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FULL PAPER

Parasitology

Molecular and serological survey of carnivore pathogens in free-roaming domestic cats of rural communities in southern Chile

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ABSTRACT. Owned, free-roaming domestic cats are abundant in the Chilean countryside, having high probability of contact with wildlife and potentially participating as reservoirs of zoonotic pathogens. In the present study, 131 cats from two remote study areas (Valdivia and Chiloe Island) in southern Chile were analyzed for infection/exposure to eight pathogens. Serum samples from 112 cats were tested for antigens against feline leukemia virus (FeLV antigen-ELISA) and antibodies against feline immunodeficiency virus (FIV-ELISA) and canine distemper virus (CDV-serum neutralization), yielded occurrence of 8.9, 1.7 and 0.8% respectively. The presence of DNA of five vector-borne pathogens, piroplasmids, *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp. and *Bartonella* spp. was investigated in thirty cats. Overall observed occurrence was 6.6% (2/30) for both *Anaplasma platys*, and *B. henselae*, and 3.3% (1/30) for both *Bartonella* sp. and *Theileria equi*. Observed occurrence for all vector-borne pathogens in Valdivia area was significantly higher than in Chiloe Island (5/15 vs 0/15; P=0.04). Our results represent the first description of exposure to CDV and DNA detection of *T. equi* and *A. platys* in domestic cats in Chile. The results highlight the importance of performing pathogen screening in owned, free-roaming rural cats to evaluate their potential role as reservoirs of infection and vectors for disease transmission to wildlife.

KEY WORDS: feline and canine virus, PCR, rural free-roaming domestic cat, serology, vector-borne pathogen

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The domestic cat (*Felis silvestris catus*) is one of the most widely distributed terrestrial mammals [7, 23] due to their association with humans, and it has been listed among the 100 worst non-native invasive species in the world [36]. Owned, free-roaming domestic cats are abundant in rural Chile, where they are usually allowed to wander, sometimes around natural preserved areas. Their ability to roam increases their probability of interacting with other domestic animals and wildlife species, and their chances

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of contact with a range of pathogens. These owned free-roaming cats are rarely subjected to any prophylactic programs or receive any type of veterinary care, further increasing their probability of pathogen infection [44, 64]. This makes them a potential reservoir of pathogens of relevance for wildlife [21, 40, 42, 45].

Examples of pathogen spillover from a domestic species to a wild counterpart have been described elsewhere [14, 25, 45, 48, 57, 73]. Canine distemper virus (CDV) has caused several fatal epidemics in wild canids such as African wild dogs (*Lycaon pictus*) [73] and wild felids such as Serengeti lions (*Panthera leo*) [15, 57]; and more recently domestic dogs have been identified as the origin of CDV infection in Iberian wolves (*Canis lupus signatus*) [47] and jackals (*Canis mesomelas*) [25]. Likewise, possible cross-species transmission of feline leukemia virus (FeLV) from domestic cats has been recorded in guigna (*Leopardus guigna*) [45] and bobcats (*Felis rufus*) [65] and FeLV outbreaks have been reported in Florida panthers (*Puma concolor coryi*) [14] and Iberian lynxes (*Lynx pardinus*) causing high mortalities [40, 45]. Regarding feline immunodeficiency virus (FIV), it is considered endemic in some wild felid populations such as African lions (*Panthera leo*) and many of the South American felids such as pumas (*Puma concolor*), jaguars (*Panthera once*), ocelots (*Leopardus pardalis*), margays (*Leopardus wiedii*), Geoffroy's cat (*Leopardus geoffroyi*), and oncilla (*Leopardus tigrinus*) [69]. However, high FIV prevalence have been described in some species of Asian wild felids like Palla's cat (*Otocolobus manul*) or leopard cat (*Prionailurus bengalensis*) [75] and possible spillover from domestic cats to wild felids has been described in guignas from Chile [45] and the Tsushima cat (*Felis bengalensis euptilura*) from Japan [50]. Epidemics associated with vector-borne agents such as piroplasmids have also been recorded in wildlife. It was proposed that the death of one third of the Serengueti lion population was due to the co-occurrence of *Babesia* and CDV infection [49]. Piroplasmids are considered the second most commonly found parasites in the blood of mammals after trypanosomes [61], making them important vector-borne agents in those species [78]. In addition to conservation implications, owned, free-roaming domestic cats could be a reservoir of zoonotic agents due to their poor health care and free-ranging behavior. *Bartonella henselae* is currently the most commonly encountered *Bartonella* zoonosis [27]. Asymptomatic infection with *Bartonella* spp. is frequently reported in cats, which are therefore considered to be a major reservoir for human infection. Cats can also be infected by members of Anaplasmataceae, which are rickettsial organisms that infect human and animal leukocytes [19].

