INTRODUCTION

Parasites of the genus Babesia are apicomplexan protozoa of the order Piroplasmida, which parasitize erythrocytes of wild and domestic birds and mammals (Alvarado-Rybak, Solano-Gallego, & Millán, 2016; Penzhorn, 2006). These parasites can cause a wide spectrum of clinical signs, from subclinical signs to intense fever, haemolytic anaemia, splenomegaly and even death (Greene, 2012). The principal species capable of causing pathology in canines are Babesia canis, B. rossi, B. gibsoni and B. vogeli. Their transmission route involves different species of ticks; Dermacentor spp. is responsible for the transmission of B. canis, Haemaphysalis spp. for B. rossi and B. gibsoni, and Rhipicephalus sanguineus for B. vogeli (Schnittger, Rodriguez, Florin-Christensen, & Morrison, 2012). All are distributed worldwide, but B. vogeli is the only one described in South America (Passos, Geiger, Ribeiro, Pfister, & Zahler-Rinder, 2005). Most of the reports in dogs in this continent came from Brazil (Dantas-Torres & Figueredo, 2006; Passos et al., 2005; Santos et al., 2009). Outside Brazil, B. vogeli is known to be endemic in Argentina (Eiras, Basabe, Mesplet, & Schnittger, 2008; Mascarelli, Tartara, Pereyra, & Maggi, 2016), Colombia (Vargas-Hernández et al., 2012) and Venezuela (Criado-Fornelio et al., 2007). Despite its proximity to other countries where B. vogeli is endemic and the nationwide presence of its vector, R. sanguineus (González-Acuña & Guglielmone, 2005), neither this parasite nor any other piroplasmid has ever been reported in Chile, either for dogs or for wild canids. Herein, we report for the first time endemic B. vogeli in naturally infected dogs in Chile.

MATERIALS AND METHODS

In November 2018, 40 owned rural dogs without permanent confinement were sampled as a part of a sanitary survey in Vicuña,
Coquimbo Region, Chile (30°01′54.9″ S, 70°42′28.19″ W). Vicuña has a typical steppe climate with a mean annual temperature of 16 °C and average annual precipitation of 95 mm. Dogs were sampled after written consent of the owners. Samples included blood and ectoparasites. As a prerequisite to be included in the survey, none of the sampled dogs had ever travelled away from this locality. A second campaign was carried out in November 2019, when 25 dogs were sampled. Samples taken in 2019 included blood, ectoparasites and blood smears. In the second campaign, we were able to resample two of the dogs sampled during the first campaign. Body condition was assessed through the five-point body condition scoring (German et al., 2006). Blood was obtained from the cephalic vein and placed into EDTA and preserved at 4°C until arrival to the laboratory. Ectoparasites were retrieved through a 5-min examination protocol and stored in 90% ethanol until identification. Blood smears were fixed with methanol and stained soon after arrival to the laboratory.

Twelve haematological parameters including red and white blood cell counts, haematocrit, platelet count, mean corpuscular volume and haemoglobin were calculated using a HumaCount 80TS cell counter (Human GmbH). Thirteen biochemical parameters were analysed with a BA400 Analyzer (Biosystems S.A.): albumin, ALP, AMP, ALT-GPT, AST-GOT, gamma-GT, calcium, cholesterol, creatinine, glucose, phosphorus, protein-total, urea-BUN-UV and BUN. Blood smears were stained with Giemsa solution and observed under an optical microscope to search for merozoites and trophozoites of Babesia. DNA was extracted from 100 μl of entire blood using the DNeasy® Blood & Tissue Kit (Qiagen). As internal control for canine DNA, the RPS19 gene was targeted using the primers RPS19F (5′ CTTCCTCTAAAA/AGTCTGGG1 3′) and RPS19-R (5′ GTTCTCATCGTAGGGAGCAAG 3′) (95 bp) (Brinkhof, Spee, Rothuizen, & Penning, 2006). We performed conventional PCR to amplify 551 bp of the 18S rRNA gene of the genus Babesia using the primers BAB143-167 (5′ CCGTGCATATTGAGCTGTAATACA 3′) and BAB694-667 (5′ GCTTCGAACTACCTTTTCTCAAG 3′) (Almeida, 2011). Each PCR was repeated three times to ensure accurate results. Ultrapure water was used as a negative control, and positive controls (Babesia bovis) were obtained from previously sequenced cow blood samples. Positive PCR products were sequenced by Macrogen®, and the sequences obtained were compared to the sequences deposited in the GenBank® database (NCBI). To determine phylogenetic relationships, a maximum-likelihood phylogenetic tree was performed with 1,000 replicates. The model was performed by the program MEGA 7, resulting in the T92 + G model. The sequence alignment was performed with ClustalW implemented in Geneious Prime® 2019.2.1 (Biomatters Ltd.), and the tree was generated in MEGA 7.0.26 (Kumar, Tamura, Jakobsen, & Nei, 2002). The new sequence obtained in the present study was submitted to GenBank with accession number MN931918.

