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

Tahar Kernif, Meriem Aissi, Salah-Eddine Doumandji, Bruno B. Chomel, Didier Raoult,* and Idir Bitam

Département de zoologie, Institut National d'Agronomie, El Harrach, Algiers, Algeria; École Nationale Vétérinaire d'El Harrach, Algiers, Algeria;

Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California;

Unité de recherche en maladies infectieuses tropicales émergentes, URMITE CNRS-IRD, Faculté de Médecine,

Université de la Méditerranée, Marseille, France; Service d'Ecologie des Systèmes Vectoriels, Institut Pasteur d'Algérie, Algiers, Algeria

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 clarridgeiae*, and *Bartonella 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.¹ 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 clarridgeiae*, *Bartonella elizabethae*, *Bartonella washoensis*, *Bartonella henselae*, *Bartonella quintana*, and *Bartonella rochalimae*.² *Bartonella* are usually vector-borne infections transmitted by various arthropods, including sand flies, lice, fleas, and potentially ticks.³ Nevertheless, the role of fleas and ticks in the transmission of *Bartonella* species to dogs is not definitively established.^{4,5} Recently, distribution and diversity of *Bartonella* species in humans, rodents, and hedgehogs in Algeria were reported.^{6,7} These animals (rodents and hedgehogs) were suspected of serving as wildlife reservoirs.⁷ 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'-CTTCGTTTCTCTTTCTTCA-3') and URB2 (5'-CTTCTCTTCACAATTTCAAT-3'), as described previously.^{8,9} The samples positive with ITS primers were then confirmed by PCR of a *ftsZ* fragment (cell division protein-encoding gene) using primers BaftsZF (5'-GCTAATCGTATTCGCGAAGAA-3') and BaftsZR (5'-GCTGGTATTTCCAAYTGATCT-3'), which amplifies an approximately 240-bp fragment of the *ftsZ*, as described previously.¹⁰ 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.^{8–10} 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

*Address correspondence to Didier Raoult, Unité des Rickettsies, CNRS UMR 6020, Faculté de Médecine, Université de la Méditerranée, 27 Bd. Jean Moulin, 13385 Marseille, Cedex 5, France. E-mail: didier.raoult@medecine.univ-mrs.fr

TABLE 1

Age, sex, breed, clinical status, and presence of fleas or ticks for five dogs polymerase chain reaction (PCR)-positive for *Bartonella* spp.*

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

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

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 the five *Bartonella* infected dogs are presented in Table 1. *Bartonella vinsonii* subsp. *berkhoffii* was detected in two of the five dogs. These two dogs lived in the same city district of Algiers. One dog was sick with severe apathy, loss of weight, and pale mucous membranes and was infested with ticks. The other dog was healthy and not parasitized by arthropods at the time of examination (Table 1). However, the owner had indicated on the written questionnaire that he was not routinely using flea and tick control products. The DNA sequencing confirmed infection with *B. clarridgeiae* in one dog, a 5-year-old Bleu de Gascogne that was healthy on physical examination. Presence of *B. elizabethae* DNA was confirmed in two sick dogs. One dog was an 8-year-old German shepherd that was emaciated and infested by fleas. The second dog was apathic, cachectic, and was infested by ticks (Table 1).

DISCUSSION

Historically, *Bartonella vinsonii* subsp. *berkhoffii* was first isolated from a dog with endocarditis in 1993.¹¹ Subsequently, this organism was amplified and sequenced from the blood of several dogs with endocarditis.^{12,13} *Bartonella clarridgeiae* was first isolated from a domestic cat by Clarridge and others in 1995,¹⁴ and domestic cats appear to be the natural reservoir for this *Bartonella* species.² However, *B. clarridgeiae* was isolated from the blood and amplified and sequenced from the heart valve of a dog with aortic endocarditis.¹⁵ *Bartonella clarridgeiae* has also been isolated from dogs from Gabon.¹⁶ *Bartonella elizabethae* was isolated for the first time in 1986 from the blood of a human patient with endocarditis.¹⁷ *Bartonella eliza-*

bethae DNA was amplified for the first time by PCR in a dog blood sample in 2002.¹⁸ 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.^{6,19} One serological study performed in Morocco reported 38% (56 of 147) of the dogs being seropositive for *B. v. berkhoffii*.²⁰ 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 Zimbabwe²¹ and Gabon.¹⁶

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.^{2,12} *Bartonella clarridgeiae* was associated with endocarditis and lymphocytic hepatitis in a few sick dogs,^{15,22} and *B. elizabethae* has also been detected in dogs with various clinical abnormalities including lethargy, anemia, and weight loss.¹⁸ 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.² 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).²³ 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.²

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.

