Veterinary Parasitology: Regional Studies and Reports 30 (2022) 100721

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original Article

Large-scale survey for canine vector-borne parasites in free-ranging dogs and foxes from six diverse bioclimatic regions of Chile

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ARTICLE INFO

Keywords:
Protozoan
Piroplasmida
Nematode
Risk factor
South America

ABSTRACT

Chile is a large country with a marked range of climate conditions that make it an ideal scenario for the study of vector-borne parasites (VBPs); however, knowledge about their distribution is limited to a few confined areas of this country. The presence of *Hepatozoon* spp., piroplasmids, *Leishmania* spp. and filarioids was investigated through molecular and serological methods in blood and serum samples of 764 free-ranging rural dogs, 154 Andean foxes (*Lycalopex culpaeus*), and 91 South American grey foxes (*Lycalopex griseus*) from six bioclimatic regions across Chile. *Hepatozoon* spp. DNA was exclusively detected in foxes (43% prevalence), including sequences closely related to *Hepatozoon felis* (24.1%; only Andean foxes), *Hepatozoon americanum* (16.2%; only grey foxes), and *Hepatozoon canis* (1.25%; in one grey fox). Risk factor assessment identified a higher probability of *Hepatozoon* infection in juvenile foxes. DNA of piroplasmids was detected in 0.7% of dogs (*Babesia vogeli*) but in no fox, whilst antibodies against *Babesia* sp. were detected in 24% of the dogs and 25% of the foxes, suggesting a wider circulation of canine piroplasmids than previously believed. A positive association between the presence of antibodies against *Babesia* and high *Rhipicephalus sanguineus* sensu lato burden was observed in dogs. *Leishmania* spp. DNA and antibodies were detected in 0.8% and 4.4% of the dogs, respectively. *Acanthocheilonema reconditum* was the only blood nematode detected (1.5% of the dogs and no fox). Differences in prevalence among bioregions were observed for some of the VBPs. These results expand our knowledge about the occurrence of vector-borne parasites in Chile, some of which are firstly reported herein. This information will facilitate the

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https://doi.org/10.1016/j.vprsr.2022.100721
Received 14 September 2021; Received in revised form 21 February 2022; Accepted 21 March 2022
Available online 24 March 2022

diagnosis of vector-borne diseases in domestic dogs and improve the control measures for both domestic and wild canids.

1. Introduction

Vector-borne parasites (VBPs) comprise a complex group of parasites that can harm animal health and welfare (Maggi and Krämer, 2019), and for which the knowledge in Chile about their distribution and prevalence in dogs and other hosts is scant. Moreover, the current scenario of climate change is expanding the distribution range and favoring the life cycle of some arthropod vectors capable of pathogen transmission (Semenza and Suk, 2018). In addition, landscape anthropization, as well as illegal wildlife trade (Bezerra-Santos et al., 2021), have increased the interactions between wildlife and domestic animals, favoring the circulation of parasites among these species (Daszak et al., 2000). VBPs are particularly suited for circulation within domestic and wild populations of carnivores because no direct contact between hosts is necessary for transmission (Otranto et al., 2019). Furthermore, it has been documented that free-ranging dogs can act as major reservoirs of ticks and their associated VBPs (Filipovic et al., 2018). For example, *Ixodes* ticks were described infesting both dogs and red foxes (*Vulpes vulpes*), being proposed as the link in the transmission of *Babesia vulpes* between these species (Camacho et al., 2003; Koneval et al., 2017). Recently, Cevidanes et al. (2021) showed that dogs and foxes do share some vectors of VBP such as *Rhipicephalus sanguineous* sensu stricto (s.s.) in Chile, although this was not a frequent situation.

In Chile, there is a growing number of free-ranging dogs, a phenomenon that intensifies in rural settlements (Villatoro et al., 2016). These dogs often lack of veterinary care and may pose a risk for the health of humans and domestic animals (Astorga et al., 2015; Otranto et al., 2017; Vanak and Gompper, 2009). Additionally, these rural dogs frequently invade natural habitats (Villatoro et al., 2019), resulting in possible interspecific pathogen transmission with native foxes (Acosta-Jamett et al., 2011; Di Cataldo et al., 2020a). Andean fox (*Lycalopex culpaeus*) and South American grey fox (*L. griseus*) are the two more widely distributed species throughout Chile, being present in all bioclimatic regions of the country, as are dogs.

Up to date, the knowledge about the presence of VBPs in Chilean canine populations is circumscribed to the description of *Trypanosoma cruzi* in dogs (Correa et al., 2020), and diverse nematode species in dogs and foxes (López et al., 2012; Oyarzún-Ruiz et al., 2020). A survey in Darwin's foxes from southern Chile failed to detect the most relevant VBPs, probably explained by the climatic conditions of the study area (Cabello et al., 2013). Finally, *Babesia vogeli* was recently reported in a village in the Steppe bioregion of the country (Di Cataldo et al., 2020b).

Chile is a large country (over 4000 km in longitude) that presents a range of climate conditions from desertic environments in the North, to temperate and ice fields in central and southern areas, respectively (INE, 2014), that makes it an ideal scenario for the study of VBPs distribution (Sutherst, 1993). This bioclimatic variability provides more or less appropriate conditions for the establishment and distribution of arthropods capable of parasite transmission (Sutherst, 1993). Indeed, it has been shown that the distribution of *R. sanguineus* sensu lato (s.l.) is restricted to suitable bioregions of Chile (Di Cataldo et al., 2021; Díaz et al., 2018).

