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Molecular Prevalence and Epidemiological Risk Factors of *Bartonella henselae* Infection in Owned and Non-Owned Cats in Ahvaz district, Iran

Sara Hajizadeh[1], Bahman Mosallanejad[2*], Darioush Gharibi[3], Mohammad Razi Jalali[2] and Mahdi Pourmahdi Borujeni[4]

[1] DVSc Student of Small Animal Internal Medicine, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[4] Professor, Department of Food Hygiene, Section of Epidemiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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Abstract

Bartonella henselae (*B. henselae*) is a zoonotic bacterium for which domestic cats serve as the primary reservoir host, maintaining persistent bacteremia and facilitating transmission mainly through the cat flea, *Ctenocephalides felis*. Given the public health significance of feline infection and the limited molecular epidemiological data available from southwestern Iran, this study aimed to determine the molecular prevalence of *B. henselae* and identify independent epidemiological risk factors associated with infection in owned and non-owned cats in Ahvaz, Iran. A cross-sectional study was conducted involving 160 cats, equally distributed between owned and non-owned populations. Adult cats constituted 65% of the study population, whereas juveniles accounted for 35%; 55% were male and 45% were female. Domestic Shorthair cats represented the predominant breed type (51.9%). Whole-blood, oral-swab, and nail samples were collected and analyzed using conventional polymerase chain reaction (PCR) targeting a 414-bp fragment of the htrA , followed by sequencing confirmation of representative amplicons. Associations between potential risk factors and infection were assessed using univariable and multivariable logistic regression analyses, and population attributable fractions (PAFs) were estimated. *B. henselae* DNA was detected in 16.9% of cats (95% CI: 11.4–23.7). The prevalence of infection was significantly higher in non-owned cats (26.3%) than in owned cats (7.5%) (OR = 4.39, 95% CI: 1.66–11.58; $p = 0.002$). Population attributable fraction analysis indicated that 67% and 36% of infections were attributable to outdoor exposure and flea infestation, respectively. These findings demonstrate that environmental exposure and ectoparasite infestation are major determinants of *B. henselae* infection, highlighting the importance of effective flea control and restricting outdoor access to reduce the burden of infection and mitigate zoonotic risk.

Key words: *Bartonella henselae*; Cats; Molecular prevalence; Epidemiology; Zoonosis

Introduction

Bartonella henselae (*B. henselae*) is a globally distributed zoonotic bacterium of considerable veterinary and public health importance. It is a small, fastidious, Gram-negative, facultative intracellular bacterium belonging to the genus *Bartonella* within the family *Bartonellaceae*. Members of this genus are vector-borne pathogens that

* **Corresponding Author**: Bahman Mosallanejad, Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: bmosallanejad@scu.ac.ir

infect a wide range of mammalian hosts and are characterized by their ability to invade erythrocytes and endothelial cells, resulting in persistent bloodstream infections (Osikowicz et al, 2021).

Domestic cats serve as the primary reservoir host, maintaining long-term intraerythrocytic bacteremia with limited or no clinical manifestations. Transmission among cats occurs primarily through hematophagous arthropods, particularly the cat flea, *Ctenocephalides felis*, which plays a central role in maintaining the bacterium within feline populations (Mazurek et al, 2020). Human infection may result in cat scratch disease and, in susceptible individuals, more severe systemic manifestations (Yakubovsky et al, 2026). The close association between cats and humans, particularly in urban environments, underscores the need to better understand infection dynamics within feline reservoirs.

The reported prevalence of *B. henselae* infection in cats varies widely across geographic regions, reflecting differences in climate, vector ecology, population structure, and management practices (Zarea et al, 2023). Studies conducted in Europe, Asia, and the Americas have identified several epidemiological factors associated with an increased risk of infection. Outdoor access has consistently been linked to higher infection rates because of increased exposure to vectors and contact with infected cats (Stepanić et al, 2024; Lopes et al, 2026). Stray or free-roaming status is frequently associated with greater environmental exposure and reduced access to preventive care (Sepúlveda-García et al, 2023). Flea infestation remains the most biologically plausible and consistently reported risk factor (Kokkinaki et al., 2022). Age has also been implicated, with adult cats often exhibiting higher prevalence, likely reflecting cumulative exposure over time (Álvarez-Fernández et al, 2022; Ebani et al, 2021). Additional studies from Europe and North America have further documented the circulation of *Bartonella* spp. in domestic cat populations (Mazurek et al, 2020; Osikowicz et al, 2021).

In the Middle East, molecular evidence of *B. henselae* infection has been reported in cats and humans in Egypt (Sayed et al, 2022). In Iran, data on feline bartonellosis remain limited. An earlier investigation conducted in Tehran confirmed the presence of *B. henselae* among domestic cats (Oskoueizadeh et al, 2008). Furthermore, a study conducted in Ahvaz identified *B. henselae* infection in dogs, suggesting the possibility of shared transmission cycles within the region (Oskoueizadeh et al, 2013). However, information from southwestern Iran remains scarce. Ahvaz is characterized by a hot climate and prolonged warm seasons that may favor vector survival and reproduction. Urban expansion, a substantial stray cat population, and variable standards of preventive veterinary care may further influence transmission dynamics. Molecular epidemiological studies investigating infection prevalence and associated risk factors in this region are therefore warranted.

