Molecular Evidence of *Bartonella* Infection in Domestic Dogs from Algeria, North Africa, by Polymerase Chain Reaction (PCR)

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Abstract. *Bartonella* species are being recognized as important bacterial human and canine pathogens, and are associated with multiple arthropod vectors. *Bartonella* DNA extracted from blood samples was obtained from domestic dogs in Algiers, Algeria. Polymerase chain reaction (PCR) and DNA sequence analyses of the ftsZ gene and the 16S-23S intergenic spacer region (ITS) were performed. Three *Bartonella* species: *Bartonella vinsonii* subsp. *berkhoffii*, *Bartonella claridgeiae*, and *Bartonells elizabethae* were detected infecting Algerian dogs. To our knowledge, this study is the first report of detection by PCR amplification of *Bartonella* in dogs in North Africa.

INTRODUCTION

*Bartonella* are fastidious, hemotropic, and oxydase-negative bacteria, with more than 20 described species or subspecies and are recognized as emerging human and canine pathogens. Among the 11 zoonotic *Bartonella* species, six species and one subspecies have been associated with infections in domestic dogs, including *Bartonella vinsonii* subsp. *berkhoffii*, *Bartonella claridgeiae*, *Bartonella elizabethae*, *Bartonella washoeensis*, *Bartonella henselea*, *Bartonella quintana*, and *Bartonella rochalimae*. *Bartonella* are usually vector-borne infections transmitted by various arthropods, including sand flies, lice, fleas, and potentially ticks. Nevertheless, the role of fleas and ticks in the transmission of *Bartonella* species to dogs is not definitively established. Recently, distribution and diversity of *Bartonella* species in humans, rodents, and hedgehogs in Algeria were reported. These animals (rodents and hedgehogs) were suspected of serving as wildlife reservoirs. In this study, we report the first molecular detection of *Bartonella* species by polymerase chain reaction (PCR) in domestic dogs from Algiers, the capital of Algeria.

MATERIALS AND METHODS

**Dogs sampled.** Eighty domestic dogs from Algiers (latitude = 36°46’N and longitude = 3°02’E) were enrolled between April and June 2006. Blood samples were obtained aseptically from the cephalic vein, and drawn in 5-mL tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Information concerning age, sex, breed, clinical status on physical examination, and presence of fleas and ticks were collected through a questionnaire administered by private veterinarians. The owners were also asked about the use of arthropod control products. A dog was recorded as sick if at least two or three of the following signs (apathy, fever, listlessness, loss of weight, pale mucous membranes, lymphadenomegaly, and cutaneous lesions) were observed at the time of blood sample collection.

**DNA extraction and PCR screening.** The DNA was extracted with QIAmp blood Kit (Qiagen, Gmbh, Hilden, Germany) from 200 μL of each uncoagulated blood sample, according to the manufacturer’s instructions. The PCR method used for sample analysis aimed at amplifying a 639–722-bp fragment of the 16S-23S ribosomal RNA (rRNA) intergenic transcribed sequence (ITS) using primers URB1 (5′-CTTCCGTGTTCTTCTTCTCA-3′) and URB2 (5′-CTTCTCTCCAATTTCAAT-3′), as described previously. The samples positive with ITS primers were then confirmed by PCR of a ftsZ fragment (cell division protein-encoding gene) using primers BaftsZF (5′-GCTAATCTGTTATGCAGAA-3′) and BaftsZR (5′-GCTGTTATTTCCAAAYTGATCT-3′), which amplifies an approximately 240-bp fragment of the ftsZ, as described previously. Negative controls consisted of DNA extracted from serum of an uninfected patient to *Bartonella* sp. A positive control (*Bartonella tribocorum*) was included in each test. The products were verified by 1% agarose gel electrophoresis.

**PCR amplification and sequencing.** All positive PCR products used for DNA sequencing were purified with QIAquick purification kit (Qiagen). The DNA sequencing reagents were obtained with BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI PRISM, PE Applied Biosystems, Foster City, CA) and sequenced for the ITS and cell division protein-encoding gene (ftsZ) fragments. The sequences were then analyzed by Basic Local Alignment Search Tool (BLAST) sequencing and were compared with *Bartonella* sequences available in the GenBank.

RESULTS

**Dogs sampled.** Blood samples were collected from 80 pet dogs between April and June 2006. Forty-two (52.5%) of the dogs were male. Dogs ranged in age from 3 months to 12 years (median age: 3.3 years), and included 14 dogs that were ≥ 4 years old. Various breeds were represented, the most common breeds being German shepherd and shepherd crosses (45 dogs), Rottweiler (11 dogs), mixed-breeds (9 dogs), and other breeds (15 dogs). Most of the dogs lived outdoors (yard) during the day and at night, except for small breed dogs, which were kept indoors at night. Twenty-three (28.75%) dogs were parasitized with ticks and two (2.5%) dogs were infested by fleas. Information about tick and flea infestation was not available for 17 of the 80 dogs. Among these 80 dogs, 26 (32.5%) were sick on physical examination and 54 clinically healthy. Algiers surface equals 809 km² and is divided into

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57 districts (communes). In this study, dogs originated from 18 of the 57 districts of Algiers.