3 | RESULTS AND DISCUSSION

In November 2018, DNA of Babesia sp. was detected in four dogs (7.5%, 95% confidence interval = 0.0%–16.0%). Three sequences presented good quality electrophoregrams. The alignment confirmed 100% nucleotide sequence identity among them, 99.8% nucleotide sequence identity with previous published B. vogeli sequences from Argentina (KY290977) and 99.6% nucleotide sequence identity with previous sequences of this pathogen from Algeria (MK645941). In November 2019, DNA of Babesia sp. was detected in one dog (4.0%, 95% CI = 0.0%–12.0%). The sequence was 100% identical to the sequences from 2018. The phylogenetic tree obtained confirmed the sequencing results, with the consensus sequence placed in the B. vogeli clade (Figure 1). Overall observed prevalence for the two study years was 6.3% (95% CI = 0.3–12.4). During the second campaign, one of the resampled dogs that was positive the year before was negative for Babesia. It is worth noting that this dog did not receive any treatment against Babesia. This could be due to a low undetectable level of parasitaemia at the resampling time (Dantas-Torres & Figueredo, 2006). Irwin (2010) indicated that false-negative PCR results are not rare during chronic stages of babesiosis. The second resampled dog, which lived in the same household as one of the positive animals in the first campaign, was negative in both events. The other two positive dogs in the first campaign were dead (unknown causes) when the second campaign was performed.

The positive dogs had never travelled abroad and were detected in a circumscriptive locality with a year of difference, suggesting a possible endemicity of the pathogen in the area. The observed prevalence detected in this study is in the range of other studies in dogs of South America, which range from 0.20% in Argentina (Eiras et al., 2008), 2% in Venezuela (Criado-Fornelo et al., 2007), 5.5% in Colombia (Vargas-Hernández et al., 2012) to 9.9% in Brazil (Costa-Júnior et al., 2012). The reported prevalence in dogs is also low in Europe (Solano-Gallego, Sainz, Roura, Estrada-Peña, & Miró, 2016), especially compared with B. canis.

Most of the surveyed dogs hosted ticks. Observed tick prevalence was 87.3% (95% CI = 79.1%–95.5%), with a mean

FIGURE 1 Maximum-likelihood tree of the 18S rRNA gene (~551bp) of Babesia vogeli for free-ranging rural dogs. An Hepatozoon canis sequence was used as out-group. Bootstrap values of ≥ 70 are given at the nodes of the tree. A diamond marks the aligned sequence from our study
The low sensitivity of molecular detection of Babesia spp. in chronic infections has been reported. To the best of our knowledge, this is the first report of Babesia vogeli or any other canine piroplasmid in Chile. Vector-borne pathogens such as Babesia are distributed in certain areas with climatic features where the vector is able to persist and reproduce (Schnittger et al., 2012). Possible explanations for this new report include lack of surveillance in the country or recent introduction of the parasite. The known high abundance in the country of its vector, R. sanguineus, which is the recognized vector for B. vogeli. In Chile, there are no reports of the presence of either Haemaphysalis spp. or Dermacentor spp. (Abarca, Gárate, López, & Acosta-Jamett, 2016; González-Acuña & Guglielmone, 2005). None of the positive dogs of this study showed clinical signs associated with babesiosis. This is in agreement with previous observations indicating that B. vogeli causes subclinical disease in adult dogs (Cacciò et al., 2002; Schnittger et al., 2012; Solano-Gallego & Baneth, 2011). The only positive dog for which haematological and serum chemistry parameters were available presented values in the reference range for dogs except for the platelet count, which was below the reference range (77,000 mm$^3$, reference value 145,000–500,000 mm$^3$). This finding is frequently associated with babesiosis (Irwin, 2010). Kuleš, Gotič, Mrlijak, and Barić Rafaj (2017) reported alteration in the coagulation parameters in dogs with babesiosis, which could be linked to the depletion of platelets in the active parasite dog detected in this study. No trophozoites or merozoites were found in the blood smears of the positive dog of the second campaign.

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The low sensitivity of molecular detection of Babesia spp. in chronic cases highlights the importance of periodic medical assessment of dogs. Considering all these aspects and the notorious lack of monitoring in the country, it is crucial to increase the awareness of veterinary practitioners about this piroplasmid and other vector-borne pathogens of animal health and zoonotic interest. Chile possesses a variety of bioclimatic regions that provide an ideal scenario for the development of these agents (Mann, 1960). However, the information about the presence, distribution and impact of these pathogens in the country is very scarce, as confirmed by the fact that B. vogeli was never reported before. The distribution of B. vogeli in Chile should be further defined.

Table 1: Personal data of Babesia vogeli-positive dogs from this study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sampling year</th>
<th>Sex</th>
<th>Age</th>
<th>Breed</th>
<th>Body condition (1 to 5)</th>
<th>R. sanguineus count</th>
<th>Haematological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>2018</td>
<td>Male</td>
<td>1 year</td>
<td>Mix breed</td>
<td>3</td>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td>Dog 2</td>
<td>2018</td>
<td>Male</td>
<td>20 years</td>
<td>Mix breed</td>
<td>2</td>
<td>7</td>
<td>None</td>
</tr>
<tr>
<td>Dog 3</td>
<td>2018</td>
<td>Female</td>
<td>1 year</td>
<td>Mix breed</td>
<td>2</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>Dog 4</td>
<td>2019</td>
<td>Male</td>
<td>3 years</td>
<td>Border Collie</td>
<td>3</td>
<td>11</td>
<td>Thrombocytopenia</td>
</tr>
</tbody>
</table>


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