This study aimed to determine the molecular prevalence of *B. henselae* infection in owned and non-owned cats in Ahvaz, Iran, and to identify independent epidemiological risk factors associated with infection. By focusing on molecular detection and the analytical assessment of demographic and management-related variables, this investigation sought to clarify the epidemiological drivers of infection within an urban feline population in southwestern Iran.

Materials and Methods

Study Design, Population, and Ethical Approval

This cross-sectional study was conducted between March 2024 and March 2025 in Ahvaz, Khuzestan Province, Iran. A total of 160 domestic cats were enrolled. The sampling framework was designed to

ensure equal representation of owned and non-owned animals, comprising 80 owned cats presented to the Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz and 80 non-owned cats. The non-owned group included 60 free-roaming stray cats captured humanely within urban districts and 20 cats housed in municipal shelters.

Owned cats were defined as animals living under direct human care with an identifiable caretaker. These were further categorized according to management conditions as indoor-only, referring to cats permanently confined within the household environment, and indoor-outdoor, referring to cats with regular or intermittent access to external environments. Non-owned cats were classified as stray when free-roaming without permanent human guardianship and as shelter-housed when maintained in organized municipal or private shelter facilities.

All animals underwent a complete physical examination prior to sampling. Cats showed no clinical signs suggestive of severe systemic or acute infectious disease and were considered clinically stable at the time of sampling. Inclusion criteria required apparent clinical stability and the absence of severe systemic disease requiring emergency intervention. No animals were excluded based on sex, breed, or age.

All procedures were conducted in accordance with institutional guidelines for animal care and use. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Shahid Chamran University of Ahvaz. Written informed consent was obtained from owners prior to enrollment of privately owned cats. Sampling of stray and shelter cats was performed under the supervision of municipal veterinary authorities, ensuring minimal stress and humane handling.

For each cat, demographic and management data were recorded using a standardized questionnaire designed by the research team. For owned cats, information was obtained through structured owner interviews. For non-owned cats, data were obtained through direct assessment and shelter records when available.

Age was categorized as juvenile (younger than 12 months) and adult (12 months of age or older), based on dental examination and available history. Sex was recorded as male or female. Breed was classified as Domestic Shorthair, Domestic Longhair, or purebred according to phenotypic characteristics and owner reports when available.

Outdoor access was coded as a binary variable and defined as present when a cat had any degree of access to outdoor environments and absent when it was permanently confined indoors. Flea infestation was assessed through direct visual inspection and combing using a fine-toothed flea comb (Hauptner Herberholz, Solingen, Germany). The presence of live fleas or flea dirt was recorded as positive. Tick infestation was evaluated by systematic palpation and visual examination of the entire body surface and coded as positive when at least one tick was detected.

Dietary regimen was categorized into three groups: commercial dry food, home-cooked food, and raw diet or active hunting behavior. History of systemic illness within the previous six months was recorded as present or absent based on owner reports or shelter documentation. Antibiotic administration during the preceding three months was documented and coded as yes or no.

All categorical variables were numerically coded prior to statistical analysis. Binary variables were coded as 1 for presence and 0 for absence. Multilevel categorical variables were transformed into dummy variables for inclusion in regression modeling where appropriate.

From each enrolled cat, three biological samples were collected under aseptic conditions, resulting in a total of 480 samples. Whole blood was obtained via

cephalic or saphenous venipuncture using sterile disposable needles and syringes and collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes (Teif Azma Tajhiz Company, Tehran, Iran). These peripheral veins were selected to minimize invasiveness and animal discomfort, particularly in non-owned and stray cats where restraint is challenging. Approximately 1.5–2.0 mL of blood was collected from each animal.

Oral samples were collected using sterile nylon flocked swabs (Zist Fanavaran Mehr Company, Tehran, Iran). The swab was gently rotated along the gingival margin and buccal mucosa for approximately 10 seconds and then placed into sterile transport tubes.

Nail samples consisted of freshly clipped distal claw tips obtained using sterile stainless-steel nail clippers (Rahpooyan Dampezeshki Company, Tehran, Iran). Each clipping was transferred into a sterile 1.5-mL microcentrifuge tube (Arvin Tajhiz Azma Company, Tehran, Iran).

All samples were transported to the Molecular Microbiology Laboratory within two hours of collection in insulated containers and stored at −20 °C until DNA extraction.

Genomic DNA was extracted from whole blood, oral swab, and nail samples using a silica membrane based commercial DNA extraction kit (Sinacolon, Tehran, Iran) according to the manufacturer's standard instructions. Because of the different physical and biological characteristics of the sample types, sample specific pretreatment protocols were applied as follows before proceeding with the main extraction procedure.