However, despite the possibility of pathogen spillover from domestic cats to wildlife, and their zoonotic implications, data on pathogens infecting rural, owned, free-roaming domestic cats is still scarce [23, 44, 45, 55, 62]. Previous serological and molecular surveys in Chile have reported the presence of different pathogens, although these studies were mainly focused on owned domestic cats from urban areas [6, 20, 30, 48, 71, 79].

Therefore, our goal was to determine the occurrence of important carnivore pathogens in owned, free-roaming domestic cats from remote isolate rural communities in southern Chile, assessing the exposure and/or infection with bacteria and viruses with different transmission modes. The free roaming behavior of these cats in rural areas increases the possibilities of contact with wildlife. Thus, the results of this study evaluate how owned, free-roaming domestic cats could act as reservoir of pathogens to wildlife.

MATERIALS AND METHODS

Sampling

During 2015 and 2016 a cross-sectional study was conducted. A total of 131 owned, free-roaming cats were sampled in six rural communities adjacent to protected areas located in two different regions of southern Chile: four in the Valdivian coastal area (Los Ríos region, 39° S 73° W) and two on Chiloe Island (Los Lagos region, 43° S 73° W) (Fig. 1). These two regions were chosen since they contain remote and isolated rural communities, where prophylactic management and health care of domestic cats and dogs (*Canis familiaris*) is scarce or nonexistent [64].

Domestic cats were selected based on their non-vaccinated and non-neutered status as well as their owned, free-roaming behavior. All of them were mix-breed, domestic short-haired cats. EDTA-anticoagulated blood and serum samples collected through manual restraint, by cephalic or jugular venipuncture were preserved at −20°C until further processing. The collection of samples was performed with the informed consent of the owners and under considerations of animal welfare and ethical aspects under the approval of Animal Ethics committee of the Institute of Ecology and Biodiversity in Universidad de Chile, resolution of 20 November 2015.

Serum analysis

Serum samples from 112 cats (37 from the Valdivia area, 75 from Chiloe Island) were tested for FeLV, FIV and CDV. Serum samples from the remaining 19 cats were not available. Progressive FeLV infection was determined by detection of the p27 antigen using a commercial ELISA kit (sensitivity 94% and specificity 100%) (INgezim FeLV DAS; Ingenasa, Madrid, Spain), and antibodies against FIV were detected using a commercial enzyme-linked immunosorbent assay (ELISA) kit INgezim FIV (sensitivity 99% and specificity 100%) (Ingenasa), following the manufacturer's instructions. Due to the low volume of serum available, samples were pre-screened for CDV antibodies using an immunofluorescence assay (IFA). In brief, Vero cells (CCL-81) were infected with the Onderstepoort strain of CDV with a multiplicity of infection (MOI) of 0.1 or were mock-infected with Dulbecco's modified Eagle medium (DMEM) to generate a negative control. After one hr of infection at 37°C the inoculum was removed, cells were washed once with phosphate-buffered saline (PBS) and DMEM supplemented with 2% (v/v). Heat-inactivated fetal bovine serum (FBS) was added for further cultivation at 37°C and 5% CO_2 for 48 hr. Cells were then washed once with PBS, fixed with 80% (v/v) acetone at −20°C for 10 min and rinsed twice with PBS. To prevent non-specific binding, a blocking