The aim of this study was to assess, by molecular and serological methods, the presence, distribution, and risk factors of infection/exposure of four groups of pathogenic canine VBPs, including *Hepatozoon* sp., piroplasmids, *Leishmania* sp., and filarial worms in rural dogs and the two widespread species of fox present in Chile, the South American grey fox and the Andean fox, in Chile.

2. Materials and methods

2.1. Animal sampling

Free-ranging rural dogs ($n = 764$) and wild foxes ($n = 245$, including 154 Andean foxes and 91 South American grey foxes) were sampled from six different bioclimatic areas of Chile (CONAMA, 2008), namely Coastal Desert, Mountain Desert, Steppe, Mediterranean, Temperate warm rainy (TWR) and Temperate maritime rainy (TMR) (Fig. 1).

Rural dogs were sampled between 2015 and 2019 with the consent of their owners. Requisite to be included in the survey were to be free-ranging and had not traveled to other localities during the last years. Whole blood and serum were obtained by venipuncture of the cephalic vein and collected in EDTA and clean tubes, respectively. In addition, ectoparasites were collected during a 5-min examination (Marchiondo et al., 2013) and stored in 90% ethanol until species identification. Dogs were classified as juveniles (less than a year) or adults (older than a year), and individual data (i.e., sex, age, and sampling location) was recorded. Some dogs from the Steppe region were included in a previous brief communication (Di Cataldo et al., 2020b).

Foxes were sampled between 2006 and 2019 either by active or passive surveillance. Active capture of foxes was carried out with leghold traps (Oneida Victor Soft Catch No. 1.5, USA) baited with tuna or chicken, and anesthesia was performed following Chirife et al. (2020) and Acosta-Jamett et al. (2010) protocols. For passive surveillance, animals admitted to rehabilitation centers or road killed were included. Whole blood, spleen, and serum were obtained, upon availability. Ectoparasites in foxes were recovered when possible, and information regarding sex or age -juvenile/adult, based on teeth eruption (Iriarte and Jaksic, 2012) was recorded. The trapping and sampling were approved by the authorities in bioethics from Universidad Andres Bello under authorization 08/2016. Fox capture and sampling permits were granted by Servicio Agrícola y Ganadero (Resolutions no: 1355/2015, 1878–2016, 4469–2016, 3379–2017, 3380–2017, 8153–2017, 2655–2018).

2.2. Laboratory analyses

DNA was extracted from whole blood samples or spleen of 747 dogs and 234 foxes, using a DNeasy blood and tissue kit (Qiagen) according to the manufacturer's instructions. All samples were subjected to internal control for canine genomic DNA. The DNA presence of *Hepatozoon* sp., *Leishmania* spp., piroplasmids, and nematodes was screened using a series of conventional PCR and/or quantitative PCR protocols (Table 1). Positive PCR controls were obtained from previously sequenced blood samples (Table 1). Ultrapure water was used as a template negative PCR control. Two percent agarose gel electrophoresis was performed, and PCR products were visualized under a UV transilluminator. All positive samples obtained were sequenced by Macrogen, and the sequences were compared with those deposited in the GenBank® database (https://www.ncbi.nlm.nih.gov/genbank/).

Sera samples from 527 dogs and 20 foxes were analyzed for IgG antibodies using two immunofluorescence antibody tests (IFAT): for *Babesia canis* by Fuller Laboratories® (Fullerton, California, USA), and for *Leishmania infantum* by bioMérieux SA (Marcy L'Etoile, France) following the manufacturer instructions. The *B. canis* and the L. *infantum* IFA IgG Antibody Kits detect antibodies against *Babesia* species at a 1:50 dilution, and against *Leishmania* at a 1:40 dilution, respectively. A fluorescence microscope was used to interpret the results, and each sample was compared with the positive and negative control sera provided by the kits.

Ectoparasites were classified based on morphological criteria following taxonomic keys (Beaucournu and Gonzalez-Acuña, 2014; Nava et al., 2017; Supplementary Table 1) and confirmed by molecular methods whenever the head of the ectoparasite was absent (Table 1). Complete information regarding tick sampling and identification can be found in Di Cataldo et al. (2021).

2.3. Phylogenetic analysis

All sequence alignments were performed with ClustalW executed in Geneious Prime® 2020.1.2 (Biomatters Limited, 2020). Determination of nucleotide sequences types (ntST) and nucleotide polymorphism were performed using DnaSP.6 (Rozas et al., 2017). To infer genetic relationships, we constructed a median-joining network using the software PopART (Bandelt et al., 1999) using several sequences of *Hepatozoon* spp. from wild and domestic carnivores worldwide obtained from the GenBank® database. Genetic structure was estimated through the pairwise Phi_{st} test with 1000 permutations, performed in Arlequin v.3.5.2.2 (Excoffier and Lischer, 2010) and the nearest neighbor statistic S_{nn} (Hudson, 2000) using DnaSP.6. Phylogenetic relationships were assessed with a Maximum Likelihood method performed with 1000 replicates using MEGA 7.0.26 (Kumar et al., 2016). The best models Hasegawa-Kishino-Yano with Gamma distribution for *Hepatozoon* sp. were selected with jModelTest 2.1.6 (Darriba et al., 2012).

2.4. Statistical analysis

All statistical analyses were performed in R version 4.0.2 (R.Core. Team, 2017). Estimated prevalence and seroprevalence of the assessed pathogens in each species and bioregion, as well as mean abundance and mean intensity of ectoparasites were calculated using the "epiR" package (Stevenson et al., 2013).

