Molecular detection of *Bartonella henselae* in blood samples obtained from owned cats in Kerman city using Nested-PCR

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Abstract

Bartonella henselae is an intracellular bacterium that causes zoonotic cat scratch disease (CSD). This disease has a global spread and is mainly transmitted through arthropod vectors. Since cats are identified as the main source of *B. henselae* infection, the aim of this study was to evaluate molecular detection of *B. henselae* in blood samples obtained from pet cats in Kerman using Nested-PCR. Seventy-two blood samples were collected from pet cats that were referred to the Shahid Bahonor University Veterinary Hospital. A Nested-polymerase chain reaction (PCR) was used to analyze the samples, with the 16S–23S rRNA internal transcribed spacer region as the target. *B. henselae* DNA was found in 5.5% (4/72) of blood samples. The results of this study showed that owned cats in Kerman could be one of the potential reservoirs of cat scratch disease, but to achieve definitive results, complementary studies must be performed on cat owners.

Keywords: *Bartonella henselae,* Iran, Kerman, Cat, Nested-PCR, CSD

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Introduction

Bartonella henselae is a gram-negative facultative intracellular bacterium, which causes a worldwide zoonotic disease named cat scratch disease (CSD) (Sayed *et al.,* 2022). Five to eighty-six percent of cats, as the main reservoir, are infected with *B. henselae*, globally (Wechtaisong *et al*., 2020).

A wide range of blood-sucking arthropods such as ticks (mostly *Ixodes ricinus*), fleas (*Ctenocephalides felis felis*), mosquitos, and lice are considered as vectors of this bacterium (Cotté *et al*., 2008; Iannino *et al*., 2018; Wechtaisong *et al*., 2020). *B. henselae* could be entered into the flea feces and inoculated by contaminated cat claws (Telford III and Wormser, 2010; Sayed *et al*., 2022). The majority of infected cats show no symptoms, however in rare instances, some animals might reveal ocular problems, endocarditis, myocarditis, lymphadenopathy, neurological dysfunction, etc. (Grippi *et al*., 2021; Sayed *et al*., 2022). Cases of CSD in humans are asymptomatic to symptomatic including cutaneous inflammations, fever and lymphadenopathy (Iannino *et al.,* 2018). CSD is usually self-limiting in humans and occurs mainly in children and adolescents with different clinical symptoms (Mazur-Melewska *et al*., 2015). Also, rare vascular proliferative lesions including bacillary angiomatosis and peliosis hepatitis are recorded in immunocompromised patients with *B. henselae* infection (Arisoy *et al.,* 1999; Chian *et al*., 2002).

Diagnosis in humans is usually based on clinical findings, skin tests, serological techniques based on IgG and IgM detection, indirect fluorescent antibody methods (IFA) and recently polymerase chain reaction (PCR) of patient tissues such as lymph nodes (Ksiaa *et al*., 2019). The gold-standard diagnostic techniques for *Bartonella* infection in human and animals include bacterial culture of blood samples, PCR, and DNA sequencing of the isolates (Sato *et al*., 2017). Bacterial culture of blood samples should be repeated several times due to the recurrent nature of the disease and fastidious nature of the organism. Primary culture of *Bartonella* requires a long time that might last up to 4 weeks (Brenner *et al*., 1997; Breitschwerdt *et al*., 2008). Culture, serological and molecular techniques are frequently used to diagnose this infection in cats (Hansmann *et al*., 2005; Girma *et al*., 2019). Diagnosis is often difficult due to the subclinical nature of the disease. Therefore, it is very important to use fast and definitive diagnostic methods in the feline population (Iannino *et al*., 2018). Molecular methods are one of the most accurate methods for identifying pathogens (Galluzzi *et al*., 2007). Nested-PCR is employed when it is essential to increase the specificity and/or sensitivity of PCR (Green and Sambrook, 2019). Nested-PCR method has been described as an appropriate technique for *B. hensellae* detection with high sensitivity and specificity (Sato *et al*., 2017; Green and Sambrook, 2019).

Since *Bartonella* pathogens can use multiple vectors and can infect a diverse range of hosts, diverse research should be conducted on *Bartonella* prevalence, infection risks, and pathobiology in hosts in different geographic regions. So, the purpose of this research is molecular detection of *B. henselae* in blood samples obtained from pet cats in Kerman city using Nested-PCR.

Materials and methods

Sampling and DNA extraction

In this study, a total of 72 blood samples were collected from pet cats in Kerman province between July and September 2022 in veterinary hospital of the Shahid Bahonar University with the consent of their owners. All cats were clinically healthy. Anticoagulant tubes with EDTA were used to collect 3 cc of blood from the cephalic vein. Then, the blood samples were transferred to the microbiology laboratory at -20°C for DNA extraction using Microcalman kit (Cat. NO. A101201, ParsTous, Mashhad, Iran) based on the instructions.