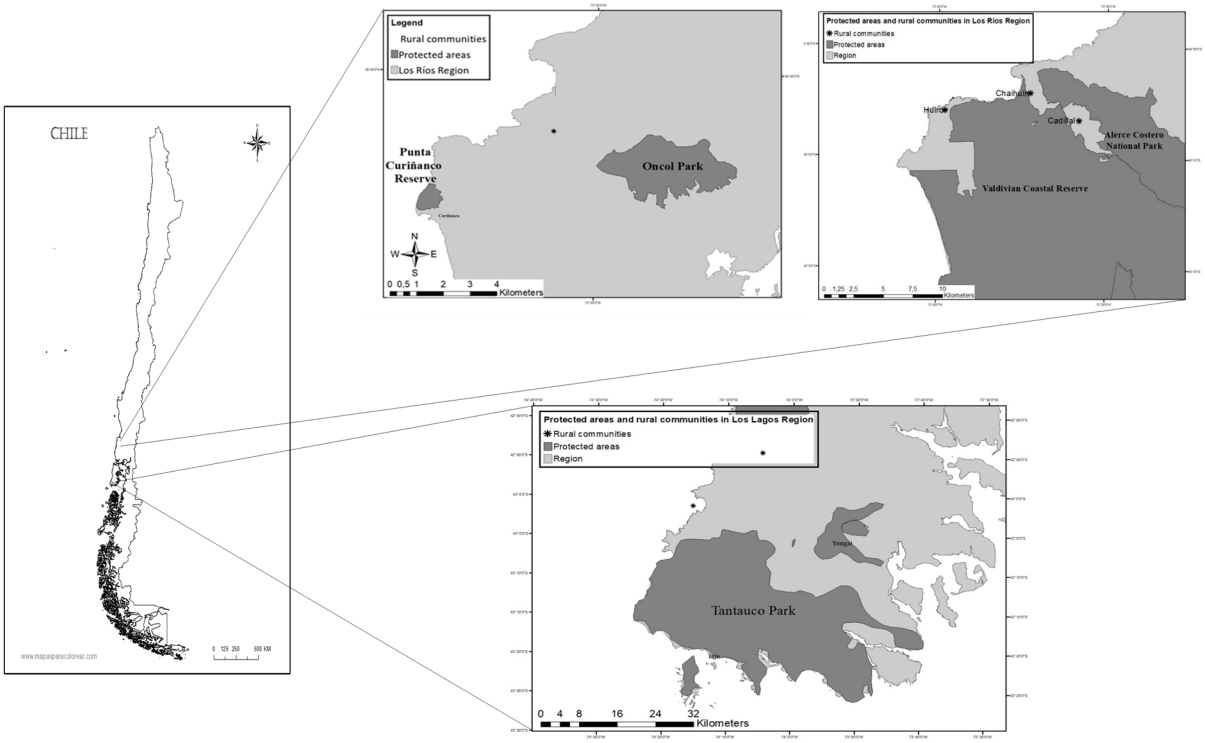

Fig. 1. Study area. Rural communities near protected areas of Valdivia, Los Ríos region, and Chiloe Island, Los Lagos region, in southern Chile.

step using 5% (w/v) bovine serum albumin (BSA) in PBS for 30 min at 37°C was performed. Serum samples diluted 1:100 in 1% (w/v) BSA in PBS were added and incubated for two hr at 37°C. Cells were then washed two times with PBS and CDV-specific antibodies were visualized by adding a fluorochrome-labeled secondary antibody (anti cat-IgG-Alexafluor488; Jackson ImmunoResearch Laboratories, West Grove, PA, U.S.A.), diluted 1:500 in 1% BSA in PBS for one hr at 37°C. Finally, cells were washed twice with PBS and analyzed using a fluorescence microscope (IX70, Olympus, Hamburg, Germany) [26]. Titers higher than 1:16 were considered positive.

IFA test shows high sensitivity (97%) but low specificity (70%) in comparison to an ELISA test [26]. Consequently, IFA positive serum samples were further investigated using a serum neutralization test (SNT) with high specificity [52], an accepted gold standard for the diagnosis of CDV-antibodies as described by [34], thereby avoiding the possible cross-reaction between CDV antibodies and other morbilliviruses.

