plays an important role in the conservation of the Vulnerable guiña *Leopardus guigna*. *Oryx*, *47*(2), 251–258.

Goddard, A., & Leisewitz, A. L. (2010). Canine parvovirus. *Veterinary Clinics: Small Animal Practice*, *40*(6), 1041–1053. https://doi.org/10.1016/j.cvsm.2010.07.007

Google Inc. (2013). *Google EarthTM. Imagery date August 2013*. Google Inc.

Hansen, M. C., Potapov, P. V., Moore, R., Hancher, M., Turubanova, S. A. A., Tyukavina, A., Thau, D., Stehman, S. V., Goetz, S. J., & Loveland, T. R. (2013). High-resolution global maps of 21st-century forest cover change. *Science*, *342*(6160), 850–853.

Harder, T., Kenter, M., Appel, M., Roelke-Parker, M., Barrett, T., & Osterhaus, A. (1995). Phylogenetic evidence of canine distemper virus in Serengeti's lions. *Vaccine*, *13*(6), 521–523. https://doi.org/10.1016/0264-410X(95)00024-U

Heilmayr, R., Echeverria, C., Fuentes, R., & Lambin, E. F. (2016). A plantation-dominated forest transition in Chile. *Applied Geography*, *75*, 71–82. https://doi.org/10.1016/j.apgeog.2016.07.014

Hofmann-Lehmann, R., Fehr, D., Grob, M., Elgizoli, M., Packer, C., Martenson, J. S., O'Brien, S. J., & Lutz, H. (1996). Prevalence of antibodies to feline parvovirus, calicivirus, herpesvirus, coronavirus, and immunodeficiency virus and of feline leukemia virus antigen and the interrelationship of these viral infections in free-ranging lions in east Africa. *Clinical and Diagnostic Laboratory Immunology*, *3*(5), 554–562. https://doi.org/10.1128/CDLI.3.5.554-562.1996

Hong, C., Decaro, N., Desario, C., Tanner, P., Pardo, M. C., Sanchez, S., Buonavoglia, C., & Saliki, J. T. (2007). Occurrence of canine parvovirus type 2c in the United States. *Journal of Veterinary Diagnostic Investigation*, *19*(5), 535–539. https://doi.org/10.1177/104063870701900512

Hosmer Jr, H., David, W., Lemeshow, S., & Rodney, X. (2013). Sturdivant. In *Applied logistic regression* (Vol. *398*). John Wiley & Sons.

Ikeda, Y. (2002). Feline host range of canine parvovirus: Recent emergence of new antigenic types in cats. *Emerging Infectious Diseases*, *8*(4), 341–346. https://doi.org/10.3201/eid0804.010228

Instituto Nacional de Estadística (INE). (2017). http://www.ine.cl/estadisticas/medioambiente/informes-anuales. Cited, August 2019. n.d

Kapil, S., Cooper, E., Lamm, C., Murray, B., Rezabek, G., Johnston, L., Campbell, G., & Johnson, B. (2007). Canine parvovirus types 2c and 2b circulating in North American dogs in 2006 and 2007. *Journal of Clinical Microbiology*, *45*(12), 4044–4047. https://doi.org/10.1128/JCM.01300-07

Laurenson, K., Sillero-Zubiri, C., Thompson, H., Shiferaw, F., Thirgood, S., & Malcolm, J. (1998). Disease as a threat to endangered species: Ethiopian wolves, domestic dogs and canine pathogens. *Animal Conservation*, *1*(4), 273–280. https://doi.org/10.1111/j.1469-1795.1998.tb00038.x

Llanos-Soto, S., González-Acuña, D., Llanos-Soto, S., & González-Acuña, D. (2019). Conocimiento acerca de los patógenos virales y bacterianos presentes en mamíferos silvestres en Chile: Una revisión sistemática. *Revista Chilena de Infectología*, *36*(1), 43–67. https://doi.org/10.4067/S0716-10182019000100043

Meli, M. L., Cattori, V., Martínez, F., López, G., Vargas, A., Simón, M. A., Zorrilla, I., Muñoz, A., Palomares, F., López-Bao, J. V., Pastor, J., Tandon, R., Willi, B., Hofmann-Lehmann, R., & Lutz, H. (2009). Feline leukemia virus and other pathogens as important threats to the survival of the critically endangered Iberian Lynx (*Lynx pardinus*). *PLoS One*, *4*(3), e4744. https://doi.org/10.1371/journal.pone.0004744

