Case Report

Detection of a *Brucella*-like (Alphaproteobacteria) Bacterium in *Boophilus* spp. (Acari: Ixodidae) from Iran

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Ticks harbor many pathogenic, as well as endosymbiotic and non-pathogenic agents. They are host of a variety of as yet unidentified microbes that continue to be described. In the present study, a *Brucella*-like *bacterium* was detected in a *Boophilus* tick by PCR amplification of a partial fragment of 16S rRNA locus followed sequencing. Our results show that the members of the genus *Boophilus* may act as vectors of brucellosis in nature, but further studies are required to confirm the real role of ticks as vector or reservoirs of specific *Brucella* species. *J Med Microbiol Infect Dis, 2017, 5 (3-4): 66-68. DOI: 10.29252/JoMMID.5.3.4.66*

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Regarding medical and veterinary medicine, the ticks of the genus *Boophilus* harbor many arthropod-borne pathogens such as the species belonging to the genera *Babesia*, *Borrelia*, and *Anaplasma* worldwide [1]. Also, the members of this genus are frequently associated with various endosymbiotic, non-pathogenic and vertically transmitted bacteria of *Coxiella*-, *Francisella*- and *Rickettsia*-like organisms. The endosymbionts of the ticks are very similar to the tick-transmitted pathogens suggesting that the ancestral origin of these endosymbionts might have been vertebrate pathogens acquired by the ticks while feeding on infected hosts [2, 3]. Ticks are hosts of various as yet unidentified microbes [4]. The family *Brucellaceae* from the order Rhizobiales, class Alphaproteobacteria, phylum Proteobacteria, comprises the type genus *Brucella* and six other genera including *Ochrobactrum*, *Pseudochrobactrum*, and *Mycoplana* [5, 6]. Some species of the genus *Brucella* cause *brucellosis* in human and different animals and are almost invariably transmitted by direct or indirect contact with infected animals or their products [7]. The role of Argasid and Ixodid ticks as vectors or reservoirs of *Brucella* have already been speculated [8]. Previously, the existence of *Brucella abortus* in sucking lice of several ruminants was reported by using real-time PCR [9].

In the present study, we collected ticks from Talesh county, Lisar protected area, Guilan Province, north of Iran. The specimens were identified based on morphological features described in a taxonomical key [10]. DNA of a single adult female tick belonging to the genus *Boophilus* was extracted using phenol-chloroform method [11], and a nested PCR to amplify a partial fragment of 16S rRNA was performed under a touchdown temperature profile using the primers designed in this study, (forward 1: 5′- ACC ATT TGC TAC GGA ATA ACT CAG -3′, reverse 1: 5′- CAG GCG GAA TGT TTA ATG CG -3′, forward 2: 5′- CCA AGG CGA CGA TCC ATA G -3′, reverse 2: 5′- CAC CTC AGC GTC AGT AAT GG -3′). Amplification program included an initial denaturation of 4 min at 95˚C, 11 cycles of denaturation at 94˚C for 50 sec, annealing at 60˚C for 60 sec with 1˚C decrease per cycle until 50˚C, extension for 60 sec at 72˚C, followed by 25 cycles of denaturation at 94˚C for 60 sec, annealing at 50˚C for 50 sec , extension at 72˚C for 60 sec and a final extension of 72˚C for 5 min. The PCR reactions (25 µl) contained 1.5 U of *Taq* DNA polymerase enzyme, 2.5 µl PCR 10x buffer, 2 mM $MgCl_2$, 200 µM dNTPs (all ingredients were from SinaClon®, Tehran, Iran), 0.5 µl forward and reverse primers (10 mM), template DNA (50-100 ng/µl) and 14.8 µl sterile water. The PCR products were visualized on 1% agarose gel electrophoresis under UV light. The amplicons were purified using a commercial gel extraction kit (GeneJET, Thermo Fisher Scientific) and were sequenced using the primer forward 2 used in the amplification. The resulting 468 bp was edited manually and BLASTed against the sequences deposited in GenBank database. The genetic distance among the sequence obtained in this study and 13 similar sequences from the