DNA extraction and PCR amplification

The DNA of 30 cats (15 from the Valdivia area, 15 from Chiloe Island) was extracted from 0.3 m*l* peripheral whole blood samples as described by [22]. The eukaryotic 18S RNA Pre-Developed TaqMan Assay Reagents (Applied Biosystems, Foster City, CA, U.S.A.) were used as internal reference for cat genomic DNA amplification, to ensure the quality of each sample for PCR amplification and that negative results corresponded to true negative samples rather than to a problem with DNA loading, sample degradation or PCR inhibition. Real-time PCR (qPCR) targeting *Ehrlichia/Anaplasma* spp., piroplasmids, *Rickettsia* spp. and *Bartonella* spp. was performed as previously described [13, 38]. The thermal cycling profile was 50°C 2 min and 95°C 10 min followed by 40 cycles at 95°C 15 sec and 60°C 1 min, using a QuantStudioTM 12K Flex Real-Time PCR System (Life Technologies, Carlsbad, CA, U.S.A.). Sterile water was used as a negative PCR control and positive controls were obtained from commercial slides coated with cells infected with the pathogens (MegaScreen® 118 FLUOEHRLICHIA c., MegaScreen® FLUOBABESIA canis, MegaScreen® FLUORICKETTSIA 119 ri., MegaScreen® BARTONELLA h. (Megacor, Hörbranz, Vorarlberg, Austria). Target genes amplified for each pathogen, primers used and sequencing of each positive qPCR product was conducted as previously described [53] (Table 1). Positive samples were sequenced with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (AB, Life Technologies). The sequences obtained were compared with sequences from the GenBank database (www.ncbi.nlm.nih.gov/BLAST). Ultrapure water was employed as negative control in each PCR run and sequenced positive cat samples were used as positive controls.

Statistical analyses

The R program [68] was used to perform statistical analyses. Fisher's exact test was performed, and statistical significance was set at $P<0.05$ to compare pathogen occurrence between areas, ages and sexes.

Table 1. Primers used in pathogen detection, target genes amplified and size of the amplified product for each pathogen using real time PCR

Pathogen	Region amplified	Primer Forward (5′-3′)	Primer Reverse (5′-3′)	Final [primer] (μM)	PCR product (bp)
Ehrlichia/ Anaplasma spp.	16S rRNA	GCAAGCYTAACACATGCAAGTCG	CTACTAGGTAGATTCCTAYGCATTACTCACC	0.5	102[a)]
Piroplasmid	18S rRNA	GACGATCAGATACCGTCGTAGTCC	CAGAACCCAAAGACTTTGATTTCTCTC	0.3	114[a)]
Rickettsia spp.	ITS1	GCTCGATTGRTTTACTTTGCTGTGAG	CATGCTATAACCACCAAGCTAGCAATAC	0.5/0.3	300[a)]
Bartonella spp.	ITS1	AGATGATGATCCCAAGCCTTCTG	CCTCCGACCTCACGCTTATCA	0.3	180[a)]

a) Targeted size could vary depending on the species.

RESULTS

Of the total 131 sampled cats, 58 were males and 73 were females; 115 were adults and 16 were juveniles. All animals were mix-bred, short-haired cats. Forty-six cats belonged to rural communities in the Valdivia area and 85 cats to rural communities in Chiloe Island. All cats were evaluated by a veterinarian: 116 cats were clinically healthy, and 15 cats presented different clinical signs of illness (gingivitis, conjunctivitis, paraplegia, ascites or ocular secretion) (Table 2).

Serum analysis

Of the 112 cat serum samples tested, 10 individuals (8.9%, 95% CI=3.5–14.29) were FeLV-positive; two individuals (1.7% 95% CI=0.07–4.2) were FIV-positive and one individual was CDV-serum neutralization positive, as a reciprocal titer of 1:160 (0.8%, 95% CI=0.08–2.6) (Table 3). No coinfections or significant differences between ages, sex groups or areas were found related to positive status.