Millán, J., López-Bao, J. V., García, E. J., Oleaga, Á., Llaneza, L., Palacios, V., de la Torre, A., Rodríguez, A., Dubovi, E. J., & Esperón, F. (2016). Patterns of exposure of Iberian Wolves (*Canis lupus*) to canine viruses in human-dominated landscapes. *EcoHealth*, *13*(1), 123–134. https://doi.org/10.1007/s10393-015-1074-8

Millán, J., & Rodríguez, A. (2009). A serological survey of common feline pathogens in free-living European wildcats (*Felis silvestris*) in central Spain. *European Journal of Wildlife Research*, *55*(3), 285–291. https://doi.org/10.1007/s10344-008-0246-z

Ministerio de Bienes Nacionales website. (2019). http://www.ide.cl/descarga/capas/advanced-search/92.html. Cited June. 2020. n.d

Mira, F., Canuti, M., Purpari, G., Cannella, V., Di Bella, S., Occhiogrosso, L., Schirò, G., Chiaramonte, G., Barreca, S., Pisano, P., Lastra, A., Decaro, N., & Guercio, A. (2019). Molecular characterization and evolutionary analyses of carnivore protoparvovirus 1 NS1 gene. *Viruses*, *11*(4), 308. https://doi.org/10.3390/v11040308

Mochizuki, M., Horiuchi, M., Hiragi, H., San Gabriel, M. C., Yasuda, N., & Uno, T. (1996). Isolation of canine parvovirus from a cat manifesting clinical signs of feline panleukopenia. *Journal of Clinical Microbiology*, *34*(9), 2101–2105. https://doi.org/10.1128/JCM.34.9.2101-2105.1996

Mochizuki, M., San Gabriel, M. C., Nakatani, H., Yoshida, M., & Harasawa, R. (1993). Comparison of polymerase chain reaction with virus isolation and haemagglutination assays for the detection of canine parvoviruses in faecal specimens. *Research in Veterinary Science*, *50*, 60–63. https://doi.org/10.1016/0034-5288(93)90035-E

Mora, M., Napolitano, C., Ortega, R., Poulin, E., & Pizarro-Lucero, J. (2015). Feline immunodeficiency virus and feline leukemia virus infection in free-ranging guignas (*Leopardus guigna*) and sympatric domestic cats in human perturbed landscapes on Chiloé Island, Chila. *Journal of Wildlife Diseases*, *51*(1), 199–208.

Nakamura, M., Tohya, Y., Miyazawa, T., Mochizuki, M., Phung, H. T. T., Nguyen, N. H., Huynh, L. M. T., Nguyen, L. T., Nguyen, P. N., Nguyen, P. V., Nguyen, N. P. T., & Akashi, H. (2004). A novel antigenic variant of canine parvovirus from a Vietnamese dog. *Archives of Virology*, *149*(11), 2261–2269. https://doi.org/10.1007/s00705-004-0367-y

Napolitano, C., Díaz, D., Sanderson, J., Johnson, W. E., Ritland, K., Ritland, C. E., & Poulin, E. (2015). Reduced Genetic Diversity and Increased Dispersal in Guigna (*Leopardus guigna*) in Chilean Fragmented Landscapes. *Journal of Heredity*, *106*(S1), 522–536.

Napolitano, C., Gálvez, N., Bennett, M., Acosta-Jamett, G., & Sanderson, J. (2015). *Leopardus guigna*. The IUCN Red List of Threatened Species 2015. e.T15311A50657245. https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T15311A50657245.en

Napolitano, C., Johnson, W. E., Sanderson, J., O'Brien, S. J., Rus Hoelzel, A., Freer, R., Dunstone, N., Ritland, K., Ritland, C. E., & Poulin, E. (2014). Phylogeography and population history of *Leopardus guigna*, the smallest American felid. *Conservation. Genetics*, *15*(3), 631–653. https://doi.org/10.1007/s10592-014-0566-3

Ortega, R., Mena, J., Grecco, S., Pérez, R., Panzera, Y., Napolitano, C., Zegpi, N., Sandoval, A., Sandoval, D., González-Acuña, D., Cofré, S., Neira, V., & Castillo-Aliaga, C. (2020). Domestic dog origin of Carnivore Protoparvovirus 1 infection in a rescued free-ranging guiña (*Leopardus guigna*) in Chile. *Transboundary and Emerging Diseases*, *00*, 1–7.