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GenBank database were calculated using maximum composite likelihood (MCL) model in MEGA7 software [12], and a phylogenetic tree was constructed using Bayesian inferences method (BI) in BEAST software (Fig. 1). The generated sequence was submitted to the GenBank under the accession number MG282918. The *Brucellaceae* family clade that included our sequence had 3% and 5% genetic differences with the *Rhizobiaceae* and *Bartonellaceae* families, respectively. The BLAST results showed the isolated bacterium from the tick was a *Brucella*-like species (Table 1). The members of the genus *Boophilus* seems to act as vectors of brucellosis in nature. Previously, the vectorial capacity of a soft tick, *Ornithodoros lahorensis*, in the transmission of *Brucella* agents was reported [13, 14]. Recently, DNA and RNA of *B. abortus* were detected in the sucking louse *Haematopinus tuberculatus* [9]. Also, the flies belonging to the genus *Stomoxys* and the family Tabanidae were reported as the mechanical vectors of *Brucella* [15, 16]. Further studies are required to confirm the real role of ticks as vectors or reservoirs of specific *Brucella* species in the epidemiology of brucellosis among animal and human populations.

Table 1. Similarity of the partial 16S rRNA sequence generated in this study with similar sequences from GenBank database

Species	Accession number	Identity	Query cover	Total score
Brucella suis	MF173089	98.3	98	752
***Brucella* sp.**	KC998937	98.3	98	752
Brucella melitensis	KC342250	98.3	98	752
Brucella canis	CP016977	98.1	98	747
Brucella ceti	KU324483	98.1	98	747
Brucella pinnipedialis	CP007743	98.1	98	747
Brucella microti	CP001578	98.1	98	747
Brucella ovis	CP000708	98.1	98	747
Brucella vulpis	NR_149245	97.8	98	741
Brucella neotomae	L26167	97.8	98	741
***Rhizobiaceae* bacterium**	MF526754	96.1	97	695
***Bartonellaceae* bacterium**	LT694673	93.1	98	630

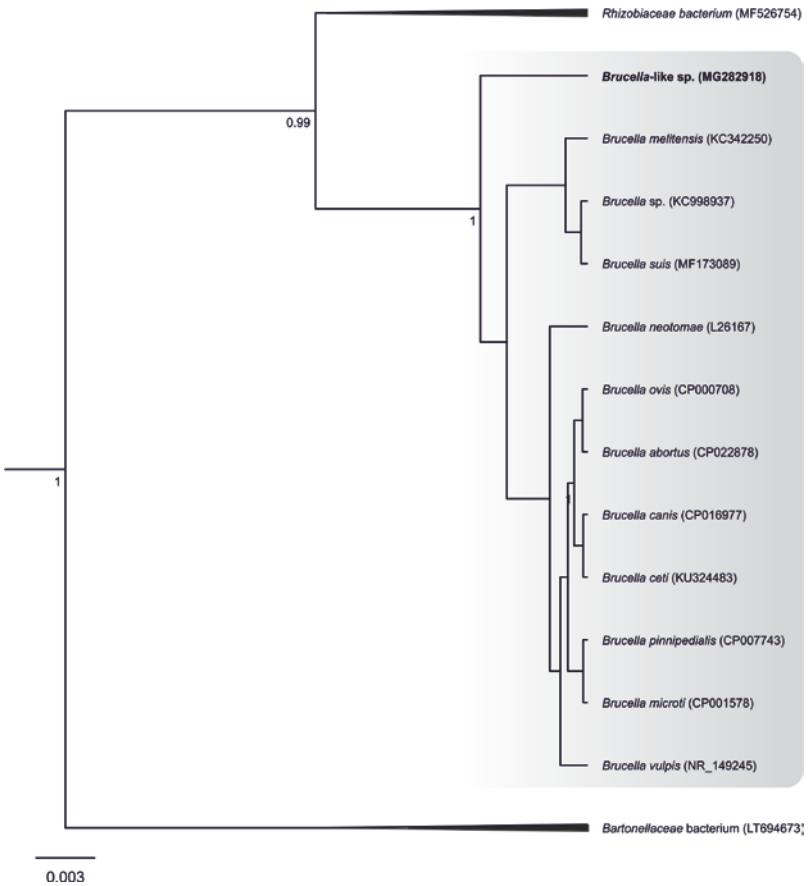

Fig. 1. Phylogenetic tree generated based on 16S rRNA sequence data of the *Brucella*-like species generated in this study and similar sequences from GenBank database constructed using Bayesian Inference method. The main clade of tree separated by a rectangular shape. The taxon of the present study is bold and defined with a name and GenBank accession number. Posterior probability values inserted in the place of nodes. Branch lengths are proportional to the evolutionary changes. The members of the families *Rhizobiaceae* and *Bartonellaceae* are included as outgroups.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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