Bartonella vinsonii subsp. berkhoffii and B. henselae in dogs

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Abstract
This study aimed to molecularly survey Bartonella in dogs from Chile. Quantitative real-time PCR (qPCR) for Bartonella spp. based on nuoG gene was performed in 139 blood samples taken from dogs belonging to rural localities of the Valdivia Province, Los Ríos region, southern Chile. nuoG qPCR-positive samples were submitted to conventional PCR assays for ftsZ, gltA, rpoB and nuoG genes and sequencing for speciation and phylogenetic analysis. Based upon qPCR results, Bartonella spp. occurrence in dogs was 4.3% (6/139). Out of six nuoG qPCR-positive samples, six, three, two and none showed positive results in cPCR assays based on gltA, ftsZ, rpoB and nuoG genes, respectively. Consistent sequencing results were obtained only for the ftsZ gene from sample #1532 (GeneBank accession number: MG252490), and gltA gene from samples #1535 (MG252490) and #1532 (148 bp fragment that was not deposited in GenBank). Phylogenetic analysis of ftsZ and gltA genes allowed speciation of two nuoG-positive samples, one as Bartonella vinsonii subsp. berkhoffii and the other as B. henselae. Bartonella vinsonii subsp. berkhoffii and B. henselae are detected for the first time in dogs from Chile, highlighting the importance of the canine population as a source of zoonotic agents and potential infection risk to humans.

The Bartonella genus includes fastidious Haemotropic Gram-negative bacteria transmitted by arthropod vectors [1]. In Chile, Bartonella henselae, B. clarridgeiae and B. koehlerae DNA were previously detected in cats [2]; B. rochalimae in Pulex irritans from dogs and B. henselae and B. clarridgeiae in Ctenocephalides felis from cats [3]. No data concerning Bartonella spp. blood stream infection in dogs have been previously reported in the country so far. This study aimed to perform a molecular survey of Bartonella in dogs from Chile.

The study was approved by the Universidad Austral de Chile Bioethics Committee (250/2016). Blood samples were collected from 139 client-owned dogs from rural localities (Chaihuín (39°56′15″S, 73°35′19″W), Cadillal Alto (39°59′18″S, 73°31′11″W), Hupe (39°54′51″S, 73°30′55″W) and Hupe (39°54′51″S, 73°30′55″W) in the Valdivia Province, Southern Chile, including most of the dogs located in each of the communities. DNA extraction was performed (DNAeasy® Blood & Tissue Kit; QiAGEN®, Valencia, CA, USA) and DNA concentration and purity were determined (NanoDrop ND-1000 spectrophotometer; Thermo Scientific®, Waltham, MA, USA). The RPS19 was used as an endogenous gene [4]. Samples were screened by nuoG real-time PCR (qPCR) for Bartonella spp. [5] (CFX96 Thermal Cycler; BioRad®, Hercules, CA, USA). nuoG qPCR-positive samples were subsequently tested using conventional PCR assays based on ftsZ [6], gltA [7], rpoB [6] and nuoG [5] genes (T100 BioRad termocycler; BioRad®). cPCR-positive samples were sequenced (Sanger method; ABI Prism 310 genetic analyser; Applied Biosystems®/Perkin-Elmer, Foster City, CA, USA) for speciation and phylogenetic analysis. Phylogenetic inference based on maximum likelihood criterion (ML) was inferred with RAxML-HPC BlackBox 7.6.3 (CIPRES Science Gateway).

