High Prevalence of *Bartonella* sp. in Dogs from Hamadan, Iran

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Abstract. Bartonellae are emerging vector-borne pathogens infecting various domestic and wild mammals. Blood samples were collected from 66 dogs at two locations near Hamedan, Iran. Twenty dogs were rescued stray dogs and 46 dogs were from a breeding colony, with many of them infested with fleas, ticks, or lice. Serology was performed using an indirect immunofluorescent antibody test for *Bartonella henselae*, *Bartonella clarridgeiae*, and *Bartonella vinsonii* subsp. *berkhoffii*. Seroprevalence was 74.2% (range: 65.2–95%). *Bartonella* DNA amplification and sequencing identified *B. vinsonii* subsp. *berkhoffii* type III in seven dogs, including five rescued dogs. Two dogs were infected with *Bartonella rochalimae* and three dogs with *Candidatus B. merieuxii*, including two of the stray dogs coinfecting with *Bartonella vinsonii* subsp. *berkhoffii*. Rescued stray dogs were 10 times (odds ratio (OR) = 10.13, 95% CI: 1.24–82.7; *P* = 0.03) more likely to be seropositive and eight times (OR = 8.82, 95% CI: 2.68–29.11; *P* = 0.0004) more likely to be flea-infested than breeding dogs, confirming that arthropod infestation is a major risk factor for these infections.

INTRODUCTION

Bartonella are vector-borne blood-borne pathogens that have mainly been recognized within the last 30 years as a source of human zoonoses, especially for *Bartonella henselae*, the agent of cat scratch disease.¹ These bacteria have been isolated from a wide range of mammalian species, including dogs.² In both humans and dogs, it can cause from mild infection to severe and life-threatening endocarditis.³ In dogs, several *Bartonella* species have been identified, the most common ones being *B. henselae*, *Bartonella vinsonii* subsp. *berkhoffii*, and *Bartonella rochalimae*.⁴ Infection is usually more prevalent in stray dogs, which are frequently infested by fleas and/or ticks.²

In the Middle East, a few studies have reported the presence of *Bartonella* in dogs in Israel,^{5,6} Turkey,^{7,8} and Iraq.⁹ However, no information on *Bartonella* infection in humans or animals is available from Iran, besides a few studies in cats from Tehran^{10,11} and Shahrekord.¹¹

In one study of 70 domestic cats from Tehran (Small Animal Teaching Hospital, University of Tehran), for which nail and saliva samples were collected, one (1.42%) saliva swab and five (7.14%) nail clips were PCR positive for *B. henselae*.¹⁰ The other study¹¹ demonstrated the presence of *B. henselae* in 12 (11%) saliva samples of 110 samples collected from pet cats. Bacteremia was detected by PCR in five (16.6%) of 30 stray cats, but not in the 110 pet cats.

The objective of the present study was to determine the prevalence of *Bartonella* infection in domestic dogs, as they are excellent sentinels for human infection.¹²

MATERIAL AND METHODS

Animals. In October 2018, blood samples (1.5 mL) were collected from 20 rescued stray dogs from Ganj Tappeh, Hamadan (34.9327°N, 48.4644°E) and from 46 animals in a dog-breeding facility at Cheshmeh Qassaban, Hamadan

(34.856°N, 48.419°E). Hamedan Province is located in western Iran, with a cool temperate climate. The age of dogs was estimated on tooth wear by a practicing veterinarian from Bu-Ali Sina University, Hamadan. Blood samples were taken with official permission and under supervision of the Research Committee of Faculty of Veterinary Science, Bu-Ali Sina University, in accordance with the veterinary laws of I.R. Iran.

Serology. Antibodies against *B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae*, and *B. henselae* were detected using an indirect immunofluorescent antibody assay, as previously described.¹³ These antigens were selected because *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, and *B. rochalimae* have been frequently detected in domestic dogs. Furthermore, *B. clarridgeiae* is also a good substitute for the detection of *B. rochalimae* antibodies because of a reported cross-reaction between the two antigens.