For whole blood samples (100 microL), samples were mixed directly with lysis buffer and Proteinase K according to the standard kit protocol without any additional pretreatment.

For oral swab samples, each swab was first immersed in 200 microL of sterile distilled water and incubated at 55 degrees Celsius for 30 minutes with vortexing every 5 minutes to facilitate detachment of cellular material. Thereafter, 100 microL of the resulting suspension was used for DNA extraction according to the same kit protocol.

For nail samples, nail clippings (approximately 10 to 20 mg) were first pulverized using a sterile mortar and pestle under liquid nitrogen to obtain a fine powder. The powdered tissue was then incubated with 40 microL of Proteinase K instead of the standard 20 microL, along with 200 microL of lysis buffer, at 56 degrees Celsius for 1 hour with intermittent shaking before proceeding with the standard kit protocol.

After these pretreatment steps, the extraction protocol continued according to the manufacturer's instructions. Briefly, samples were lysed using lysis buffer and Proteinase K, followed by incubation and binding of DNA to a silica membrane column. After sequential washing steps to remove impurities, purified DNA was eluted using 50 microL of elution buffer.

DNA concentration and purity were assessed spectrophotometrically using a microvolume spectrophotometer (Parto Tandis Company, Tehran, Iran). Extracted DNA was stored at minus 20 degrees Celsius until amplification.

Detection of Bartonella henselae DNA was performed by conventional polymerase chain reaction (PCR) targeting a 414 bp fragment of the htrA , as previously described (Diniz et al, 2007; Anderson and Neuman, 2000). All extraction kits used in this study were obtained from Sinacolon Company (Tehran, Iran).

Oligonucleotide primers were synthesized commercially by Sinacolon Company (Tehran, Iran). The primer sequences were as follows: forward primer BartF (5' to 3'): GATTCAATTGGTTTGAAGGAGGCT and reverse primer BartR (5' to 3'): TCACATCACCAGGACGTATTC.

Each reaction was carried out in a total volume of 25 microL containing 12.5 microL of 2 times PCR Master Mix (BioTeb Company, Tehran, Iran), 0.5 microM of each primer, 5 microL of template DNA, and nuclease free water (Parto Tandis Company, Tehran, Iran). Amplification was performed in a thermal cycler (BIO RAD T100, Bio Rad Laboratories, USA). Cycling conditions consisted of an initial denaturation at 95 degrees Celsius for 5 minutes, followed by 35 cycles of denaturation at 94 degrees Celsius for 30 seconds, annealing at 58 degrees Celsius for 30 seconds, and extension at 72 degrees Celsius for 45 seconds, with a final extension step at 72 degrees Celsius for 7 minutes.

A reference strain of B. henselae (ATCC 49793) was obtained from the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, and served as a positive control in each run. Sterile nuclease free water was used as a negative control. Amplified products were separated by electrophoresis on 1.5 percent agarose gels stained with GelRed and visualized under ultraviolet illumination using a gel documentation system (Ariya Mabna Tajhiz Company, Tehran, Iran).

Five representative positive amplicons exhibiting clear and distinct bands were selected for sequencing (Figure 1). PCR products were purified using a commercial purification kit (Codon Genetics Company, Tehran, Iran). Bidirectional Sanger sequencing was performed by a commercial sequencing provider (Codon Genetics Company, Tehran, Iran).

Raw chromatograms were examined and edited using sequence analysis software (Technelysium Pty Ltd, South Brisbane, Australia). Consensus sequences were generated and compared with reference sequences available in the National Center for Biotechnology Information (NCBI) database using the BLASTn algorithm. All analyzed sequences demonstrated 99–100% nucleotide identity to reference *B. henselae* sequences, confirming species-level identification. The validated sequences were submitted to GenBank, and accession numbers were obtained.

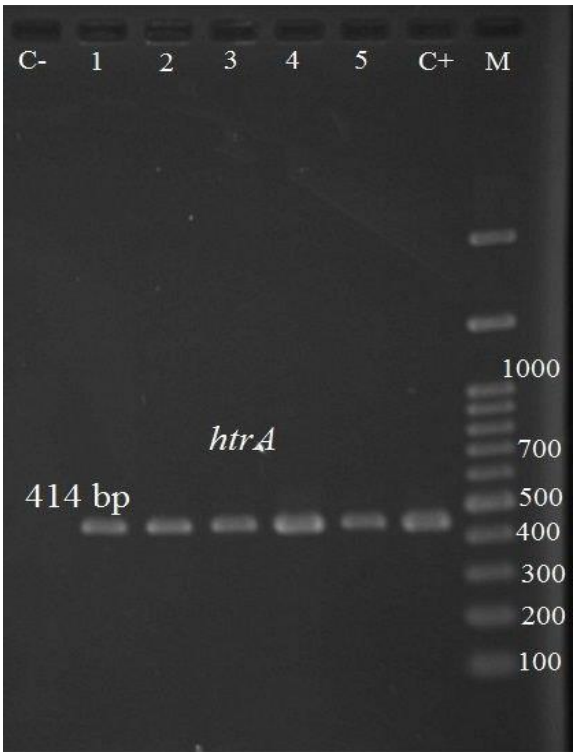

Figure 1: Representative agarose gel electrophoresis of PCR products showing the expected 414-bp amplicon. M: 100-bp DNA ladder; C−: negative control; C+: positive control; lanes 1–5: representative positive clinical samples.