Table 2. PCR results with correlated serological results and clinical signs information

Sample	Clinical signs	Location	Sex	Age	FIV-ELISA	FeLV-ELISA	CDV-IFT/ SNT	Piro-plasmid	*H. felis*	*Bartonella* spp.	*Ehrlichia/ Anaplasma* spp.	*Rickettsia* spp.
16046	Asymptomatic	Chiloe Island	Female	Adult	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
15221	Asymptomatic	Valdivia	Female	Adult	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
15225	Asymptomatic	Valdivia	Female	Adult	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
15226	Asymptomatic	Valdivia	Female	Adult	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
15007	Asymptomatic	Chiloe Island	Male	Adult	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
15231	Asymptomatic	Valdivia	Male	Adult	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
15240	**Fever**	Valdivia	Male	Adult	Negative	Negative	Negative	Negative	Negative	***Bartonella* sp.**	***Anaplasma platys***	Negative
16011	**Paraplegia**	Chiloe Island	Female	Adult	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative
15004	**Gingivitis**	Chiloe Island	Female	Adult	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative
15002	Asymptomatic	Chiloe Island	Female	Adult	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
16013	Asymptomatic	Chiloe Island	Female	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
16014	Asymptomatic	Chiloe Island	Female	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15029	Asymptomatic	Chiloe Island	Female	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15191	Asymptomatic	Valdivia	Female	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15207	Asymptomatic	Valdivia	Female	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15212	Asymptomatic	Valdivia	Female	Juvenile	NE	NE	NE	Negative	Negative	***B. henselae***	Negative	Negative
15223	Asymptomatic	Valdivia	Female	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15227	Asymptomatic	Valdivia	Female	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
16031	Asymptomatic	Chiloe Island	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
16037	Asymptomatic	Chiloe Island	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15014	Asymptomatic	Chiloe Island	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15022	Asymptomatic	Chiloe Island	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15030	Asymptomatic	Chiloe Island	Male	Juvenile	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15034	**Respiratory symptoms**	Chiloe Island	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15038	Asymptomatic	Chiloe Island	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15184	Asymptomatic	Valdivia	Male	Adult	NE	NE	NE	Negative	Negative	Negative	***Anaplasma platys***	Negative
15195	Ocular secretion	Valdivia	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15201	Asymptomatic	Valdivia	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative
15203	Asymptomatic	Valdivia	Male	Adult	NE	NE	NE	Negative	Negative	Negative	Negative	Negative

NE, not explored; FIV, feline immunodeficiency virus; FeLV, feline leukemia virus; CDV, canine distemper virus.

Table 3. Molecular and serological screening results of pathogens in owned, free-roaming domestic cats. Pathogen occurrence by study area and sex

	Serology								
	ELISA						IFA/SNT		
	FeLV			FIV			CDV		
	n/total	%	95%CI	n	%	95%CI	n/total	%	95%CI
Area									
Valdivia	1/37	2.7	2.7–8.1	0/37	0.0	0.0	1/37	2.7	1.6–2.7
Chiloe	9/75	12.0	3.7–32.7	2/75	2.6	1.0–6.3	0/75	0.0	0.0
Total	10/112	8.9	3.5–14.2	2/112	1.7	0.7–4.2	1/112	0.8	0.08–2.6
Sex									
Male	4/47	8.5	0.2–16.7	0/47	0.0	0.0	1/47	2.1	2.1–6.4
Female	6/65	9.23	2.0–16.4	2/65	3.0	1.2–7.3	0/65	0.0	0.0

	Molecular analyses											
	Piroplasmida			*Bartonella* spp.			*Ehrlichia/Anaplasma*			*Rickettsia* spp.		
	n/total	%	95%CI	n/total	%	95%CI	n/total	%	95%CI	n/total	%	95%CI
Area												
Valdivia	1/15	6.6	7.6–20.9	3/15	20.0	2.9–42.9	2/15	13.3	6.1–32.82	0/15	0.0	0.0
Chiloe	0/15	0.0	0.0	0/15	0.0	0.0	0/15	0.0	0.0	0/15	0.0	0.0
Total	1/30	3.3	3.4–10.15	3/30	10.0	1.3–21.39	2/30	6.6	2.8–16.14	0/30	0.0	0.0
Sex												
Male	0/14	0.0	0.0	1/14	7.1	8.2–22.5	2/14	14.2	6.6–35.2	0.0	0.0	0.0
Female	1/16	6.2	7.7–19.5	2/16	12.5	5.7–30.7	0/16	0.0	0.0	0.0	0.0	0.0

IFA, immunofluorescence assay; SNT, serum neutralization test; C.I.,Confidence intervals (lower-upper); FIV, feline immunodeficiency virus; FeLV, feline leukemia virus; CDV, canine distemper virus.