Analyses of risk factors associated with the presence of *Hepatozoon* sp. DNA in foxes and antibodies against *Babesia* in dogs were calculated using GLM models. The presence of pathogens was binary coded and compared with intrinsic individual variables. For *Hepatozoon* sp., the variables assessed were age, sex, and fox species, whilst for *Babesia* sp., these were age, sex, and tick abundance (defined as: none = zero ticks, low = 1–10 ticks, and high = over 10 ticks). In both cases, the best model was selected using the "dredge" function from the "MuMIn" package (Barton, 2020). Differences in *Hepatozoon* and *Babesia* presence between bioregions in foxes and dogs respectively were established by nonparametric analyses using χ-square and Fisher's exact tests.

2.5. Data accessibility

All newly obtained sequences were submitted to GenBank® under the accession numbers (a.n.) MW633709-MW633713 (*Hepatozoon* sp.), MT747439 (*Amblyomma tigrinum*).

3. Results

3.1. Prevalence of DNA and antibodies

DNA of *Hepatozoon* sp. was detected in 96 foxes (observed prevalence = 42.7%; 95% Confidence Intervals (CI) = 36.4–49.2%; Table 2), of which 70 were Andean and 26 were grey foxes. All dogs were negative for *Hepatozoon* sp. DNA. Prevalence in foxes ranged from 0% in the Coastal Desert to 57.0% in the Mediterranean bioregion. The sequencing of 49 amplicons of 670 bp revealed the presence of five ntSTs presenting 97.3–100% identity with previous *Hepatozoon* sp. sequences deposited in GenBank® (Table 3, Fig. 2). Overall, *Hepatozoon* sp. sequence polymorphisms revealed a haplotype diversity (Hd) of 0.494 and a nucleotide diversity (π) of 0.0135 between fox species and between bioregions (Table 4). Phylogenetic analyses grouped all sequences obtained for each *Hepatozoon* species in three paraphyletic clades corresponding to *H. felis* (all in Andean foxes), *H. americanum* (all in grey foxes), and *H. canis* (in one grey fox, Fig. 2), respectively. Network analysis confirmed this classification (Fig. 3). In consequence, the prevalence of *H. americanum* and *H. canis* in grey fox was of 16.2% and 1.25%, respectively, and the prevalence of *H. felis*-like in Andean fox was 24.1%.

DNA of Piroplasmida (A.N. KY290977) was detected in four dogs

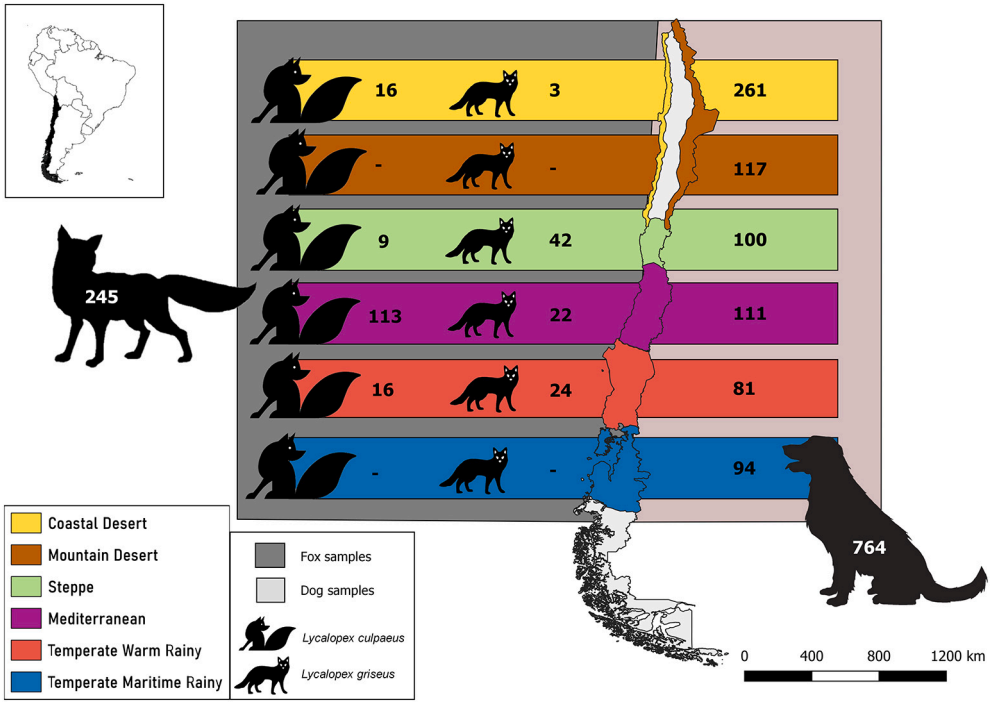

Fig. 1. Map of the study areas and total number of animals sampled in each bioclimatic region evaluated. Sample sizes for foxes and dogs are shown on the left and the right of the map, respectively.

(0.7%; 95% CI = 0.2–1.8; Table 5). No further cases were found apart from the four previously reported positive dogs from the Steppe region (Di Cataldo et al., 2020b), and all foxes were negative for Piroplasmida DNA. The IFAT revealed antibodies against *Babesia* in 128 dogs (24.3%). Three of the four DNA-positive dogs were positive for antibodies against *Babesia*. In addition, three grey and two Andean foxes were seropositive for *Babesia* (with an overall seroprevalence in foxes = 25.0%; Table 5).

