Received: 9 March 2020 | Revised: 2 April 2020 | Accepted: 27 April 2020

DOI: 10.1111/tbed.13609



RAPID COMMUNICATION

Transboundary and Emerging Diseases WILEY

# *Babesia vogeli* in dogs in Chile

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**Funding information**
Fondo Nacional de Desarrollo Científico y Tecnológico, Grant/Award Number: 1161593

## Abstract

We report the presence of *Babesia vogeli* in dogs in Chile. During two surveillance campaigns separated by a year, sixty-three blood samples from free-ranging rural dogs in Coquimbo, Chile, were analysed through conventional PCR screening of the 18S rRNA for *Babesia* species. Sequencing confirmed the presence of *Babesia vogeli* in 6.3% of the tested animals, with positive cases in both years. All the sequences showed 100% nucleotide sequence identity among them and 99.8% with other previously published sequences from dogs. No clinical signs or haematological abnormalities other than thrombocytopenia were found in the parasitized individuals. This is the first report of a canine piroplasmid in Chile.

**KEYWORDS**

canine, piroplasmid, South America

## 1 | 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 & Fig ueredo, 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.

## 2 | 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 RPS19-*F* (5′ CCTTCCTCAAAAA/GTCTGGG1 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′ CCGTGCTAATTGTAGGGCTAATACA 3′) and BAB694-667 (5′ GCTTGAAACACTCTARTTTTCTCAAAG 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

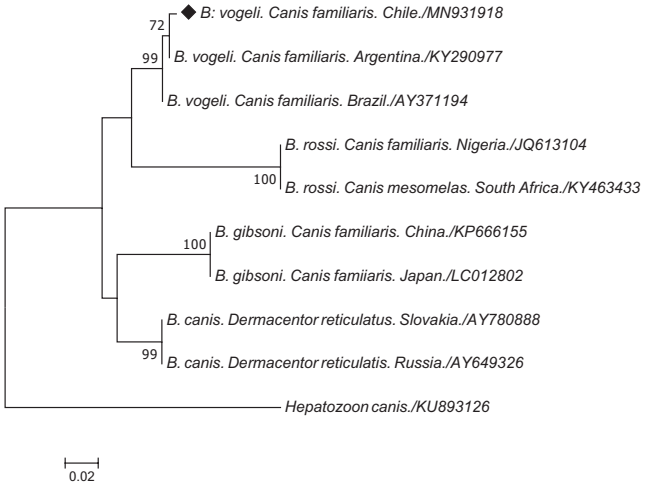


**FIGURE 1** Maximum-likelihood tree of the 18S rRNA gene (~551bp) of *Babesia vogeli* for free-ranging rural dogs. An *Hepatozoon canis* sequence was used as out-group. Bootstrap values of ≥ 70 are given at the nodes of the tree. A diamond marks the aligned sequence from our study

presented good quality electropherograms. 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-Fornelio 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

**TABLE 1** Personal data of *Babesia vogeli*-positive dogs from this study

| Sample | Sampling year | Sex | Age | Breed | Body condition (1 to 5) | *R. sanguineus* count | Haematological findings |
|---|---|---|---|---|---|---|---|
| Dog 1 | 2018 | Male | 1 year | Mix breed | 3 | 6 | None |
| Dog 2 | 2018 | Male | 20 years | Mix breed | 2 | 7 | None |
| Dog 3 | 2018 | Female | 1 year | Mix breed | 2 | 5 | None |
| Dog 4 | 2019 | Male | 3 years | Border Collie | 3 | 11 | Thrombocytopenia |

intensity of 12.1 ± 9.2. The four *Babesia*-positive dogs hosted between five and 11 ticks (Table 1). All the retrieved ticks were identified as *Rhipicephalus sanguineus* sensu lato. This tick 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, Gotić, Mrljak, 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.

To the best of our knowledge, this is the first report of *B. 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* (Díaz, Martínez-Valdebenito, López, Weitzel, & Abarca, 2018), and the abundant population of free-ranging dogs (Astorga, Escobar, Poo-Muñoz, & Medina-Vogel, 2015) that often lack any veterinary health control provide the ideal scenario for the spread of this piroplasmid. Moreover, it has been shown that long-distance movement of rural dogs by their owners is frequent in Chile (Villatoro, Sepúlveda, Stowhas, & Silva-Rodríguez, 2016), which can favour the spread of parasites and pathogens.

