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Bartonella vinsonii subsp. berkhoffii and *B. henselae* in dogs

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Short Paper

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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: MG252491), 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), Huiro (39°54′51″S, 73°30′55″W) and Huape (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 (DNeasy® 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; ABIPrism310 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 s.d. efficiency: 96·2 ± 0·81%, r^2 = 0·998 ± 0·00046) 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 *B. 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.

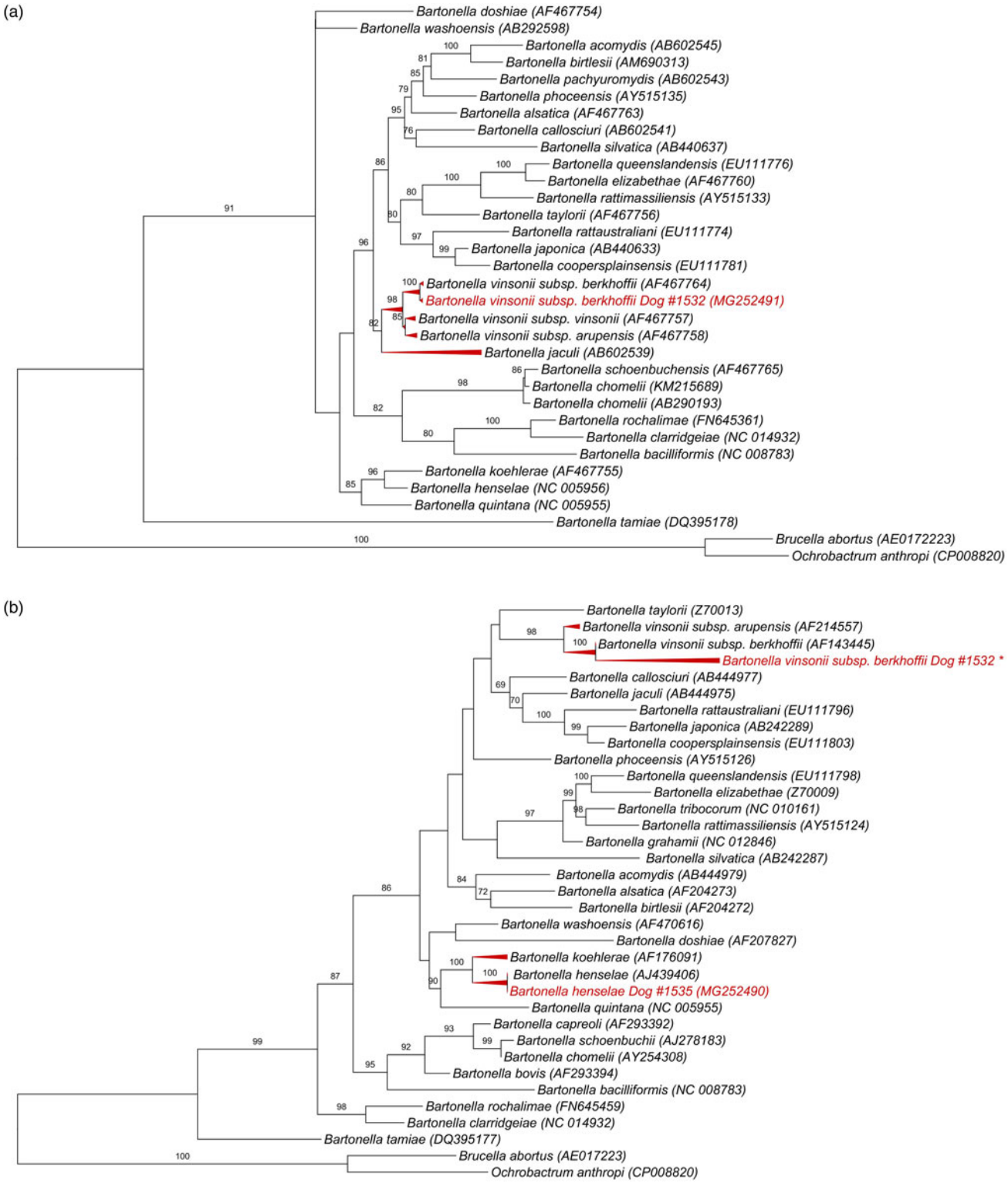

Fig. 1. Phylogenetic relationships within the *Bartonella* genus based on: (a) 950 pb fragment of the *ftsZ* gene after alignment including *B. vinsonii* subsp. berkhoffii from dog #1532 (GeneBank accession number: MG252491), (b) 1320 pb fragment of the *gltA* gene after alignment, including *B. henselae gltA* gene (305 bp) from sample #1535 (MG252490) and **B. vinsonii* subsp. berkhoffii from #1532 (148 bp fragment that was not deposited in GenBank due to low length). The tree was inferred by using the ML method and evolutive model GTR + G + I. The numbers at the nodes correspond to the bootstrap values higher than 50% obtained with 1000 replicates. *Brucella abortus* and *Ochrobactrum anthropi* were used as out groups.

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 sensibility 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 and vasoproliferative 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* bacteraemic 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.

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