Statistical analyses were performed using IBM SPSS Statistics version 26. Molecular prevalence was calculated as the proportion of PCR-positive animals among the total examined population, and exact binomial 95% confidence intervals were computed. Associations between categorical variables and PCR status were initially evaluated using the chi-square and Fisher's exact test when expected cell counts were less than five, and crude odds ratios with corresponding 95% confidence intervals were calculated.

Variables with *p*-values < 0.10 in univariable analyses were considered eligible for inclusion in the multivariable logistic regression model, and a backward stepwise procedure was applied to construct

the final model while maintaining an appropriate events-per-variable ratio. Multicollinearity was assessed using variance inflation factor and tolerance statistics, and highly correlated variables were not entered simultaneously into the model. Adjusted odds ratios with 95% confidence intervals were reported.

Model calibration was evaluated using the Hosmer–Lemeshow goodness-of-fit test, and explanatory performance was assessed using Nagelkerke R^2. Population attributable fractions (PAFs) were calculated for significant independent risk factors according to the formula PAF = Pe (AOR − 1) / [Pe (AOR − 1) + 1], where Pe represents the prevalence of exposure in the study population. Statistical significance was defined at a two-sided alpha level of 0.05.

Results

Population Characteristics

A total of 160 cats were included in the study, comprising 80 owned and 80 non-owned animals. The demographic and management characteristics of the study population are presented in Table 1. Adult cats represented 65.0% (104/160) of the population, whereas 35.0% (56/160) were classified as juveniles. The sex distribution was balanced, with 55.0% males (88/160) and 45.0% females (72/160). Domestic Shorthair cats were the most common breed type, accounting for 51.9% (83/160) of the study population, followed by purebred cats (31.2%; 50/160) and Domestic Longhair cats (16.9%; 27/160).

With respect to housing conditions, 32.5% (52/160) of cats were kept exclusively indoors, 17.5% (28/160) were classified as indoor-outdoor, 12.5% (20/160) were shelter-housed, and 37.5% (60/160) were classified as stray. Overall, 67.5% (108/160) of cats had some degree of outdoor access. Flea infestation was detected in 28.7% (46/160) of animals, whereas tick infestation was uncommon, occurring in only 1.9% (3/160) of cats. Approximately 27.5% (44/160) of cats were reported to consume a raw diet or engage in hunting behavior.

Molecular Prevalence of *B. henselae*

Molecular analysis identified 27 PCR-positive animals among the 160 cats examined, resulting in an overall prevalence of 16.9% (27/160; 95% CI: 11.4–23.7) (Table 2).

Table 1: Baseline Characteristics of the Study Population (n = 160)

Variable	Category	n (%)
Age	Juvenile (<12 months)	56 (35.0)
	Adult (≥12 months)	104 (65.0)
Sex	Male	88 (55.0)
	Female	72 (45.0)
Breed	Domestic Shorthair	83 (51.9)
	Domestic Longhair	27 (16.9)
	Purebred	50 (31.2)
Ownership status	Owned	80 (50.0)
	Non-owned	80 (50.0)
Housing category	Indoor-only	52 (32.5)
	Indoor–outdoor	28 (17.5)
	Shelter-housed	20 (12.5)
	Stray	60 (37.5)
Outdoor access	Yes	108 (67.5)
	No	52 (32.5)
Flea infestation	Positive	46 (28.7)
	Negative	114 (71.3)
Tick infestation	Positive	3 (1.9)
	Negative	157 (98.1)
Diet	Commercial dry food	74 (46.3)
	Home-cooked food	42 (26.2)
	Raw/hunting	44 (27.5)
Recent illness (6 months)	Yes	38 (23.7)
	No	122 (76.3)
Antibiotic use (3 months)	Yes	29 (18.1)
	No	131 (81.9)

Note: Values are presented as number and percentage of total population. Flea infestation was defined as detection of live fleas or flea dirt during clinical examination.

Table 2: Molecular Detection of *Bartonella henselae* by PCR

A. Animal-Level Prevalence

PCR Status	n	%	95% CI
Positive	27	16.9	11.4–23.7
Negative	133	83.1	—
Total	160	100	—

B. PCR Positivity by Sample Type

Sample Type	Positive (n)	Total (n)	% Positive
Blood	27	160	16.9
Saliva	0	160	0
Nail	3	160	1.9

Note: PCR targeted a 414 bp fragment of the htrA. Confidence intervals were calculated using the exact binomial method. All PCR-positive animals yielded detectable *Bartonella henselae* DNA in whole blood samples.