DNA of *Leishmania* sp. was detected in five dogs (0.8%; 95% = 0.3–1.9%), all corresponding to the Mediterranean region (4.5%, 95% = 1.9–10.1%, Table 6). No readable sequences were obtained by cPCR products. All foxes tested negative for this parasite. The IFAT revealed antibodies against *Leishmania* in 23 dogs (4.4%, 95% C. I. = 2.9–6.5%) with a seroprevalence up to 9.7% in Coastal Desert. No foxes showed exposure to *Leishmania* (Table 6). None of the dogs that presented antibodies against *Leishmania* were DNA-positive. DNA of filarioids was detected in nine dogs, one from the Mediterranean region and eight from the Temperate Warm Rainy region, and in no foxes (Table 7). All the sequences obtained showed 100% nucleotide identity with *Acanthocheilonema reconditum* (A.N. JF461460). Mixed infections with more than one VBP were not confirmed in any dog or fox.

Table 2
Prevalence of DNA of *Hepatozoon* sp. detected in rural dogs and foxes per bioclimatic region in Chile.

Bioregion	Dog		Andean fox		South American grey fox	
	n	Prev. % (C.I.)	n	Prev. % (C. I.)	n	Prev. % (C. I.)
Coastal Desert	113	0 (0–3.3)	16	0 (0–19.4)	3	0 (0–56.1)
Mountain Desert	95	0 (0–3.9)	–	–	–	–
Steppe	100	0 (0–3.7)	7	71.4 (35.9–91.8)	42	45.2 (31.2–60.0)
Mediterranean	111	0 (0–3.3)	108	57.4 (47.9–66.3)	13	53.8 (29.1–76.8)
Temperate Warm Rainy	79	0 (0–4.6)	14	21.4 (7.6–47.6)	22	0 (0–14.9)
Temperate Maritime Rainy	80	0 (0–4.6)	–	–	–	–
Overall	578	0 (0–0.7)	145	48.3 (40.3–56.3)	80	32.5 (23.2–43.4)

n: sample size, Prev.: prevalence; C.I.: 95% Confidence intervals.

3.2. Risk factors assessment

Risk infection models performed in foxes revealed a significantly higher prevalence of *Hepatozoon* sp. in Andean than in grey foxes (48.3% vs 32.5%; z = 2.41, $p < 0.05$). Considering both fox species together, the prevalence was higher in juveniles than in adults (56.9% vs 39.7%; z = 2.11, $p < 0.05$). Analyses per bioregion indicated that *Hepatozoon* sp. prevalence was higher in the Mediterranean than in the Temperate Warm Rainy region for both Andean and grey foxes ($\chi^2 = 5.08$, $p < 0.05$; and $\chi^2 = 11.63$, $p < 0.001$, respectively). Models performed in dogs showed lower *Babesia* seroprevalence in dogs with no ticks (24.5%) than in dogs with high (35.2%; z = 2.19, $p < 0.01$) or low *R. sanguineus* s.l. abundance (32.4%; z = 2.97, $p < 0.05$). At a bioregional scale, *Babesia* seroprevalence was lower in TWR than in all the other regions studied (in all cases, $\chi^2 \geq 3.97$, $p < 0.05$). Seroprevalence was also significantly higher in Steppe when compared with Mountain Desert ($\chi^2 = 3.97$, $p < 0.05$).

4. Discussion

This study updates the previously insufficient information about the presence and distribution of the main VBPs in wild and domestic canines in Chile, and to the authors' best knowledge, represents the first report of *Hepatozoon* species in Chilean canines. The remarkable high *Hepatozoon* spp. prevalence detected resembles the one reported in grey foxes in the Argentinean Patagonia (Millán et al., 2019), indicating that *Hepatozoon* may be enzootic in foxes from the southern cone of South America. Two of the detected species in our study, namely *H. felis*-like and *H. americanum*, were also found in the grey foxes from Argentina (Millán et al., 2019). We observed that all the grey foxes studied were almost exclusively infected by *H. americanum*, whereas all Andean foxes were infected by *H. felis*-like. This finding highlights the existence of several variants of this protozoan (Inokuma et al., 2002) and suggests a possible host preference for each variant. It is remarkable that the majority of sequences detected in grey foxes from Argentina in the abovementioned study were *H. felis*-like (Millán et al., 2019), whereas, in the present study, grey foxes were predominantly parasitized by *H. americanum*. This further supports the hypothesis that *Hepatozoon* presents some degree of geographical and/or per-host genetic heterogeneity (Gabrielli et al., 2010). However, the high variability of the 18S rRNA of *Hepatozoon* species obscures the determinations of species among this genera (Modrý et al., 2017). Further molecular characterization of *Hepatozoon* spp. in South American canids is warranted.

Although several ticks, and some fleas, have been proposed as competent vectors for *Hepatozoon* species (de Sousa et al., 2017), other transmission routes have been reported. For example, *H. americanum* and *H. felis* have shown a predilection to muscle tissues, inducing the formation of cysts in the infested host and later ingested by another animal, with the consequent protozoan development (Baneth and Cohn, 2016). Rodents have been confirmed as hosts in the life cycle of

Table 1
Primers used in this study.