Received January 28, 2009. Accepted for publication January 2, 2010.

Acknowledgments: We are grateful to Khaled Harhoura from the National School of Veterinary Medicine in Algiers, Algeria and to private veterinarians in Algiers for their help in the investigation, and to Harrat Zoubir, Eco-epidemiology Parasitological and Genetic of population

Pasteur Institute, Algiers, for her constant support. In memory of Doctor Baziz Belkacem from the Department of zoology, National Agronomic Institute, Algiers, Algeria.

Authors' addresses: Tahar Kernif, Department de Zoologie, Institut National d'Agronomie, Algiers, Algeria and Service d'Ecologie des Systèmes Vectoriels, Institut Pasteur d'Alger, Annexe Ruisseau, Algiers, Algeria, E-mail: taharscorse@hotmail.com. Meriem Aissi, École Nationale Vétérinaire d'El Harrach, Algiers, Algeria, E-mail: aissimiriem@yahoo.fr. Salah-Eddine Doumandji, Department de Zoologie, Institut National d'Agronomie, Algiers, Algeria, E-mail: dmndjisldn@yahoo.fr. Bruno B. Chomel, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, E-mail: bbchomel@ucdavis.edu. Didier Raoult, Unité des Rickettsies, CNRS UMR 6020, Faculté de Médecine, Université de la Méditerranée, Marseille, Cedex 5, France, E-mail: didier.Raoult@medecine.univ-mrs.fr. Idir Bitam, Service d'Ecologie des Systèmes Vectoriels, Institut Pasteur d'Alger, Annexe Ruisseau, Algiers, Algeria, E-mail: idirbitam@yahoo.fr.

Reprint requests: Idir Bitam, Service d'Ecologie des Systèmes Vectoriels, Institut Pasteur d'Alger, Annexe Ruisseau, Algiers, Algeria, E-mail: idirbitam@yahoo.fr.