Molecular analysis

DNA of the following vector-borne pathogens was amplified in five individuals: (1) piroplasmids (n=1), (2) *Ehrlichia/Anaplasma* sp. (n=2), (3) *Bartonella* sp. (n=3). No *Rickettsia* sp. DNA was detected. These pathogens were identified through sequencing as: (1) *Theileria equi* (1/30, 3.3%, 95% CI=0.3–10.15), 100% identity to GenBank *T. equi* sequences in equids worldwide; (2) *Anaplasma platys* (2/30, 6.6%, 95% CI=2.8–16.14), 100% identity to GenBank *A. platys* sequences in dogs worldwide; (3) *Bartonella* sp. (1/30, 3.3%, 95% CI=3.4–10.15), 100% identity to GenBank *Bartonella* spp. sequences detected in fleas worldwide, and *Bartonella henselae* (2/30, 6.6%, 95% CI=2.8–16.14), 100% identity to GenBank *Bartonella henselae* sequences in lions from Namibia, and cats from Malaysia, Guatemala and Brazil. The new *T. equi*, *A. platys*, *Bartonella* sp. and *B. henselae* nucleotide sequences were submitted to the GenBank database under accession numbers MK774808, MK791257, MK791259 and MK791258 respectively. Four of the five vector-borne infected individuals were healthy and carried only one pathogen, whereas one cat was pyrexic at the time of sampling and coinfected with *B. henselae* and *A. platys* (Tables 2 and 3).

All PCR-positive individuals were from the Valdivia area. Overall vector-borne occurrence in Valdivia (5/15, 33.3%, 95% CI=6.3–60.3) was higher than on Chiloe Island (0/15, 0.0%, 95% CI=0–0) (P=0.04). Age and sex were not significantly related to the probability of infection from any of the vector-borne pathogens evaluated in this study.

DISCUSSION

FeLV and FIV infection in cats lead to immunosuppressive wasting syndromes. Both viruses are among the most common infectious diseases affecting domestic cats, and have a worldwide distribution [12, 31, 37, 56, 75]. Worldwide prevalences of FeLV and FIV are estimated between 2–18 and 1.2–43%, respectively [12, 31, 37, 56, 75], varying widely depending on lifestyle, gender, health condition and other variables [31, 37, 77]. In the current study, FeLV and FIV seroprevalences were relatively low compared to other studies conducted in large cities in Chile (FeLV=13.5% (Valdivia city), 23% (Concepción city); FIV=10.3% (Valdivia city), 4% (Concepción city)) [6, 71, 72] and South America [29, 66, 69, 70]. These differences could be attributed to the rural origin of the cats from this study, where cat abundance and aggregation is lower than in urban areas.

Canine distemper virus is one of the most important infectious disease of carnivores worldwide and has the second highest fatality rate of any infectious disease after rabies in domestic dogs [17]. Evidence of CDV infection has been reported in several families of terrestrial carnivores, including Canidae, Felidae, Hyaenidae, Mustelidae, Mephitidae, Procyonidae, Ursidae, Ailuridae and Viverridae [8]. In the last decades, CDV has occurred as large-scale epidemics in felids [5, 57]. Both *in vivo* and *in vitro* studies have demonstrated that domestic cats can be efficiently infected by CDV [39]. However, few reports of CDV in domestic cats have been described worldwide [39], with seroprevalences ranging between 4.5 and 23% [32, 43]. To the best of our

knowledge, this is the first report of CDV exposure in a domestic cat in Chile.