When evaluated by sample type, all positive animals were detected in whole-blood samples, corresponding to a blood positivity rate of 16.9% (27/160). No cat tested positive exclusively in oral-swab samples without concurrent blood positivity. Specifically, none of the 160 oral-swab samples were PCR-positive. Three nail samples (3/160; 1.9%) tested positive; however, all corresponding animals were also positive in blood samples. These findings indicate that whole blood was the most sensitive sample type for molecular detection in this study (Table 2).

Stratified prevalence according to housing category is illustrated in Figure 2. The highest prevalence was observed among stray cats (31.7%; 19/60), followed by indoor-outdoor cats (17.9%; 5/28) and shelter-housed cats (10.0%; 2/20). Indoor-only cats exhibited the lowest prevalence (1.9%; 1/52). When stratified by ownership status, infection prevalence was significantly higher in non-owned cats than in owned cats. Among owned cats, 6 of 80 animals (7.5%) were PCR-positive, whereas 21 of 80 non-owned cats (26.3%) tested positive. This difference was statistically significant (OR = 4.39, 95% CI: 1.66–11.58; p = 0.002).

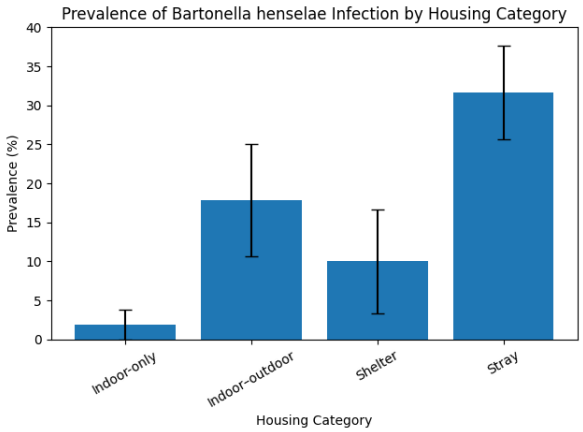

Figure 2: Prevalence of molecularly confirmed *Bartonella henselae* infection among cats according to housing category in Ahvaz, Iran. Bars represent the proportion of PCR-positive animals within each group. Error bars indicate the standard error of the proportion. The highest prevalence was observed in stray cats, followed by indoor–outdoor and shelter-housed cats, whereas indoor-only cats showed the lowest prevalence.

Univariable Analysis of Risk Factors

The results of the univariable analysis are presented in Table 3. Adult cats exhibited a significantly higher prevalence of infection than juveniles (21.2%; 22/104 versus 8.9%; 5/56), corresponding to a crude odds ratio of 2.75 (95% CI: 1.01–7.46; p = 0.048). Sex was not significantly associated with infection status.

Table 3: Univariate Analysis of Risk Factors Associated with PCR Positivity (n = 160)

Variable	Category	Positive/Total	Prevalence (%)	Crude OR	95% CI	p-value
Ownership status	Owned	6/80	7.5	1 (Ref)	—	—
	Non-owned	21/80	26.3	4.39	1.66–11.58	0.002
Age	Juvenile	5/56	8.9	1 (Ref)	—	—
	Adult	22/104	21.2	2.75	1.01–7.46	0.048
Sex	Female	11/72	15.3	1 (Ref)	—	—
	Male	16/88	18.2	1.23	0.53–2.87	0.52
Flea infestation	Negative	6/114	5.3	1 (Ref)	—	—
	Positive	21/46	45.7	15.12	5.38–42.47	<0.001
Tick infestation	Negative	26/157	16.6	1 (Ref)	—	—
	Positive	1/3	33.3	2.53	0.21–29.9	0.34
Outdoor access	No	1/52	1.9	1 (Ref)	—	—
	Yes	26/108	24.1	16.17	2.11–123.8	<0.001
Diet	Commercial dry	8/74	10.8	1 (Ref)	—	—
	Home-cooked	5/42	11.9	1.12	0.34–3.70	0.85
	Raw/hunting	14/44	31.8	3.83	1.48–9.94	0.018

Note: Crude odds ratios were calculated using binary logistic regression. Chi-square or Fisher exact tests were applied as appropriate. Ref indicates reference category.

Outdoor access was strongly associated with PCR positivity. Cats with outdoor access had a prevalence of 24.1% (26/108), compared with 1.9% (1/52) among indoor-confined cats, yielding a crude odds ratio of 16.17 (95% CI: 2.11–123.8; $p < 0.001$).

Flea infestation was also strongly associated with infection. Cats infested with fleas had a prevalence of 45.7% (21/46), compared with 5.3% (6/114) among flea-negative animals, corresponding to a crude odds ratio of 15.12 (95% CI: 5.38–42.47; $p < 0.001$).