Target	PCR type	Primer names	Primer sequences 5′-3′	Fragment length (bp)	Reference
Canine endogenous control (RPS19)	Conventional	RPS19F RPS19R	CCTTCCTCAAAAA/GTCTGGG1 GTTCTCATCGTAGGGAGCAAG	95	Brinkhof et al. (2006)
Hepatozoon sp. (18S rRNA)	Conventional	HEP-1 mod F HEP-4 R	CGCGAAATTACCCAATTCTA TAAGGTGCTGAAGGAGTCGTTTAT	670	Spolidorio et al. (2009)
Babesia sp. (18S rRNA)	Conventional	BAB143–167 BAB694–667	CCGTGCTAATTGTAGGGCTAATACA GCTTGAAACACTCTARTTTTCTCAAAG	500	Almeida (2011)
Leishmania spp. (kDNA)	Real-time	LEISH-1 LEISH-2 TaqMan probe	AACTTTTCTGGTCCTCCGGGTAG ACCCCCAGTTTCCCGCC AAAAATGGGTGCAGAAAT	120	Francino et al. (2006) Cortes et al. (2004)
	Conventional	MC1 MC2	GTTAGCCGATGGTGGTCTTG3 CACCCATTTTTCCGATTTTG	447	
Filarioids (12S rRNA)	Conventional	D.imm-12S D.rep-12S	ATTTGTTGTAATATTACGA ATGTTTTGATTTTTTTGTAT	330	Casiraghi et al. (2006)
Filarioids (cox1)	Conventional	ArCox1F CbCox1F	ATCTTTGTTTATGGTGTATC CGGGTCTTTGTTGTTTTTATTGC	689	Otranto et al. (2011)
Tick endogenous control (16S rRNA)	Conventional	16S-F 16S-R1	TTAAATTGCTGTRGTATT CCGGTCTGAACTCASAWC	455	Lv et al. (2013)

Table 3
Hepatozoon nucleotide sequence types (ntST) of 670 bp 18S rRNA from foxes across the different bioclimatic regions of Chile, and their closest sequence deposited in GenBank®.

Nucleotide sequence type	Group	Host (n)	Bioregion	Percentage of identity by BLAST® analysis
ntST1	*H. americanum*	*Lycalopex griseus* (12)	Steppe	100% identity with a *Hepatozoon* sp. of a *L. griseus* from Argentina (MK049949)
ntST2	*H. americanum*	*L. griseus* (1)	Steppe	98.8% identity with a *Hepatozoon* sp. of a *L. griseus* from Argentina (MK049949)
ntST3	*H. canis*	*L. griseus* (1)	Mediterranean	100% identity with a *Hepatozoon canis* of a *Canis lupus familiaris* from Algeria (MK645966)
ntST4	*H. felis*	*Lycalopex culpaeus* (2)	Steppe and Mediterranean	100% identity with a *Hepatozoon* sp. of a *L. griseus* from Argentina (MK049948)

H. americanum (Johnson et al., 2009), and possibly of *H. felis* (Baneth et al., 2013). Thus, the detection of a high prevalence of *Hepatozoon* species in foxes herein examined suggests that carnivorism may be an important transmission route. Likewise, this live-prey feeding behavior of foxes would make them more prone to the accidental ingestion of arthropods and the consequent transmission of *Hepatozoon* (Criado-Fornelio et al., 2003). The higher prevalence of *Hepatozoon* in younger foxes could be associated with the vertical transmission of this parasite (Murata et al., 1993). Additionally, *A. tigrinum* ticks were proposed as a potential vector for *Hepatozoon* spp. (Millán et al., 2019). Further studies, including the screening of fox's prey in Chile and experimental studies with *A. americanum*, should be carried out to elucidate possible ways of transmission of these parasites.

On the other hand, the detection in our study of *H. canis* was unexpected because this parasite, typically found in dogs, was never reported in Chile before, where dogs are extremely abundant. *Hepatozoon canis* in foxes from South America has only been described in the pampas fox (*Lycalopex gymnocercus*) in Brazil (Criado-Fornelio et al., 2006), while in the Northern hemisphere it appears to be well established in the red fox (*V. vulpes*) (Alvarado-Rybak et al., 2016). Potential reasons for this finding can be diverse, such as the lack of research in the field in Chile, a recent introduction of the parasite, or a combination of both. The absence of *H. canis* in dogs in Chile is singular because dogs are a host of *Hepatozoon* species worldwide (Ivanov and Tsachev, 2008; Maggi and Krämer, 2019), and their known vector, *R. sanguineus* s.l., are abundant in the country (Di Cataldo et al., 2021). The causes of this result might be: (i) genetic variants of the protozoan in association with a specific host species or geographical region (Gabrielli et al., 2010); (ii) a marked scavenger behavior in foxes, leading to an increased probability of tick ingestion and consequent protozoan transmission (Gabrielli et al., 2010); (iii) a reduced vectorial capacity of *R. sanguineus* ticks for some strains of *H. canis* (Criado-Fornelio et al., 2007). This last hypothesis is further supported by the fact that dogs from the country were infested mostly with *R. sanguineus* s.l. while, in foxes, the most prevalent tick was *A. tigrinum* (Cevidanes et al., 2021).