Identification of Bartonella spp. Five (6.25%) of the 80 dogs were PCR positive after amplification using primers for the ITS region and the ftsZ gene of Bartonella spp. Sequencing of the 5 positive PCR products revealed gene sequences matching those of *B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae*, and *B. elizabethae* (Table 1). A search in GenBank of similar partial sequences from the 16S-23S rDNA (689 bp) and cell division protein *ftsZ*, indicated that two samples derived from dogs 1 and 2 (Table 1) were identical to *B. vinsonii* subsp. *berkhoffii* type IV (GenBank accession nos. DQ059765 and AF467764 for ITS and *ftsZ*, respectively, with 100% similarity). The ITS and *ftsZ* amplicons with DNA extracted from the blood of dog 3 shared a complete partial sequence identity with *B. clarridgeiae* (accession nos. DQ003029 for ITS and AF141018 for *ftsZ*, respectively, with 100% similarity). The amplicons from dogs 4 and 5 contained 16S-23S rDNA and *ftsZ* that were in complete homology with the sequences for *B. elizabethae* (100% similarity with accession nos. L35103 for ITS and AF467760 for *ftsZ*).

**Characteristics of dogs PCR positive for Bartonella spp.** Age, gender, breed, clinical status, and presence of ticks or fleas for five dogs polymerase chain reaction (PCR)-positive for *Bartonella* spp. *B. vinsonii* DNA was amplified for the first time by PCR in a dog blood sample in 2002. This study is the first report of detection of *Bartonella* in canine peripheral blood samples from North Africa.

In North Africa, there is limited information describing bartonellosis. One serological study performed in Morocco reported 38% (56 of 147) of the dogs being seropositive for *B. v. berkhoffii*. In Algeria, 31% of 100 stray dogs were seropositive for *B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae* and/or *B. henselae* antigens (Kernif T and others, unpublished data). In other African countries, seroreactivity against *Bartonella* species (*B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae* or *B. henselae*) has been reported in dogs from Zimbabwe and Gabon.

Clinically, *B. vinsonii* subsp. *berkhoffii* has been associated with a wide variety of clinical symptoms in dogs, including cardiac arrhythmias, endocarditis, myocarditis, granulomatous lymphadenitis, granulomatous rhinitis, and epistaxis. *Bartonella clarridgeiae* was associated with endocarditis and lymphocytic hepatitis in a few sick dogs and *B. elizabethae* has also been detected in dogs with various clinical abnormalities including lethargy, anemia, and weight loss. In this study, two dogs infected with *Bartonella* were healthy on physical examination. However, we could not establish a definitive diagnosis without further analyses, such as hematologic and biochemical analyses, echocardiogram and electrocardiography tests to exclude any cardiac lesion.

It is important to identify vector-borne bartonellosis in domestic dogs, because *Bartonella* infections are recognized as a cause of severe clinical illness in both humans and dogs. In addition, dogs sub-clinically infected and living in the proximity of people could serve as carriers of infected arthropods (such as fleas and ticks). In this study, more than 50% of the *Bartonella* PCR positive-dogs were infested by ticks or fleas. Nevertheless, the role of dogs as a major reservoir of *Bartonella* species is still not clear and needs to be further investigated in countries where *Bartonella* infection in dogs and humans is common.

In conclusion, this study provides the first molecular detection for canine exposure to *Bartonella* in North Africa. Our results should strongly encourage veterinarians in Algeria and other North African countries to investigate the medical aspects of bartonellosis in dogs and to use appropriate diagnostic tests to confirm this etiology. Further studies are needed to establish the prevalence of flea- and tick-borne infections in dogs in Algeria and to better understand the mode of transmission of *Bartonella* species in dogs.

**DISCUSSION**

Historically, *Bartonella vinsonii* subsp. *berkhoffii* was first isolated from a dog with endocarditis in 1993. Subsequently, this organism was amplified and sequenced from the blood of several dogs with endocarditis. *Bartonella clarridgeiae* was first isolated from a domestic cat by Clarridge and others in 1995, and domestic cats appear to be the natural reservoir for this *Bartonella* species. However, *B. clarridgeiae* was isolated from the blood and sequenced from the heart valve of a dog with aortic endocarditis. *Bartonella clarridgeiae* has also been isolated from dogs from Gabon. *Bartonella elizabethae* was isolated for the first time in 1986 from the blood of a human patient with endocarditis.

**Table 1**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Breed</th>
<th>Clinic status</th>
<th>Presence ticks or fleas</th>
<th>PCR sequencing results</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>4</td>
<td>M</td>
<td>Pointer</td>
<td>H</td>
<td>None</td>
<td><em>B. vinsonii</em> subsp. <em>berkhoffii</em></td>
</tr>
<tr>
<td>02</td>
<td>1</td>
<td>F</td>
<td>Mixed-breed</td>
<td>S</td>
<td>Ticks</td>
<td><em>B. vinsonii</em> subsp. <em>berkhoffii</em></td>
</tr>
<tr>
<td>03</td>
<td>5</td>
<td>M</td>
<td>Bleu de Gascogne</td>
<td>H</td>
<td>NR</td>
<td><em>B. clarridgeiae</em></td>
</tr>
<tr>
<td>04</td>
<td>8</td>
<td>M</td>
<td>German shepherd</td>
<td>S</td>
<td>Fleas</td>
<td><em>B. elizabethae</em></td>
</tr>
<tr>
<td>05</td>
<td>4</td>
<td>M</td>
<td>Griffon/poodle</td>
<td>S</td>
<td>Ticks</td>
<td><em>B. elizabethae</em></td>
</tr>
</tbody>
</table>

*M = male; F = female; H = healthy; S = sick; NR = not registered.
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