Dietary regimen was significantly associated with infection status. Cats consuming a raw diet or engaging in hunting behavior showed a prevalence of 31.8% (14/44), which was significantly higher than that observed in cats fed commercial dry food (10.8%; 8/74), corresponding to a crude odds ratio of 3.83 (95% CI: 1.48–9.94; $p = 0.018$). Tick infestation (1/3; 33.3%) and recent antibiotic administration were not significantly associated with PCR positivity.

Multivariable Logistic Regression

Variables with p-values < 0.10 in the univariable analysis were entered into the multivariable logistic regression model. In the final model, outdoor access and flea infestation remained independently associated with *B. henselae* infection (Table 4).

Table 4: Multivariable Logistic Regression Model for Independent Risk Factors

Variable	Adjusted OR	95% CI	p-value
Outdoor access	4.05	1.37–11.98	0.011
Flea infestation	2.94	1.04–8.28	0.042
Adult age	2.18	0.90–5.26	0.082

Note: Model fit was acceptable, as indicated by a non-significant Hosmer–Lemeshow test ($p = 0.73$). The model showed moderate explanatory power with a Nagelkerke R^2 of 0.34, and the −2 log likelihood was 78.6. Variables with $p < 0.10$ in univariate analysis were entered into the multivariable model using backward stepwise elimination, and multicollinearity was assessed prior to model construction.

Outdoor access was the strongest independent predictor of infection, with an adjusted odds ratio of 4.05 (95% CI: 1.37–11.98; $p = 0.011$). Flea infestation was also independently associated with PCR positivity (AOR = 2.94, 95% CI: 1.04–8.28; $p = 0.042$). Although adult age was associated with increased odds of infection, the association did not reach statistical significance in the multivariable model (AOR = 2.18, 95% CI: 0.90–5.26; $p = 0.082$).

Model fit was acceptable, as indicated by a non-significant Hosmer–Lemeshow goodness-of-fit test (p = 0.73). The model demonstrated moderate explanatory power, with a Nagelkerke R^2 value of 0.34. The −2 log-likelihood value was 78.6.

Population Attributable Fractions

Population attributable fractions (PAFs) for the independent risk factors are presented in Table 5. Based on the adjusted odds ratios and exposure prevalence, 67% of infections, corresponding to approximately 18 of the 27 identified cases, were attributable to outdoor access. Flea infestation accounted for an estimated 36% of infections, corresponding to approximately 10 cases. Because these exposures partially overlapped within the study population, the estimated PAFs are not additive and should not be interpreted as representing independent or mutually exclusive proportions of the total infection burden.

Table 5: Population Attributable Fraction for Independent Risk Factors

Risk Factor	Adjusted OR	Exposure Prevalence (Pe)	PAF	Attributable (%)
Outdoor access	4.05	0.675	0.67	67
Flea infestation	2.94	0.287	0.36	36

Note: Population attributable fraction was calculated using the formula PAF = Pe (AOR − 1) / [Pe (AOR − 1) + 1], where Pe represents exposure prevalence in the total study population. Values indicate the proportion of infection cases theoretically preventable if the exposure were eliminated, assuming a causal relationship.

Discussion

The present study identified a molecular prevalence of *Bartonella henselae* infection of 16.9% among cats in Ahvaz, Iran, with significantly higher prevalence observed in non-owned cats than in owned animals. This prevalence can be considered moderate to high within the global spectrum of feline bartonellosis (Zarea et al, 2023). Outdoor access emerged as the strongest independent predictor of infection, followed by flea infestation, whereas adult age showed a borderline association after adjustment. These findings indicate that environmental exposure and vector contact are the principal epidemiological drivers of infection in this urban feline population, consistent with the previous epidemiological observations in domestic cats (Kokkinaki et al, 2022; Sepúlveda-García et al, 2023).

Comparable findings have been reported in the previous studies conducted in Iran. Oskoueizadeh et al, (2008) investigated the prevalence of *B. henselae* infection in domestic cats from Tehran and reported a seroprevalence of 23% (23/100 cats) using indirect fluorescent antibody testing, although *B. henselae* was not isolated by blood culture. Their study also demonstrated significantly higher seropositivity in outdoor cats (21/50, 42%) than in indoor cats (2/50, 4%). Although the epidemiological context differed from that of the present investigation because serology and molecular detection measure different aspects of infection, their findings similarly highlighted the circulation of *B. henselae* among cats in Iranian metropolitan areas. In comparison, the molecular prevalence observed in the present study (16.9%) appears consistent with the exposure level reported in Tehran, considering that molecular detection identifies active bacteremia whereas serology reflects previous exposure. The observed differences may also reflect regional ecological variation, including climatic conditions, flea abundance, and the larger proportion of free-roaming cats included in the samples of Ahvaz population.