Andean foxes were more prone to be infected with *Hepatozoon* than

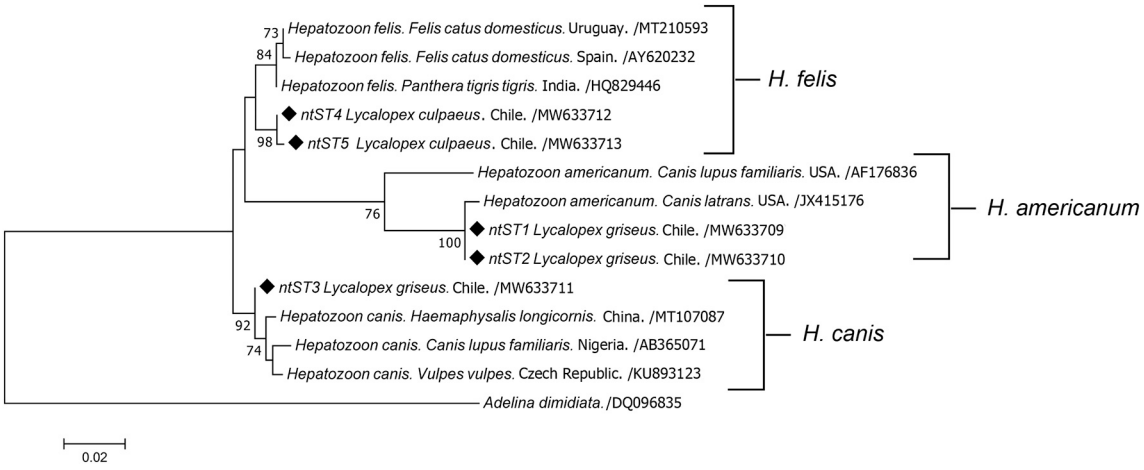

Fig. 2. Maximum likelihood tree of the 18S rRNA gene (670 bp) of *Hepatozoon* species from Andean foxes and South American grey foxes. Diamonds mark the five nucleotide sequence types (ntST) detected in foxes in this study.

Table 4
Genetic diversity and polymorphism of the 18S rRNA sequences of *Hepatozoon* sp. detected in foxes in Chile.

Bioregion	Host	Hd	π	K	S_{nn}	Phi_{st}	*p*-value
All	All	0.4940	0.01356	6.48129	0.91959	0.91025	**<0.01**
	Andean fox	0.1109	0.00023	0.11092			
	Grey fox	0.2743	0.00551	2.57143			
Steppe	All	0.4417	0.01027	4.90833	0.86990	0.76586	**<0.01**
Mediterranean	All	0.1193	0.00189	0.90530			

Hd: haplotype diversity, π: nucleotide diversity, K: average number of nucleotide differences, S_{nn}: nearest neighbor statistic, Phi_{st}: average pairwise difference. Significant *p*-values indicate genetic structure between the sequences for each group.