Exposure to the vector-borne pathogens *Bartonella* spp. and *R. felis* has been evaluated in cats in large cities in Chile. Seroreactivity for *Bartonella* spp. was 86% in cats sampled in three large cities (Coquimbo, Santiago and Valdivia) [20], and 71% in Valdivia city [79]; while for *R. felis*, it was 72.7% in cats from Santiago city [30]. Only a few studies in South America have evaluated the molecular occurrence of *Bartonella* sp. in cats, mostly in Brazil [10, 16, 41, 67]. In Chile, *Bartonella* sp. (18.1%) was molecularly detected in cats from the city of Valdivia [48]. Occurrence of *Bartonella* sp. in the present study was lower than that reported in Valdivia city [48]. Different sample size, type of sampled populations (i.e. demographics, urban *vs.* rural populations), or vector distribution may explain the difference in pathogen occurrence [48]. *B. henselae*, detected in this study, is one of the three species associated with cat-scratch disease and other syndromes in humans [9, 11]. In Chile, mandatory reporting of this disease is not required. Nevertheless, more than 200 human cases of bartonellosis were diagnosed between 1997 and 2000 in Chile [20], emphasizing the zoonotic importance of this agent in the country.

We detected an occurrence of 13.3% for *A. platys*, an obligate intracellular gram-negative bacteria of the family Anaplasmataceae, described mainly in dogs from various regions of the world such as Brazil [60], Venezuela [28] and Japan [46], causing infectious canine cyclic thrombocytopenia [18]. The tick Rhipicephalus sanguineus *sensu lato* is suspected to be involved as its principal vector [2]. The presence of *R. sanguineus* in Chile has been described from Arica in the north (18°29′01″S) to Valdivia in the south (39°49′11″S) [1, 24, 35]. Although the mode of transmission of *A. platys* in cats is unknown, *R. sanguineus* has been described parasitizing cats [63]. *Anaplasma*-like bodies have been detected in cat platelets and molecular detection of *A. platys* has been reported in cats from Brazil, Thailand and North America [33, 54, 58, 59], however the clinical implication of *A. platys* in cats is poorly understood. To our knowledge, this is the first description of *A. platys* in domestic cats in Chile.

With reference to the molecular detection of piroplasmids, DNA detection of *T. equi* was recorded in one individual. According to the World Organization for Animal Health, Central and South America are considered among the endemic regions of *Theileria equi* [76]. The disease is readily identified in all regions of South America with the exception of the southernmost areas of Chile and Argentina [76]. *T. equi* is transmitted to equids by ticks of the genera *Hyalomma* and *Rhipicephalus* [3]. A sequence from a free-roaming domestic cat caught within a Brazilian zoo showed 94% identity with *T. equi* [4]. However, reports of *T. equi* in domestic cats are rare. To our knowledge, our study constitutes the first report of *T. equi* in Chile.

The occurrence of vector-borne pathogens was higher in rural Valdivia, the northern part of the study area, compared to rural Chiloe Island, in the south [13], analyzed different vector-borne agents infecting Darwin's fox from Chiloe Island and found only one animal positive for *Rickettsia* sp. A wide range of factors (e.g., climatic differences, human and animal population dynamics) may affect the occurrence and spread of vector-borne pathogens [51]. The main factor that could explain the difference in vector borne occurrence between the two study areas is the distribution of *R. sanguineous* in Chile; Valdivia is the southern distribution limit of this tick, it is therefore absent from Chiloe Island [1, 24, 35]. Other drivers may lie behind the observed occurrence difference, such as different cat abundance or cat population migratory flows [74], which could be limited on Chiloe Island due to its intrinsic isolation, posing an obstacle for the arrival of pathogens. However, small sample size for vector-borne pathogen detection in this study suggests our results constitute only preliminary data, and therefore more analyses are needed.

The results of this study indicate that exposure to feline pathogens is lower when compared to previous urban domestic cat studies. However, due to their intrinsic behavior, rural domestic cats might transmit pathogens and parasites to sympatric threatened carnivores inhabiting nearby natural habitats. In addition, these cats can act as reservoirs of zoonotic agents. This study highlights the importance of performing pathogen screening in owned free-roaming rural cats in order to identify their pathogens. This should be the first step to establish its potential as reservoirs and the risk of disease transmission to wildlife, other domestic animals and humans, guiding the implementation of effective management and control measures in rural communities.

The present work represents a pilot study, being the first prospecting of these pathogens in owned-free, rural cats from isolate regions of Chile. This pilot study has limited capacity for sample analysis. However, the lines of evidence achieved on this first approach, will be expand in the future.

CONFLICT OF INTEREST. No potential conflict of interest was reported by the authors.

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