Furthermore, the potential role of dogs in the transmission cycle of *B. henselae* in Ahvaz merits consideration. A previous cross-sectional study conducted in Ahvaz reported the presence of *B. henselae* DNA in dogs using PCR (Oskoueizadeh et al, 2013), indicating that dogs in this region can also become infected. Although dogs are not considered primary reservoirs of *B.*

henselae and are generally regarded as incidental hosts (Mazurek et al., 2020; Osikowicz et al, 2021), their possible role in maintaining the bacterium within urban environments, particularly through shared arthropod vectors such as fleas and ticks, cannot be excluded. Given that stray dogs and cats often occupy overlapping ecological niches and may be exposed to the same flea populations, cross-species transmission remains biologically plausible. However, further studies incorporating molecular characterization of isolates and investigations of vector-borne transmission dynamics are required to clarify whether dogs contribute meaningfully to the epidemiology of feline bartonellosis in this region.

Compared with the recent European studies, the prevalence observed in Ahvaz exceeds several reported estimates. Stepanić et al, (2024) in Croatia documented a molecular prevalence of approximately 12%, with outdoor lifestyle and stray status identified as significant risk factors. Similarly, Lopes et al, (2026) in Portugal reported prevalence estimates ranging from 8% to 11% among apparently healthy cats. In both settings, outdoor exposure was associated with infection, although the overall prevalence was lower than that observed in the present study. Additional European investigations have reported similar epidemiological patterns linking environmental exposure and ectoparasite infestation with infection risk (Álvarez-Fernández et al, 2022; Ebani et al, 2021; Mazurek et al, 2020). These differences may reflect climatic variation, as Mediterranean and Central European regions experience shorter periods of vector activity than the prolonged warm conditions characteristic of southwestern Iran.

Globally, the meta-analysis conducted by Zarea et al, (2023) estimated a pooled molecular prevalence of approximately 12% among cats, with higher rates reported in free-roaming populations. The prevalence of 16.9% observed in Ahvaz exceeds the pooled global estimate and approaches the upper range reported for stray cat populations worldwide. In the Middle East, Sayed et al, (2022) reported a molecular prevalence of 10.8% in Egypt, which is lower than that observed in the present study, although similar associations with outdoor exposure were identified. In South America, Sepúlveda-García et al, (2023) reported prevalence estimates ranging from 14% to 18% in Chile, closely approximating the findings reported here. Collectively, these comparisons suggest that the infection burden in Ahvaz is comparable to that of high-transmission regions but exceeds that reported in many recent European studies, potentially because of climatic conditions that favor flea survival and sustained transmission cycles.

The biological plausibility of the identified risk factors further strengthens the validity of these findings. Outdoor access increases opportunities for contact with infected conspecifics and exposure to flea vectors. Free-roaming cats are more likely to encounter ectoparasites and engage in aggressive interactions that may facilitate transmission. Flea infestation, which remained independently associated with infection after adjustment, reflects the established role of *Ctenocephalides felis* as the principal vector of *B. henselae* (Osikowicz et al, 2021). Adult age was associated with increased odds of infection, consistent with cumulative exposure over time, although this association did not retain statistical significance in the multivariable model. Similar age-related patterns have been reported in Croatia and Chile, suggesting that repeated environmental exposure, rather than intrinsic susceptibility, accounts for this effect (Stepanić et al, 2024; Sepúlveda-García et al, 2023).

From a public health perspective, these findings highlight the importance of the stray cat population as a potential urban reservoir of infection. The markedly higher

prevalence observed among free-roaming animals suggests that uncontrolled outdoor populations may sustain transmission within urban environments. Given the zoonotic nature of *B. henselae*, close contact with infected cats, particularly those lacking routine ectoparasite control, may increase the risk of cat scratch disease and other clinical manifestations associated with this pathogen (Yakubovsky et al, 2026). Population attributable fraction analysis indicated that 67% of infections could theoretically be attributed to outdoor exposure and 36% to flea infestation, underscoring the potential impact of targeted preventive interventions. Routine flea control programs, responsible ownership practices, and restriction of uncontrolled outdoor access may substantially reduce infection burden, consistent with recent molecular evidence of vector-borne pathogens in free-roaming companion animals in Iran (Hatooth et al., 2025).

Several limitations should be acknowledged. First, the cross-sectional design precludes causal inference and does not permit assessment of temporal relationships between exposure and infection. Second, the study was conducted in a single metropolitan area, which may limit the generalizability of the findings to other ecological settings within Iran. Third, molecular detection identifies active bacteremia but does not capture previous exposure or serological status. In addition, co-infections with other vector-borne pathogens were not investigated and therefore cannot be excluded. Furthermore, although the sample size was adequate for multivariable analysis, the number of positive animals was relatively modest, which may have reduced the precision of certain estimates. Despite these limitations, the study provides robust molecular and epidemiological evidence that environmental exposure and vector infestation are central determinants of *B. henselae* infection in this urban feline population.