DNA extraction from dog blood samples. DNA was extracted from 100 µL of ethylenediaminetetraacetic acid blood of each dog by using the QIAamp Cador Kit (Qiagen, Milan, Italy)

TABLE 1
Number and characteristics of dogs from Ganj Tappeh (GT) and Cheshmeh Qassaban (CQ) investigated for *Bartonella* sp. infection

	GT*	CQ†
Dogs, <i>n</i>	20	46
Median/mode age (months) (range)	24/12 (7–96)	24/12 (8–180)
Sex, female/male, <i>n</i> (%)	10/10 (50)	30 (65.2)/16 (34.8)
Breed	–	4 (8.7)
Mixed	16 (80)	4 (8.7)
Pure	4 (20)	42‡ (91.3)
Infested with ectoparasites		
Dogs infested by fleas, <i>n</i> (%)	13 (65)	8 (17)
Dogs infested by lice, <i>n</i> (%)	3 (15)	6 (13)
Dogs infested by ticks, <i>n</i> (%)	0	32§ (70)
Clinical condition		
Dogs with good body condition, <i>n</i> (%)	16 (80)	33 (72)
Dogs with low body condition, <i>n</i> (%)	4 (20)	13 (28)

* Rescued stray dogs.

† Dogs from breeding colonies.

‡ Most of the dogs (*n* = 42) from CQ were of specific breeds, including 16 German shepherds, 12 Husky, and four Sarabi Mastiff, and two of each of the following breeds: Boxer, Doberman, Pitbull, Rottweiler, as well as one pointer and one Tazi (Afghan hound).

§ Of the 32 dogs infested by ticks, seven and six dogs were coinfecting with fleas and lice, respectively.

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TABLE 2

Bartonella serology titers for dogs from Ganj Tappeh (GT) and Cheshmeh Qassaban (CQ), Hamadan, Iran: number of dogs negative or positive at the sequential dilutions

Titers	<i>Bartonella henselae</i>		<i>Bartonella clarridgeiae</i>		<i>B. vinsonii berkhoffii</i>	
	GT	CQ	GT	CQ	GT	CQ
0	1	23	2	27	1	22
64	1	7	2	9	1	8
128	4	6	7	6	3	5
256	6	8	5	2	3	7
512	2	1	1	1	7	1
1,024	4	1	3	1	3	3
≥ 1,024	2	0	0	0	2	0
Total	20	46	20	46	20	46

according to the manufacturer's instructions. The DNA was obtained in 100 µL of elution buffer. For quality assurance, a *Bartonella*-free blood sample was used as an extraction control.

Molecular screening of *Bartonella* DNA from dog blood samples. *Bartonella* screening was performed using *ssrA* real-time PCR, as described previously.¹⁴ *ssrA* real-time PCR-positive samples were subsequently tested using conventional PCR (cPCR) assays based on *ssrA*¹⁴ and the 16S-23S internal transcribed spacer (ITS) region.¹⁵ PCR-positive samples were sequenced for speciation and phylogenetic analysis. Reference strains *B. henselae* (MH350809), *B. clarridgeiae* (MH348146), and *B. vinsonii* subsp. *berkhoffii* (local collection) were used as positive controls.

***Bartonella* species identification.** Amplicons generated from any one of the PCR assays used during the confirmatory steps were sequenced by Eurofins Genomics (Vimodrone, Italy). Chromatogram evaluation, primer deletion, and sequence alignment were performed using the software package Geneious version 10.1.3 (Biomatters Ltd., Auckland, New Zealand). *Bartonella* spp. were defined by comparing

similarities with other sequences deposited in the GenBank database using the basic local alignment search tool. The neighbor-joining method was used with data resampled 1,000 times to estimate the confidence of branching patterns. Representative sequences obtained in the study were submitted to the GenBank database.

Statistical tests. A Chi-squared test was used to evaluate categorical data, and the Fisher's exact test was used to evaluate categorical data with groups < 5. Significance was set as *P*-value < 0.05. All statistics were performed on commercially available software (SAS version 9.4 2013 SAS Institute Inc, Cary, NC).