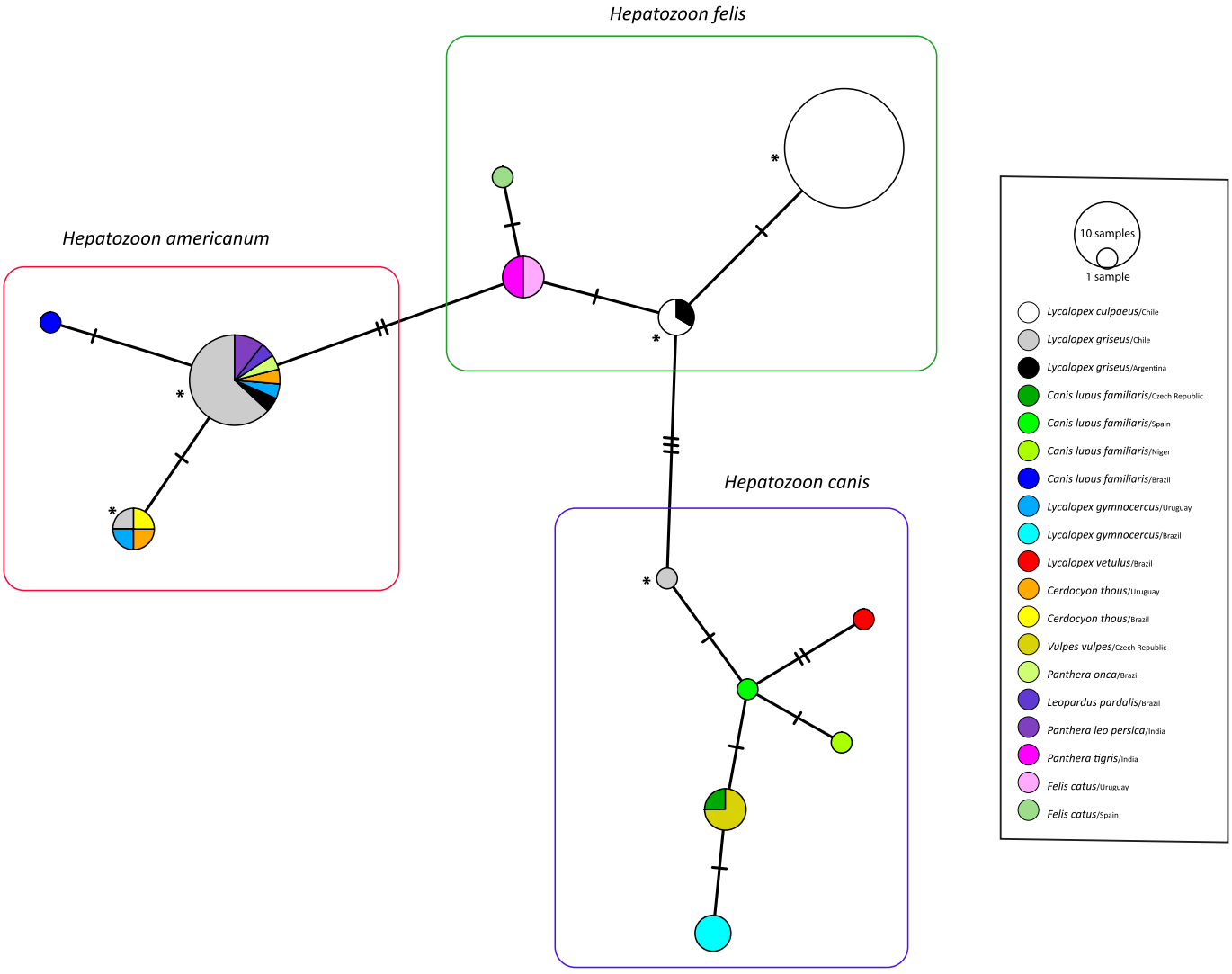

Fig. 3. Median-joining network of the 18S rRNA gene (670 bp) of *Hepatozoon* species in carnivores of the world. The color of the circles corresponds to the species addressed. Each circle in the networks corresponds to a different nucleotide sequence type (ntST), and the size of the circles corresponds to ntST frequencies. Asterisks mark the animals from our study.

Table 5
Babesia DNA and seroprevalence detected in rural dogs and foxes per bioclimatic regions in Chile.

Bioregion	Dog				Andean fox				South American grey fox			
	PCR		IFAT		PCR		IFAT		PCR		IFAT	
	n	Prev. % (C. I.)	n	Seroprev. % (C. I.)	n	Prev. % (C. I.)	n	Seroprev. % (C. I.)	n	Prev. % (C. I.)	n	Seroprev. % (C. I.)
Coastal Desert	101	0 (0–3.7)	185	25.9 (20.2–32.7)	16	0 (0–19.4)	1	0 (0–94.9)	3	0 (0–56.1)	–	–
Mountain Desert	95	0 (3.9)	82	21.9 (14.4–32.0)	–	–	–	–	–	–	–	–
Steppe	100	4.0 (1.6–9.8)	86	37.2 (27.7–47.8)	7	0 (0–35.4)	2	50.0 (2.6–97.4)	42	0 (0–8.4)	–	–
Mediterranean	111	0 (0–3.3)	82	25.6 (17.4–36.0)	113	0 (0–3.4)	5	20.0 (1.0–62.4)	15	0 (0.20–4)	12	25.0 (8.9–53.2)
Temperate Warm Rainy	79	0 (0–4.6)	81	4.9 (1.9–12.0)	14	0 (0–21.5)	–	–	22	0 (0–14.9)	–	–
Temperate Maritime Rainy	94	0 (0–3.9)	11	45.4 (21.3–72.0)	–	–	–	–	–	–	–	–
Overall	571	0.7 (0.2–1.8)	527	24.3 (20.8–28.1)	150	0 (0–2.5)	8	25.0 (7.1–59.1)	82	0 (0–4.5)	12	25.0 (8.9–53.2)

n: sample size, Prev.: prevalence; (C.I.): 95% Confidence intervals, Seroprev.: seroprevalence.

grey foxes, which may be explained by the fact that most of the Andean foxes were sampled in the Mediterranean region, where *Hepatozoon* appears to be endemic in this host. This may be related to the climate of this region, which might be more suitable for potential vectors, or to a higher population abundance of Andean foxes in this region.

Regarding piroplasmids, our large-scale survey from different bioclimatic regions resulted in no new infections other than the previously reported for the Steppe region (Di Cataldo et al., 2020b). Its presence in only a limited geographical area of the Steppe region suggests that this case represents a *Babesia* cluster. However, we did find antibodies against *Babesia* sp. in the six assessed regions. The detection of this parasite by molecular methods in chronic stages is difficult

Table 6
Leishmania spp. DNA and seroprevalence detected in rural dogs and foxes per bioclimatic regions in Chile.

Bioregion	Dog				Fox			
	PCR		IFAT		PCR		IFAT	
	n	Prev. % (C.I.)	n	Seroprev. % (C.I.)	n	Prev. % (C.I.)	n	Seroprev. % (C.I.)
Coastal Desert	261	0 (0–1.7)	186	9.7 (6.2–14.8)	19	0 (0–16.8)	1	0 (0–94.9)
Mountain Desert	117	0 (0–3.2)	92	0 (0–4.0)	–	–	–	–
Steppe	100	0 (0–3.7)	87	2.3 (0.6–8.0)	49	0 (0–7.3)	3	0 (0–56.1)
Mediterranean	111	4.5 (1.9–10.1)	82	1.2 (0.1–6.6)	25	0 (0–13.3)	14	0 (0–21.5)
Temperate Warm Rainy	81	0 (0–4.5)	80	2.5 (0.7–8.7)	36	0 (0–9.6)	–	–
Overall	625	0.8 (0.3–1.9)	527	4.4 (2.9–6.5)	129	0 (0–2.9)	18	0 (0–17.6)

n: sample size, Prev.: prevalence; (C.I.): 95% Confidence intervals, Seroprev.: seroprevalence.

Table 7
Acanthocheilonema reconditum molecular prevalence detected in rural dogs and foxes per bioclimatic regions in Chile.