REFERENCES

1. Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB, 2005. Factors associated with rapid emergence of zoonotic *Bartonella* infections. *Vet Res* 36: 383–410.
2. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB, 2006. *Bartonella* spp. in pets and effect on human health. *Emerg Infect Dis* 12: 389–394.
3. Maggi RG, Chomel BB, Hegarty B, Henn J, Breitschwerdt EB, 2006. A *Bartonella vinsonii berkhoffii* typing scheme based upon 16S–23S ITS and Pap31 sequences from dog, coyote, gray fox, and human isolates. *Mol Cell Probes* 20: 128–134.
4. Parola P, Shpynov S, Montoya M, Lopez M, Houpiqian P, Zeaiter Z, Guerra H, Raoult D, 2002. First molecular evidence of new *Bartonella* spp. in fleas and a tick from Peru. *Am J Trop Med Hyg* 67: 135–136.
5. Hinrichsen VL, Withworth UG, Breitschwerdt EB, Hegarty BC, Mather TN, 2001. Assessing the association between the geographic distribution of deer ticks and seropositivity rates to various tick-transmitted disease organisms in dogs. *J Am Vet Med Assoc* 218: 1092–1097.
6. Benslimani A, Fenollar F, Lepidi H, Raoult D, 2005. Bacterial zoonoses and infective endocarditis, Algeria. *Emerg Infect Dis* 11: 216–224.
7. Bitam I, Rolain J-M, Kernif T, Baziz B, Parola P, Raoult D, 2008. *Bartonella* species detected in rodents and hedgehogs from Algeria. *Clin Microbiol Infect* 15: 102–103.
8. Rolain JM, Franc M, Davoust B, Raoult D, 2003. Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. *Emerg Infect Dis* 9: 338–342.
9. La VD, Tran-Hung L, Aboudharam G, Raoult D, Drancourt M, 2005. *Bartonella quintana* in domestic cat. *Emerg Infect Dis* 11: 1287–1289.
10. Zeaiter Z, Liang Z, Raoult D, 2002. Genetic and differentiation of *Bartonella* species based on comparison of partial *ftsZ* gene sequences. *J Clin Microbiol* 40: 3641–3647.
11. Breitschwerdt EB, Kordick DL, Malarkey DE, Keene B, Hadfield TL, Wilson K, 1995. Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. *J Clin Microbiol* 33: 154–160.
12. Breitschwerdt EB, Atkins CE, Brown TT, Kordick DL, Snyder PS, 1999. *Bartonella vinsonii* subsp. *berkhoffii* and related members of the alpha subdivision of the *Proteobacteria* in dogs with cardiac arrhythmias, endocarditis, or myocarditis. *J Clin Microbiol* 37: 3618–3626.
13. MacDonald KA, Chomel BB, Kittleson MD, Kasten RW, Thomas WP, Pesavento P, 2004. A prospective study of canine infective endocarditis in northern California (1999–2001): emergence of *Bartonella* as a prevalent etiologic agent. *J Vet Intern Med* 18: 56–64.
14. Clarridge JE 3rd, Raich TJ, Pirwani D, Simon B, Tsai L, Rodriguez-Barradas MC, Regnery R, Zollo A, Jones DC, Rambo C, 1995. Strategy to detect and identify *Bartonella* species in routine clinical laboratory yields *Bartonella henselae* from human immunodeficiency virus-positive patient and unique *Bartonella* strain from his cat. *J Clin Microbiol* 33: 2107–2113.
15. Chomel BB, MacDonald KA, Kasten RW, Chang CC, Wey AC, Foley JE, Thomas WP, Kittleson MD, 2001. Aortic valve endocarditis in a dog due to *Bartonella clarridgeiae*. *J Clin Microbiol* 39: 3548–3554.
16. Gundi VA, Bourry O, Davoust B, Raoult D, La Scola B, 2004. *Bartonella clarridgeiae* and *B. henselae* in dogs, Gabon. *Emerg Infect Dis* 10: 2261–2262.
17. Daly JS, Worthington MG, Brenner DJ, Moss CW, Hollis DG, Weyant RS, Steigerwalt AG, Weaver RE, Daneshvar MI, O'Connor SP, 1993. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. *J Clin Microbiol* 31: 872–881.
18. Mexas AM, Hancock SI, Breitschwerdt EB, 2002. *Bartonella henselae* and *Bartonella elizabethae* as potential canine pathogens. *J Clin Microbiol* 40: 4670–4674.
19. Znazen A, Rolain JM, Hammami N, Kammoun S, Hammami A, Raoult D, 2005. High prevalence of *Bartonella quintana* endocarditis in Sfax, Tunisia. *Am J Trop Med Hyg* 72: 503–507.
20. Henn JB, VanHorn BA, Kasten RW, Kachani M, Chomel BB, 2006. *Bartonella vinsonii* subsp. *berkhoffii* antibodies in Moroccan dogs. *Am J Trop Med Hyg* 74: 222–223.
21. Kelly PJ, Eoghain GN, Raoult D, 2004. Antibodies reactive with *Bartonella henselae* and *Ehrlichia canis* in dogs from the communal lands of Zimbabwe. *J S Afr Vet Assoc* 75: 116–120.
22. Gillespie TN, Washabau RJ, Goldschmidt MH, Cullen JM, Rogala AR, Breitschwerdt EB, 2003. Detection of *Bartonella henselae* and *Bartonella clarridgeiae* DNA in hepatic specimens from two dogs with hepatic disease. *J Am Vet Med Assoc* 222: 47–51, 35.
23. Solano-Gallego L, Llull J, Osso M, Hegarty B, Breitschwerdt EB, 2006. A serological study of exposure to arthropod-borne pathogens in dogs from northeastern Spain. *Vet Res* 37: 231–244.