RESULTS

Of the 20 dogs from Ganj Tappeh, half of them were males, and age ranged from 7 months to 8 years (median: 2 years; mode: 1 year) (Table 1). The majority (80%) were of mixed breed, and the body condition was good to average for 80% of the dogs. Most of the dogs were also infested with ectoparasites, 13 (65%) had fleas and three (15%) lice. Of the 46 dogs from the breeding colony at Cheshmeh Qassaban, 16 (34.8%) were males and 30 (65.2%) were females. The age of dogs ranged from 8 months to 15 years (median: 2 years; mode: 1 year). Most dogs were from specific breeds and four dogs were mongrels. Body condition was good for the majority (72%) of the dogs. Many dogs were infested with ectoparasites: 32 (70%) had ticks, eight (17%) had fleas, and six (13%) had lice, including seven dogs with both fleas and ticks and six dogs with both ticks and lice.

Nineteen (95%) of the 20 rescued stray dogs were seropositive for *B. henselae* and *B. vinsonii* subsp. *berkhoffii*, with titers ranging from 1:64 (one dog) to > 1:1,024 (two dogs), and 18 (90%) were seropositive for *B. clarridgeiae*, with titers ranging from 1:64 (two dogs) to 1:1,024 (three dogs) (Table 2). Thirty (65.2%) of the 46 breeding dogs were seropositive for at

TABLE 3

Bartonella species molecularly detected in dog blood samples from Hamadan, Iran

Dog	Real-time PCR		cPCR		Strain/clone ID†	<i>Bartonella</i> sp. (% identity)§	Accession number
	<i>ssrA</i>		<i>ssrA</i>	ITS*			
1	+	+	+	+	Iran_GT_3a Iran_GT_3b	<i>B. vinsonii berkhoffii</i> type III (100)§ <i>Candidatus B. merieuxii</i> (100)§ Clone Ca-1 KU316211 (100)	MK773857 MK775033, MK780190
2	+	+	+	+	Iran_GT_4	<i>B. vinsonii berkhoffii</i> type III (100)§	MK773858
3	+	+	+	+	Iran_GT_5a Iran_GT_5b	<i>B. vinsonii berkhoffii</i> type III (100)§ <i>B. rochalimae</i> (100)	MK773859 MK780191
4	+	+	+	+	Iran_GT_6	<i>B. vinsonii berkhoffii</i> type III (100)§	MK773860
5	+	+	+	+	Iran_GT_7	<i>B. vinsonii berkhoffii</i> type III (100)§	MK773861
6	+	0	0	0	Iran_CQ_23		
7	+	0	0	0	Iran_CQ_25		
8	+	+	+	0	Iran_CQ_34	<i>Candidatus B. merieuxii</i> (100)§	MK775034
9	+	+	+	+	Iran_CQ_36	<i>B. vinsonii berkhoffii</i> type III (100)§	MK773862
10	+	0	0	0	Iran_CQ_44		
11	+	0	0	0	Iran_CQ_45		
12	+	0	0	0	Iran_CQ_49		
13	+	0	0	+	Iran_CQ_51	<i>B. vinsonii berkhoffii</i> type III (100)§	MK773863
14	+	0	0	0	Iran_CQ_53		
15	+	+	0	0	Iran_CQ_64	Clone Ca-1 KU316211 (100)	ns
16	+	0	+	0	Iran_CQ_65	<i>B. rochalimae</i> (100)§	MK775286

CQ = Cheshmeh Qassaban; GT = Ganj Tappeh; ITS = internal transcribed spacer; ns = not submitted to GenBank.

* PCR assay performed by using the generic primers 325 s and 1100 as.

† PCR assay performed by using the *Bartonella vinsonii* subsp. *berkhoffii* specific primers 46 s and 590 as.

‡ Dog identity from GT and CQ localities.

§ Identity based on the ITS sequence.

|| Identity based on the *ssrA* sequence.