All 139 DNA samples (mean and standard deviation (s.d.) of DNA concentration = 33 ± 22.3 ng/μl; mean and s.d. 260/280 ratio = 1.8 ± 0.07) were positive for the RPS19 gene. Bartonella-nuoG DNA (mean and standard deviation: 96.2 ± 0.81%, r² = 0.998 ± 0.0004) was detected in 4.3% (6/139) of the tested dogs. Out of six nuoG qPCR-positive samples, six, three, two and none showed results consistent with Bartonella spp. in cPCR assays based on gltA, ftsZ, rpoB and nuoG genes, respectively. Consistent sequencing results were obtained only for the ftsZ gene (550 bp) from sample #1532 (GeneBank accession number: MG252491), and gltA gene (305 bp) from sample #1535 (MG252490) and from sample #1532 (148 bp fragment that was not deposited in GenBank). While Blastn analysis of ftsZ fragment showed 99% identity with Bartonella vinsonii subsp. berkhoffii (CP003124.1, AF467764.1), gltA fragment shared 100% identity to B. henselae (HG969191.1; AJ439406). It was not possible to identify species in the other cPCR-positive samples due to low signal strength in the electropherogram. While B. vinsonii subsp.
berkhoffii sequence (MG252491) from sample #1532 clustered with an American Type Culture Collection B. vinsonii subsp. berkhoffii, B. henselae sequence obtained from sample #1535 (MG252490) grouped with a human B. henselae Houston-1 isolate (Fig. 1).

Few reports in South America have evaluated the molecular occurrence of Bartonella spp. in dogs. For instance, B. vinsonii subsp. berkhoffii was molecularly detected only in a dog from Colombia [8] and in a dog from Brazil, co-infected with
B. vinsonii subsp. berkhoffii and B. henselae [9]. To the best of our knowledge, B. vinsonii subsp. berkhoffii and B. henselae are detected for the first time in dogs from Chile. Serologic surveys of B. vinsonii subsp. berkhoffii have been performed in North America, Europe, Asia and Africa, with an exposure that ranged from 3% to 65% [1]. Nevertheless, as observed in the present study, B. vinsonii subsp. berkhoffii DNA is rarely detected by PCR from domestic dogs because dogs tend to maintain a very low level of bacteraemia, which makes molecular confirmation of blood stream infection challenging [10]. Differences between qPCR and cPCR results in this study were in accordance with other authors [11, 12], with a higher sensitivity of qPCR compared with cPCR assays, highlighting the use of multiple approaches in order to increase the sensitivity of Bartonella detection. Better performance of qPCR over cPCR in detecting low Bartonella DNA copy numbers was described before [12]. Evidence suggests that dogs from regions with cold average winter temperatures, as the observed in southern Chile, are less likely to be PCR-positive than dogs from other climatic zones [1]. Attempts to improve the detection of this Bartonella species from dog blood samples using pre-enrichment media should be addressed in the future [10], as the techniques used in this study may have resulted in a lower molecular prevalence than actually exists in the dogs from Chile. Although cats are known to play a major role as B. henselae reservoirs in Chile [13], our results suggest that this species is also circulating in domestic dogs from the country. As observed before in Brazil [9] and Colombia [8], and described in Africa and Asia [1], rural and stray dogs, such as the dogs from Valdivia Province, are more likely to be infected or seroreactive to Bartonella spp.

Bartonella vinsonii subsp. berkhoffii is an emerging bacteria that has been isolated from immunocompetent human patients with arthritis, endocarditis, neurological disease andvasoprolif-erative neoplasia [14]. Vector transmission is suspected among dogs, which are the primary reservoir hosts. Unlike the domestic cat, for which clinical manifestations of natural infection are rarely documented, a wide range of clinical abnormalities have been reported in B. vinsonii subsp. berkhoffii and B. henselae bacte-raemic dogs [1].

Since Bartonella spp. infected dogs develop pathology that is very similar to their human counterparts, natural and experimental infection in dogs could provide important information to enhance knowledge of the disease in people [15]. In Chile, B. henselae has been implicated in more than 200 human cases of bartonellosis serologically diagnosed between 1997 and 2000 [12]. The presence of B. vinsonii subsp. berkhoffii suggests the need to consider this species when testing clinical samples from suspected human cases in Chile. High similarity detected between B. vinsonii subsp. berkhoffii and B. henselae from dogs and human isolates highlights the importance of the canine population as a potential source of zoonotic agents and infection risk to humans in the country.

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Conflict of interest. None.

References