Bioregion	Dog	Andean fox		South American grey fox		
	n	Prev. % (C.I.)	*n*	Prev. % (C.I.)	*n*	Prev. % (C.I.)
Coastal Desert	209	0 (0–1-8)	16	0 (0–19.36)	3	0 (0–56.1)
Mountain Desert	117	0 (0–3.2)	–	–	–	–
Steppe	83	0 (0–4.4)	7	0 (0–35.43)	42	0 (0–8.4)
Mediterranean	111	0.9 (0.04–4.9)	5	0 (0–43.45)	20	0 (0–16.1)
Temperate Warm Rainy	81	9.9 (5.1–18.3)	14	0 (0–21.53)	22	0 (0–14.9)
Overall	601	1.5 (0.8–2.8)	42	0 (0–8.38)	87	0 (0–4.2)

n: sample size, Prev.: Prevalence, (C.I.): 95% Confidence Intervals.

(Irwin, 2010), whereas the high seroprevalence indicates animal exposure to the pathogen. We found a significant association between *Babesia* seroprevalence and the abundance of *R. sanguineus* s.l., which is not unexpected because it is the known vector of *B. vogeli* (Solano-Gallego and Baneth, 2011). The presence of antibodies in five foxes is interesting and may suggest that foxes are occasionally infected by *Babesia* (or some related piroplasmid). Infections with other *Babesia* species in foxes have been reported worldwide (Alvarado-Rybak et al., 2016) but these piroplasmids have been always associated with tick species that are absent in Chile (Cicuttin et al., 2017; González-Acuña and Guglielmone, 2005).

The molecular and serological detection of *Leishmania* spp. comprises the first report of this parasite in Chile. Although it was not possible to obtain readable sequences of the dogs to confirm the identity of the parasite, the seroprevalence detected would indicate that L. *infantum* or related parasites are actively circulating in the dog population of Chile. *Leishmania* spp. is endemic in neighboring countries such as Argentina and Brazil (Bern et al., 2008; Dantas-Torres, 2008) where sandflies vectors are present. Sandflies are present in Chile (Elgueta and Jezek, 2014), but none of the species has a known vectorial capacity. However, *Leishmania* DNA has been reported in areas where the known vectors appear not to be present (Millán et al., 2016), warranting further entomological studies.

Acanthocheilonema reconditum was previously described in Chile, both in dogs (Alcaíno and Gorman, 1999) and in Andean foxes (Oyarzún-Ruiz et al., 2020). Herein, we only detected filarial worms in a small proportion of a large sample size of dogs and foxes, which agrees with reports worldwide (Ionică et al., 2017; Otranto et al., 2019). It has been suggested that confinement of the animals parasitized with*A. reconditum* is crucial to the maintenance of the nematode in the population (Brianti et al., 2012), mostly because it is vectored only by adult fleas or lice species (Otranto et al., 2013). All our sampled canids were free-ranging and most of them were never confined, reducing, therefore, the probability of transmission of the filarioid, which could explain the low infestation detected in our study. Finally, although *Dirofilaria immitis* appears to be well established in neighboring countries (Cuervo et al., 2013), our survey confirms that Chile is probably free of this nematode (Bendas et al., 2017).

5. Conclusion

This study represents the first large-scale study regarding the main VBPs present in wild and domestic canids in Chile. *Hepatozoon* spp. belonging to up to three different groups are described for the first time in wild canids from Chile: in contrast, these parasites were not detected in over 700 samples from rural free-ranging dogs. Our findings open several new questions that should be addressed regarding the potential impact of these parasites on the health of their hosts. Additionally, experimental studies investigating the vectorial capacity of common fox ectoparasite species for these protozoans are necessary (Criado-Fornelio et al., 2007). This analysis may also contribute to elucidating the reasons for the apparent absence of *H. canis* and other *Hepatozoon* spp. in dogs from Chile. The reported seroprevalence of *Babesia* all over the bioclimatic gradient of Chile suggests that their true prevalence has been underestimated, a reason why veterinary practitioners should consider this parasite in cases compatible with babesiosis. Information, as provided in the present study, is pivotal to understanding the epidemiology of vector-borne parasites that may affect the health of domestic animals and wildlife and to designing management strategies against them.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2022.100721.

CRediT authorship contribution statement

Di Cataldo Sophia: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Cevidanes Aitor:** Conceptualization, Data curation, Methodology, Resources, Writing – review & editing. **Ulloa-Contreras Claudia:** Resources, Writing – review & editing. **Cabello Javier:** Resources, Writing – review & editing. **Gambino Delia:** Resources, Writing – review & editing. **Gargano Valeria:** Resources, Writing – review & editing. **Hidalgo-Hermoso Ezequiel:** Resources, Writing – review & editing. **Latrofa Maria Stefania:** Resources, Validation, Writing – review & editing. **Napolitano Constanza:** Funding acquisition, Resources, Writing – review & editing. **Sacristán Irene:** Formal analysis, Resources, Writing – review & editing. **Sallaberry-Pincheira Nicole:** Resources, Writing – review & editing. **A. Vianna Juliana:** Funding acquisition, Resources, Writing – review & editing. **Acosta-Jamett Gerardo:** Funding acquisition, Resources, Validation, Writing – review & editing. **Vicari Domenico:** Funding acquisition, Resources, Writing – review & editing. **Otranto Domenico:** Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. **Millán Javier:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank the rural residents for allowing us to sample their animals. We also thank Andrés Mancifesta and Andrea Chirife for field assistance, Cristian Bonacic, Sara Moore Carvacho, and Rodrigo Villalobos for providing some of the fox samples, and Carla Barría and Antonino Gentile for helping with the laboratory analyses. This study was funded by Fondecyt Regular 1161593, Fondecyt Iniciación 11100303, Fondecyt Iniciación 11150934, and ANID PAI 771900064.

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