Bartonella DNA was detected in 16 of 66 (24.24%, 95% CI: 13.90–34.58) dogs, using *ssrA* real-time PCR. However, only 10 of these 16 dogs were PCR positive through cPCR assay, targeting the ITS region or the *ssrA* gene (Table 3). All sequences obtained during the present study were deposited in the GenBank database under ITS accession numbers: MK773857–MK7738563, MK775033–MK775034, and MK775286, and *ssrA*: MK780190–MK780191. Using ITS and *ssrA* gene-combined PCR assays and sequence analyses, seven of the 66 dogs (10.61, 95% CI: 3.18–18.03) were infected by *B. vinsonii* subsp. *berkhoffii* type III, two (3.03%, 95% CI: 0.00–7.17%) were infected with *B. rochalimae*, and three (4.55%, 95% CI: 0.00–9.57%) with *Candidatus B. merieuxii* (Table 3). More specifically, five (25%) rescued stray dogs were PCR positive for *B. vinsonii* subsp. *berkhoffii* type III, with two dogs coinfecting, one with *B. rochalimae* and one with *Candidatus B. merieuxii* (Table 3, Figure 1). Four of these five dogs were 1 year old. Similarly, five (10.8%) dogs from the breeding colony were PCR positive for *Bartonella*, including two dogs

Stray dogs were 10 times (odds ratio (OR) = 10.13, 95% CI: 1.24–82.7; $P = 0.03$) more likely to be seropositive and eight times (OR = 8.82, 95% CI: 2.68–29.11; $P = 0.0004$) more likely to be flea infested than dogs from the breeding colony. They were also 2.73 times more likely to be PCR positive, but that difference was not statistically significant ($P > 0.05$).

Several zoonotic *Bartonella* species were identified in dogs from Hamadan, Iran, with *B. vinsonii* subsp. *berkhoffii* type III being the most common *Bartonella* species. A study in shelter, stray, and pet dogs in Ankara, Turkey, also reported a common infection of dogs with *B. vinsonii* subsp. *berkhoffii* type III.⁷ Therefore, this *Bartonella* species seems to be endemic in the Middle East. Bacteremia was more prevalent in dogs less than 1 year old, as observed in our study, where eight of the 10 PCR-positive dogs were one to 2 years old, suggesting initial bacteremia at an early age when the dogs are fully receptive to infection. As identified in wild carnivores (jackals and red foxes) and fleas collected on domestic dogs in Israel,^{16–18} and in Iraq,⁹ infection with *B. rochalimae* was also identified in dogs in Iran. Three dogs were infected with *Candidatus B. merieuxii*, which has been shown to be endemic in dogs living in Southern Italy and Greece^{19,20} and in Iraq and Tunisia, where it was the only

Bartonella species detected in dogs.^{9,21} As previously reported in a dog from Greece coinfecting with *Candidatus B. merieuxii* and *B. henselae* SA2,²⁰ two Iranian dogs were coinfecting with *Bartonella vinsonii* subsp. *berkhoffii* and *Candidatus B. merieuxii* or *B. rochalimae*, respectively, underlining the lack of cross-protection between these various *Bartonella* species.

One of the most important observations was the very high prevalence of *Bartonella* antibodies in the rescued stray dogs, which is one of the highest ever reported in free roaming dog populations, indicating a very high exposure rate among heavily flea-infested dogs (65%). The prevalence was also very high among the breeding colony, despite a lower level of flea infestation but a high tick infestation. In Iraq, seroprevalence was 47.4% ($n = 97$ dogs), which is still lower than the 65.2% observed in the dog breeding colony in Hamadan. Similarly, seroprevalence for *B. vinsonii* subsp. *berkhoffii* was only 12% (40/333) in rural dogs from Turkey.⁸

The present study underlines the importance of dogs as sentinels of zoonotic infections, as *B. vinsonii* subsp. *berkhoffii*, including genotype III, and *B. rochalimae* have been identified as etiological agents of endocarditis cases in dogs^{22,23} and various types of symptoms in humans.⁴ Therefore, *Bartonella* seroprevalence in Iranian dog owners and possible bacteremia in Iranian people with unexplained fever or culture-negative endocarditis requires further investigation, especially in people from rural areas where herding and guarding dogs are common.

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