Nested-PCR condition

DNA of *B. henselae* was screened via Nested-PCR. The genus *Bartonella* and the species *B. henselae* were identified by screening the specific sequences on 16s-23s rRNA ITS (intergenic spacer) region. The second PCR was conducted using the products obtained from the first PCR to identify *B. henselae*. DNA of *B. henselae* provided by Bu-Ali Sina University of Hamadan was used as positive control. Nuclease-free distilled water was used for negative control in PCR. The primer sequences and PCR conditions are shown in Table 1.

Table 1: Primer sequences and PCR condition to identify the genus *Bartonella* and the species *B. henselae*.

PCR	Target species	Primer	Sequence (5'–3')	PCR condition	Product size (bp)	Reference
First	*Bartonella* species	UR Barto1	CTTCGTTTCTCTTTCTTCA	95°C (3 m), 40 cycles: 95°C (30s), 50°C (30s), 72°C (1 m), 68°C (3 m)	700–722	Sato *et al*., 2017; Rolain *et al*., 2003)
		UR Barto2	CTTCTCTTCACAATTTCAAT			
Second	*B. henselae*	UR Bhen-f	TTGCTTCTAAAAAGCTTATCAA		254	Sato *et al*., 2017
		UR Bhen-r	CAAAAGAGGGATTACAAAATC			

PCR volume was 25 µL containing 1 µL DNA extract, 0.5 µL of each primer (from 20 µM initial concentration), 10 µL 2× ready to use Taq DNA Polymerase Master Mix (ParsTous, Iran) and sterile distilled water up to reaction volume for both stages of nested-PCR. The PCR products were electrophoresed on a 1.3 % agarose gel for 45 minutes at 110 V and The GelDoc 1000 was used for the imaging with UV (Vilber Lourmat, France).

Result

In this study, out of 72 blood samples obtained from domestic cats in Kerman city, four samples (5.5%; 95%CI, 1.5%-13.6%) were evaluated as positive for

B. henselae DNA in the Nested-PCR method (Figs. 1 to 3). All four samples were clinically completely healthy.

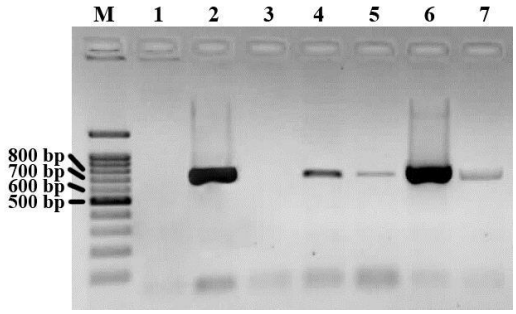

Figure 1: Nested-PCR test (first PCR) to screen *Bartonella* spp. DNA; M: marker 100 bp, 1: negative control, 2: positive control (700 bp), 3: negative sample, 4-7: positive samples.

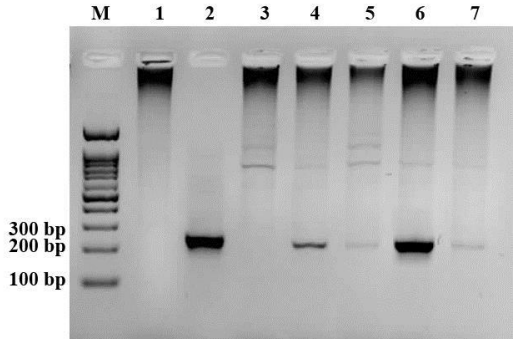

Figure 2: Nested-PCR test (second PCR) to screen the specific sequences of *B. henselae*; M: marker 100 bp, 1: negative control, 2: positive control (bp254), 3: negative sample, 4-7: positive samples.