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.

## ETHICS STATEMENT

This study was approved by the authorities on bioethics of Universidad Andres Bello, Santiago, Chile (permit no. 08/2016).

## ACKNOWLEDGEMENTS

This study received support from Fondecyt-Regular 1161593, Gobierno de Chile. We wish to thank the rural communities of Vicuña for agreeing to collaborate with this study. Dr. J. M. Venzal kindly provided the positive control.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## REFERENCES

Abarca, K., Gárate, D., López, J., & Acosta-Jamett, G. (2016). Flea and ticks species from dogs in urban and rural areas in four districts in Chile. *Archivos De Medicina Veterinaria*, *48*, 247–253. https://doi.org/10.4067/s0301-732x2016000200017

Almeida, A. P. (2011). *Pesquisa de rickettsia, ehrlichia, anaplasma, babesia, hepatozoon e leishmania em cachorro-do-mato (*Cerdocyon thous*) de vida livre do Estado do Espírito Santo Diss*. Mestrado Epidemiol. Exp. Apl. São Paulo, São Paulo.

Alvarado-Rybak, M., Solano-Gallego, L., & Millán, J. (2016). A review of piroplasmid infections in wild carnivores worldwide: Importance for domestic animal health and wildlife conservation. *Parasites and Vectors*, *9*, 1–19. https://doi.org/10.1186/s13071-016-1808-7

Astorga, F., Escobar, L. E., Poo-Muñoz, D. A., & Medina-Vogel, G. (2015). Dog ownership, abundance and potential for bat-borne rabies spillover in Chile. *Preventive Veterinary Medicine*, *118*, 397–405. https://doi.org/10.1016/j.prevetmed.2015.01.002

Brinkhof, B., Spee, B., Rothuizen, J., & Penning, L. (2006). Development and evaluation of canine reference genes for accurate quantification of gene expression. *Analytical Biochemistry*, *356*, 36–43. https://doi.org/10.1016/j.ab.2006.06.001

Cacciò, S. M., Antunovic, B., Moretti, A., Mangili, V., Marinculic, A., Baric, R. R., … Pieniazek, N. J. (2002). Molecular characterisation of *Babesia canis* canis and *Babesia canis* vogeli from naturally infected European dogs. *Veterinary Parasitology*, *106*, 285–292. https://doi.org/10.1016/S0304-4017(02)00112-7

Costa-Júnior, L. M., Zahler-Rinder, M., Ribeiro, M. F. B., Rembeck, K., Rabelo, E. M. L., Pfister, K., & Passos, L. M. F. (2012). Use of a Real Time PCR for detecting subspecies of *Babesia canis*. *Veterinary Parasitology*, *188*, 160–163. https://doi.org/10.1016/j.vetpar.2012.03.015

Criado-Fornelio, A., Rey-Valeiron, C., Buling, A., Barba-Carretero, J. C., Jefferies, R., & Irwin, P. (2007). New advances in molecular epizootiology of canine hematic protozoa from Venezuela, Thailand and Spain. *Veterinary Parasitology*, *144*, 261–269. https://doi.org/10.1016/j.vetpar.2006.09.042

Dantas-Torres, F., & Figueredo, L. A. (2006). Canine babesiosis: A Brazilian perspective. *Veterinary Parasitology*, *141*, 197–203. https://doi.org/10.1016/j.vetpar.2006.07.030

Díaz, F. E., Martínez-Valdebenito, C., López, J., Weitzel, T., & Abarca, K. (2018). Geographical distribution and phylogenetic analysis of *Rhipicephalus sanguineus* sensu lato in northern and central Chile. *Ticks and Tick-borne Diseases*, *9*, 792–797. https://doi.org/10.1016/j.ttbdis.2018.03.004

Eiras, D. F., Basabe, J., Mesplet, M., & Schnittger, L. (2008). First molecular characterization of *Babesia vogeli* in two naturally infected dogs of Buenos Aires, Argentina. *Veterinary Parasitology*, *157*, 294–298. https://doi.org/10.1016/j.vetpar.2008.07.037

German, A. J., Holden, S. L., Moxham, G. L., Holmes, K. L., Hackett, R. M., & Rawlings, J. M. (2006). A simple, reliable tool for owners to assess the body condition of their dog or cat. *Journal of Nutrition*, *136*, 2031S–2033S. https://doi.org/10.1093/jn/136.7.2031S

González-Acuña, D., & Guglielmone, A. A. (2005). Ticks (Acari: Ixodoidea: Argasidae, Ixodidae) of Chile. *Experimental and Applied Acarology*, *35*, 147–163. https://doi.org/10.1007/s10493-004-1988-2

Greene, C. E. (2012). *Infectious diseases of the dog and cat*, 4th edn. (Elsevier, Ed.). Veterinary Medicine: Penny Rudolph.