Parrish, C. R., Aquadro, C. F., Strassheim, M. L., Evermann, J. F., Sgro, J. Y., & Mohammed, H. O. (1991). Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. *Journal of Virology*, *65*(12), 6544–6552. https://doi.org/10.1128/JVI.65.12.6544-6552.1991

Parrish, C. R., Have, P., Foreyt, W. J., Evermann, J. F., Senda, M., & Carmichael, L. E. (1988). The global spread and replacement of canine parvovirus strains. *Journal of General Virology*, *69*(5), 1111–1116. https://doi.org/10.1099/0022-1317-69-5-1111

Parrish, C. R., Holmes, E. C., Morens, D. M., Park, E.-C., Burke, D. S., Calisher, C. H., Laughlin, C. A., Saif, L. J., & Daszak, P. (2008). Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiology and Molecular Biology Reviews*, *72*(3), 457–470. https://doi.org/10.1128/MMBR.00004-08

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Parrish, C. R., & Kawaoka, Y. (2005). The origins of new pandemic viruses: The acquisition of new host ranges by canine parvovirus and influenza A viruses. *Annual Review of Microbiology*, *59*, 553–586. https://doi.org/10.1146/annurev.micro.59.030804.121059

Parrish, C. R., O'Connell, P. H., Evermann, J. F., & Carmichael, L. E. (1985). Natural variation of canine parvovirus. *Science*, *230*(4729), 1046–1048.

Pénzes, J. J., Söderlund-Venermo, M., Canuti, M., Eis-Hübinger, A. M., Hughes, J., Cotmore, S. F., & Harrach, B. (2020). Reorganizing the family Parvoviridae: A revised taxonomy independent of the canonical approach based on host association. *Archives of Virology*, *165*(9), 2133–2146. https://doi.org/10.1007/s00705-020-04632-4

Pérez, R., Francia, L., Romero, V., Maya, L., López, I., & Hernández, M. (2007). First detection of canine parvovirus type 2c in South America. *Veterinary Microbiology*, *124*(1–2), 147–152. https://doi.org/10.1016/j.vetmic.2007.04.028

Pfeiffer, D., Robinson, T. P., Stevenson, M., Stevens, K. B., Rogers, D. J., & Clements, A. C. (2008). *Spatial analysis in epidemiology* (pp. 142). Oxford University Press.

Posada, D., & Buckley, T. R. (2004). Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, *53*(5), 793–808. https://doi.org/10.1080/10635150490522304

R Development Core Team 3.0.1. (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Riley, S. P., Foley, J., & Chomel, B. (2004). Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. *Journal of Wildlife Diseases*, *40*(1), 11–22. https://doi.org/10.7589/0090-3558-40.1.11

Roelke-Parker, M. E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., O'Brien, S. J., Pospischil, A., Hofmann-Lehmann, R., Lutz, H., Mwamengele, G. L. M., Mgasa, M. N., Machange, G. A., Summers, B. A., & Appel, M. J. G. (1996). A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature*, *379*(6564), 441. https://doi.org/10.1038/379441a0

Rubio, A., Fredes, F., & Bonacic, C. (2013). Serological and parasitological survey of free-ranging culpeo foxes (*Lycalopex culpaeus*) in the Mediterranean biodiversity hotspot of central Chile. *Journal of Animal and Veterinary Advances*, *12*, 1445–1449.

Sacristán, I., Acuña, F., Aguilar, E., García, S., López, M. J., Cevidanes, A., Cabello, J., Hidalgo-Hermoso, E., Johnson, W. E., Poulin, E., Millán, J., & Napolitano, C. (2019). Assessing cross-species transmission of hemoplasmas at the wild-domestic felid interface in Chile using genetic and landscape variables analysis. *Scientific Reports*, *9*, 16816.

Sanderson, J., Sunquist, M. E., & Iriarte, A. W.(2002). Natural history and landscape-use of guignas (*Oncifelis guigna*) on Isla Grande de Chiloé, Chila. *Journal of Mammalogy*, *83*(2), 608–613. https://doi.org/10.1644/1545-1542(2002)083<0608:NHALUO>2.0.CO;2

Santos, N., Almendra, C., & Tavares, L. (2009). Serologic survey for canine distemper virus and canine parvovirus in free-ranging wild carnivores from Portugal. *Journal of Wildlife Diseases*, *45*(1), 221–226. https://doi.org/10.7589/0090-3558-45.1.221