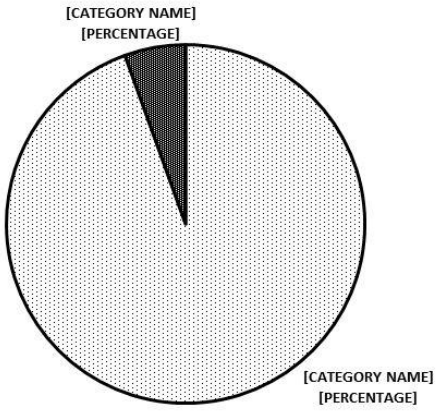

Figure 3: Prevalence of positive and negative samples for *B. Henselae.*

Discussion

Our results revealed that the prevalence of *B. henselae* in the southeast of Iran (Kerman) was lower than other findings in recent works in Iran, approximately 1.4-23% in central and northern parts (19–22). Also, according to recent studies, this rate is 9.1% in Saudi Arabia (23), 8% in Egypt (1), 2.22% in Japan (11), 3.94% in China (24), 9.1% in Cyprus (25), 4.7-58.8% in Greece (26–28), 2.72-47.3% in Italy (6,29), 31.2% in the United States (30) and 16.82% in Brazil (31) (Table 2). All positive cats for *B. henselae* in this study were found without clinical signs of the disease which is in agreement with most researches; infected cats don't display any clinical symptoms and seem to tolerate persistent bacteremia without clinical abnormalities (Raimundo *et al*., 2019). However, some studies have revealed that *Bartonella* species in rare cases may be the cause of some clinical conditions, including fevers with unclear causes, disorders of the central nervous system, endocarditis, stomatitis, pyogranulomatous myocarditis and diaphragmatic myositis (Álvarez-Fernández *et al*., 2018; Grippi *et al*., 2021; Sayed *et al*., 2022). This high prevalence of asymptomatic bacteremia suggests that *Bartonella* and their feline hosts have a long history of coevolution (Aboudharam *et al*., 2005; Raimundo *et al*., 2019). Therefore, cat owners and veterinary clinic workers in endemic areas may acquire *Bartonella* infection from these animals. Oteo *et al*. (2017) evaluated the molecular and serological prevalence of *Bartonella*

infections in veterinary personnel in Spain and found *Bartonella* infection in cats could be considered as a health risk for people related to the cats (Oteo *et al*., 2017).

Table 2: Recent prevalence of *B. Henselae* infection all around the world.

Year	Country	Prevalence	Method	Samples	Cat	Reference
2008	Iran	23 %	IFA	Blood	Domestic[1]	Oskoueizadeh *et al*., 2008
2010	Iran	10.9 %	PCR	Blood, saliva and nail	Domestic	Oskouizadeh *et al*., 2010
2011	Iran	12.5 %	PCR	Paw, mouth specimens and blood	Domestic	Oskouizadeh e*t al.*, 2011
2016	Iran	7.14 %	PCR	Nail and saliva	Domestic	Mazaheri Nezhad Fard *et al*., 2016
2017	Greece	4.7 %	PCR	Blood	Stray	Diakou *et al.*, 2017
2017	Italy	2.72 %	PCR	Blood and conjunctival swabs	Pet	Otranto *et al*., 2021
2017	Japan	2.22 %	Nested-PCR	Blood	Pet	Sato *et al*., 2017
2017	Cyprus	10.9 %	PCR	Blood	Domestic	Attipa *et al*., 2017
2018	Greek	6.38 %	PCR	Blood	Pet	Mylonakis *et al*., 2018
2019	China	3.94 %	Nested-PCR	Blood	Pet	Zhang *et al*., 2019
2021	Italy	34.5 %	Real-time PCR	Blood	Stray	Grippi *et al*., 2021
2021	USA	31.2 %	IFA	Sera	Domestic	Osikowicz *et al.*, 2021
2022	Greek	35.4 %	IFA	Sera	Domestic	Kokkinaki *et al*., 2022
2021	Saudi Arabia	9.1 %	PCR	Blood	Stray	Alanazi *et al*., 2021
2022	Egypt	8 %	PCR	Blood	Domestic	Sayed *et al*., 2022
2022	Brazil	16.82 %	PCR	Blood	Domestic	Raimundo *et al*., 2019

[1] Domestic cats include pet and stray cats

Various factors can affect the prevalence of *Bartonella* in the population of domestic cats in an area. Some of these factors include sample size, climatic conditions, environmental factors, public health surveillance, flea infestation, socio-economic status, type of diagnostic method (serological or molecular), type of under study population (stray cats versus domestic cats), primary infections. and secondary (Table 2) (Álvarez-Fernández *et al*., 2018; Kokkinaki *et al*., 2022; Sayed *et al*., 2022). For example, incidence of the infection in various climates is different (0% in Norway to 68% in the Philippines) (Álvarez-Fernández *et al*., 2018; Sayed *et al*., 2022). It is believed that a hot, humid atmosphere is ideal for the growth of ectoparasites like cat fleas. In Mediterranean and European countries, where temperature and

humidity are ideal for flea and tick infestation, seroprevalence in cats has been reported to be high (Sato *et al*., 2017; Álvarez-Fernández *et al*., 2018). It seems that the hot and dry climate of Kerman is not suitable for fleas' infestation which is the most important risk factor for *Bartonella* infection. The prevalence of infected cats in this study is less than Shahrekord and Tehran cities, which have a more humid climate.

All cats of this study were indoor pet cats with a good health condition which decrease the chance for ectoparasite infestation. Pet cats have a higher quality of life compared with stray cats and they have better health status because of veterinary surveillance and good nutrition. Besides, pet cats benefit from contraception surgeries which decrease their free roaming, deworming and vaccinations which improve their health status. Besides, routine veterinary checkup's along with prescription of anti _flea collars and repellent insecticide decrease less environmental exposure to *Bartonella* vectors so they have lower rate of *Bartonella* infection in compare with stray cats in agreement with findings reported by Eskuizadeh *et al*. (Oskoueizadeh *et al*., 2008; Oskouizadeh *et al*., 2010).

Conclusion

The results of this study showed that owned cats in Kerman could be one of the potential reservoirs of cat scratch disease, but to achieve definitive results, more extensive studies are needed. It is recommended that complementary researches were done by different clinical samples including saliva and nail to increase the screening accuracy. Surveillance of infected cat owners was also highly recommended.

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