Irwin, P. J. (2010). Canine Babesiosis. *Veterinary Clinics of North America: Small Animal Practice*, *40*, 1141–1156. https://doi.org/10.1016/j.cvsm.2010.08.001

Kuleš, J., Gotić, J., Mrljak, V., & Barić Rafaj, R. (2017). Alteration of haemostatic parameters in uncomplicated canine babesiosis. *Comparative Immunology, Microbiology and Infectious Diseases*, *53*, 1–6. https://doi.org/10.1016/j.cimid.2017.06.001

Kumar, S., Tamura, K., Jakobsen, I. B., & Nei, M. (2002). MEGA2: Molecular evolutionary genetics analysis software. *Bioinformatics*, *17*, 1244–1245. https://doi.org/10.1093/bioinformatics/17.12.1244

Mann, G. (1960). Regiones biogeográficas de Chile. *Investing Zoológicas Child*, *6*, 15–49.

Mascarelli, P. E., Tartara, G. P., Pereyra, N. B., & Maggi, R. G. (2016). Detection of *Mycoplasma haemocanis*, *Mycoplasma haematoparvum*, *Mycoplasma suis* and other vector-borne pathogens in dogs from Córdoba and Santa Fé, Argentina. *Parasites and Vectors*, *9*, 1–5. https://doi.org/10.1186/s13071-016-1920-8

Passos, L. M. F., Geiger, S. M., Ribeiro, M. F. B., Pfister, K., & Zahler-Rinder, M. (2005). First molecular detection of *Babesia vogeli* in dogs from Brazil. *Veterinary Parasitology*, *127*, 81–85. https://doi.org/10.1016/j.vetpar.2004.07.028

Penzhorn, B. L. (2006). Babesiosis of wild carnivores and ungulates. *Veterinary Parasitology*, *138*, 11–21. https://doi.org/10.1016/j.vetpar.2006.01.036

Santos, F., Coppede, J. S., Pereira, A. L. A., Oliveira, L. P., Roberto, P. G., Benedetti, R. B. R., … Marins, M. (2009). Molecular evaluation of the incidence of *Ehrlichia canis*, *Anaplasma platys* and *Babesia* spp. in dogs from Ribeirão Preto, Brazil. *The Veterinary Journal*, *179*, 145–148. https://doi.org/10.1016/j.tvjl.2007.08.017

Schnittger, L., Rodriguez, A. E., Florin-Christensen, M., & Morrison, D. A. (2012). Babesia: A world emerging. *Infection, Genetics and Evolution*, *12*, 1788–1809. https://doi.org/10.1016/j.meegid.2012.07.004

Solano-Gallego, L., & Baneth, G. (2011). Babesiosis in dogs and cats-expanding parasitological and clinical spectra. *Veterinary Parasitology*, *181*, 48–60. https://doi.org/10.1016/j.vetpar.2011.04.023

Solano-Gallego, L., Sainz, Á., Roura, X., Estrada-Peña, A., & Miró, G. (2016). A review of canine babesiosis: The European perspective. *Parasites and Vectors*, *9*, 1–18. https://doi.org/10.1186/s13071-016-1596-0

Vargas-Hernández, G., André, M. R., Faria, J. L. M., Munhoz, T. D., Hernandez-Rodriguez, M., Machado, R. Z., & Tinucci-Costa, M. (2012). Molecular and serological detection of *Ehrlichia canis* and *Babesia vogeli* in dogs in Colombia. *Veterinary Parasitology*, *186*, 254–260. https://doi.org/10.1016/j.vetpar.2011.11.011

Villatoro, F., 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

**How to cite this article:** Di Cataldo S, Ulloa-Contreras C, Cevidanes A, Hernández C, Millán J. *Babesia vogeli* in dogs in Chile. *Transbound Emerg Dis*. 2020;00:1–4. https://doi.org/10.1111/tbed.13609