Schulz, J. J., Cayuela, L., Echeverria, C., Salas, J., & Benayas, J. M. R. (2010). Monitoring land cover change of the dryland forest landscape of Central Chile (1975–2008). *Applied Geography*, *30*(3), 436–447. https://doi.org/10.1016/j.apgeog.2009.12.003

Schüttler, E., Klenke, R., Galuppo, S., Castro, R. A., Bonacic, C., Laker, J., & Henle, K. (2017). Habitat use and sensitivity to fragmentation in America's smallest wildcat. *Mammalian Biology*, *86*, 1–8. https://doi.org/10.1016/j.mambio.2016.11.013

Shackelton, L. A., Parrish, C. R., Truyen, U., & Holmes, E. C. (2005). High rate of viral evolution associated with the emergence of carnivore parvovirus. *Proceedings of the National Academy of Sciences*, *102*(2), 379–384. https://doi.org/10.1073/pnas.0406765102

Sillero-Zubiri, C., King, A. A., & Macdonald, D. W. (1996). Rabies and mortality in Ethiopian wolves (*Canis simensis*). *Journal of Wildlife Diseases*, *32*(1), 80–86. https://doi.org/10.7589/0090-3558-32.1.80

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, *57*(5), 758–771. https://doi.org/10.1080/10635150802429642

Steinel, A., Munson, L., van Vuuren, M., & Truyen, U. (2000). Genetic characterization of feline parvovirus sequences from various carnivores. *The Journal of General Virology*, *81*(Pt 2), 345–350. https://doi.org/10.1099/0022-1317-81-2-345

Steinel, A., Parrish, C. R., Bloom, M. E., & Truyen, U. (2001). Parvovirus Infections in Wild Carnivores. *Journal of Wildlife Diseases*, *37*(3), 594–607. https://doi.org/10.7589/0090-3558-37.3.594

Streck, A. F., Rüster, D., Truyen, U., & Homeier, T. (2013). An updated TaqMan real-time PCR for canine and feline parvoviruses. *Journal of Virological Methods*, *193*(1), 6–8. https://doi.org/10.1016/j.jviromet.2013.04.025

Teare, J. A. (2002). *Reference ranges for physiological values in captive wildlife*. International Species Information System.

Thorne, E. T., & Williams, E. S. (1988). Disease and endangered species: The black-footed ferret as a recent example. *Conservation Biology*, *2*(1), 66–74. https://doi.org/10.1111/j.1523-1739.1988.tb00336.x

Touihri, L., Bouzid, I., Daoud, R., Desario, C., El Goulli, A. F., Decaro, N., Ghorbel, A., Buonavoglia, C., & Bahloul, C. (2009). Molecular characterization of canine parvovirus-2 variants circulating in Tunisia. *Virus Genes*, *38*(2), 249–258. https://doi.org/10.1007/s11262-008-0314-1

Truyen, U., Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Hosie, M. J., Lloret, A., Lutz, H., Marsilio, F., Pennisi, M. G., Radford, A. D., Thiry, E., & Horzinek, M. C. (2009). Feline panleukopenia. ABCD guidelines on prevention and management. *Journal of Feline Medicine & Surgery*, *11*(7), 538–546. https://doi.org/10.1016/j.jfms.2009.05.002

Truyen, U. W. E., Evermann, J. F., Vieler, E., & Parrish, C. R. (1996). Evolution of canine parvovirus involved loss and gain of feline host range. *Virology*, *189*(0021), 186–189.

Villatoro, F. J., Sepúlveda, M. A., Stowhas, P., & Silva-Rodríguez, E. A. (2016). Urban dogs in rural areas: Human-mediated movement defines dog populations in southern Chile. *Preventive Veterinary Medicine*, *135*, 59–66. https://doi.org/10.1016/j.prevetmed.2016.11.004

Wilson, K., Newton, A., Echeverria, C., Weston, C., & Burgman, M. (2005). A vulnerability analysis of the temperate forests of south-central Chile. *Biological Conservation*, *122*(1), 9–21. https://doi.org/10.1016/j.biocon.2004.06.015

Zar, J. H. (1999). *Biostatistical analysis* (4 th ed.). Prentice Hall.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Sacristán I, Esperón F, Pérez R, et al. Epidemiology and molecular characterization of *Carnivore protoparvovirus-1* infection in the wild felid *Leopardus guigna* in Chile. *Transbound Emerg Dis*. 2021;68:3335–3348. https://doi.org/10.1111/tbed.13937

18651682, 2021, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/tbed.13937 by Institution Nac De Investig Tec Agra, Wiley Online Library on [02/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License