In conclusion, this study demonstrates that *Bartonella henselae* infection occurs at a moderate to high molecular prevalence among owned and non-owned cats in Ahvaz, Iran, with environmental exposure emerging as the principal determinant of infection. Outdoor access and flea infestation were independently associated with bacteremia and accounted for a substantial proportion of the population-level infection burden. These findings reinforce the central role of vector-mediated transmission and unrestricted roaming behavior in sustaining urban transmission cycles. Implementation of routine ectoparasite control measures and reduction of uncontrolled outdoor exposure may substantially decrease infection prevalence and mitigate potential zoonotic risk.

Ethics Approval and Consent to Participate

All procedures involving animals were conducted in accordance with institutional guidelines for animal care and use. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Shahid Chamran University of Ahvaz.Written informed consent was obtained from owners prior to enrollment of privately owned cats.

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Conflict of Interest

The authors declare that they have no competing interests.

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شیوع مولکولی و عوامل خطر اپیدمیولوژیک عفونت ناشی از *بارتونلا هنسله*، در گربه‌های صاحب‌دار و بدون صاحب شهرستان اهواز، ایران

سارا حاجی‌زاده[۱]، بهمن مصلی‌نژاد[۲*]، داریوش غریبی[۳]، محمد راضی‌جلالی[۲] و مهدی پورمهدی‌بروجنی[۴]

[۱] دانشجوی دکتری تخصصی بیماری‌های داخلی حیوانات کوچک، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۲] استاد گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۳] استاد گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۴] استاد گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

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چکیده

بارتونلا هنسله، یک باکتری زئونوز است که گربه‌های خانگی به عنوان مخزن اصلی آن عمل می‌کنند و با ایجاد باکتریمی پایدار، انتقال عامل بیماری را عمدتاً از طریق کک گربه (*کتنوسفالیدس فلیس*) تسهیل می‌کنند. با توجه به اهمیت بهداشت عمومی عفونت در گربه‌ها و محدود بودن داده‌های مولکولی از جنوب غربی ایران، این مطالعه با هدف تعیین شیوع مولکولی و شناسایی عوامل خطر اپیدمیولوژیک مستقل مرتبط با عفونت *بارتونلا هنسله*، در گربه‌های صاحب‌دار و بدون صاحب در اهواز، ایران انجام شد. در این مطالعه مقطعی، ۱۶۰ گربه مورد بررسی قرار گرفتند که به طور مساوی بین دو گروه صاحب‌دار و بدون صاحب توزیع شده بودند. گربه‌های بالغ ۶۵ درصد از جمعیت مورد مطالعه را تشکیل می‌دادند، در حالی که ۳۵ درصد نابالغ بودند؛ همچنین ۵۵ درصد نر و ۴۵ درصد ماده بودند. گربه‌های نژاد مو کوتاه اهلی، شایع‌ترین تیپ نژادی را تشکیل می‌دادند (۵۱/۹ درصد). از هر حیوان نمونه‌های خون کامل، سواب دهانی و ناخن جمع‌آوری شد و با استفاده از واکنش زنجیره‌ای پلیمراز متداول با هدف تکثیر قطعه‌ای به طول ۴۱۴ جفت باز از ناحیه فاصله‌انداز رونویسی داخلی htrA مورد بررسی قرار گرفتند و سپس برای برخی آمپلیکون‌های نماینده توالی‌یابی جهت تأیید انجام شد. ارتباط بین عوامل خطر بالقوه و عفونت با استفاده از رگرسیون لجستیک تک متغیره و چند متغیره ارزیابی و همچنین کسر منتسب به جمعیت محاسبه شد. DNA مربوط به *بارتونلا هنسله* در ۱۶/۹ درصد از گربه‌ها (فاصله اطمینان ۹۵ درصد: ۱۱/۴ تا ۲۳/۷) شناسایی شد. شیوع عفونت در گربه‌های بدون صاحب (۲۶/۳ درصد) به طور معنی‌داری بیش‌تر از گربه‌های صاحب‌دار (۷/۵ درصد) بود (OR=4.39؛ فاصله اطمینان ۹۵ درصد: ۱٫۶۶–۱۱٫۵۸؛ p=۰٫۰۰۲). تحلیل کسر منتسب به جمعیت نشان داد که به ترتیب ۶۷ درصد و ۳۶ درصد از موارد عفونت به دسترسی به محیط خارج از منزل و آلودگی به کک نسبت داده می‌شوند. این یافته‌ها نشان می‌دهد که مواجهه محیطی و آلودگی به ناقل‌ها از عوامل تعیین‌کننده اصلی عفونت هستند و اهمیت کنترل کک و محدود کردن دسترسی کنترل نشده به محیط بیرون را برای کاهش بار عفونت و خطر زئونوز برجسته می‌سازند.

کلمات کلیدی: *بارتونلا هنسله*، گربه، شیوع مولکولی، اپیدمیولوژی، زئونوز

* نویسنده مسئول: بهمن مصلی‌نژاد، استاد گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

E-mail: bmosallanejad@scu.ac.ir