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Original article

Epidemiology of feline bartonellosis and molecular characteristics of *Bartonella henselae* in Bangladesh

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ABSTRACT

Bartonellosis, a neglected vector-borne zoonotic disease transmitted from animals to humans, continues to threaten human and animal health significantly. This study aims to determine the epidemiology of feline bartonellosis and the molecular characteristics of *Bartonella* spp. in cats. From June 2018 to June 2020, 304 oral swabs were randomly collected from Bangladesh's Dhaka, Mymensingh, and Rajshahi districts. A pre-tested questionnaire was administered to collect data. Oral swabs were subjected to PCR targeting *htrA* gene to confirm *Bartonella* spp., which was subsequently validated through sequencing. Risk factors were identified using multivariable logistic regression analysis. The overall prevalence of feline bartonellosis was found to be 15.1 %. The following factors were significantly ($p < 0.05$) associated with *Bartonella* infection in risk factor analysis: cats aged ≥ 1 year (OR: 3.23, 95 % CI: 1.38–24.40), local breed cats (OR: 3.37, 95 % CI: 1.05–10.81), cats carrying fleas (OR: 2.33, 95 % CI: 1.93–13.45), antifleacidal drugs inconsistently administered cats (OR: 6.74, 95 % CI: 3.17–14.31), outdoor access cats (OR: 2.54, 95 % CI: 1.16–5.57). Notably, zoonotic *B. henselae* was confirmed through sequencing, establishing it as the causal agent of cat scratch disease. Phylogenetic analysis showed homology with *B. henselae* sequences from Brazil, Saint Kitts, and Nevis. We recommend consistent and appropriate flea control measures to curb its spread among Bangladeshi cats. Moreover, limiting outdoor exposure or implementing preventive measures for outdoor cats could reduce the disease burden. The associated human health risk can be decreased by effectively controlling this disease within the cat population.

1. Introduction

Bartonellosis is a vector-borne neglected zoonotic disease caused by *Bartonella* spp. *Bartonella* species are becoming more significant bacterial infections in veterinary and human health (Murano et al., 2001). Among the 11 species or subspecies recognized or assumed to be dangerous in human beings, eight have been discovered or isolated from domestic dogs or cats, consisting of *Bartonella henselae, B. clarridgeiae*, and *B. koehlerae* (Alvarez-Fernandez et al., 2018; Lappin et al., 2020). Feline bartonellosis is typically asymptomatic, and has the potential for rapid global spread (Breitschwerdt, 2008; Alvarez-Fernandez et al., 2018; Mazurek et al., 2018).

Bartonellosis produces asymptomatic bacteremia in natural hosts. The clinical manifestations vary, causing signs and symptoms ranging from mild flu-like disease to serious, life-threatening conditions such as arthritis, endocarditis, hepatitis, myocarditis, and arthralgia in both humans and animals (Iannino et al., 2018). *B. henselae*-associated cat scratch disease (CSD) is primarily characterized by benign lymphadenopathy (Massei et al., 2000; Chomel et al., 2003; Massei et al., 2004; Florin et al., 2008). CSD is a self-limiting human disease characterized by low-grade fever, lymphadenopathy, primary cutaneous inoculation lesion, and weight loss, typically lasting 6–12 weeks (Skerget et al., 2003).

Bartonella, a homeotropic gram-negative bacterium, colonizes

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erythrocytes and endothelial cells of mammals (Kosoy et al., 2010). These bacteria are spread by the bites of arthropod vectors, such as sandflies, lice, ticks, and fleas (Tsai et al., 2011). Cat bites, scratches, ticks, and fleas can disseminate through contaminated saliva during gum bleeding episodes (Guptill, 2010).

Pet animals play a crucial societal role, contributing to children's development and providing stress relief for their owners (Wells, 2009). The popularity of keeping pet cats is steadily growing in Bangladesh (Islam et al., 2019). Cats often share proximity to their owners and their beds. Due to limited knowledge and awareness, CSD can affect both pet owners and those without pets (Stull et al., 2012). The prevalence of bartonellosis has been documented worldwide which included saliva of the domestic cat (10.9 %), domestic cats (34.3 %), the veterinary worker population (37.1 %), vampire bats (67 %), and stray cats (45 %) (Oskouizadeh et al., 2010; Müller et al., 2017; Oteo et al., 2017; Becker et al., 2018; Köseoğlu et al., 2022). Thousands of pet owners in the United States and Europe report *Bartonella* infection annually, with many requiring hospitalization (Chomel et al., 2006). However, despite the growing cat population and popularity of pet ownership in densely populated Bangladesh, epidemiological studies on bartonellosis in cats and humans are absent. The inadequate pet owners' knowledge and diagnostic facilities in pet hospitals increase the potential health risk of such zoonotic diseases in Bangladesh. Therefore, our objective was to estimate the prevalence of bartonellosis and identify the risk factors associated with *Bartonella* infection in apparently healthy cats in Bangladesh.

2. Materials and methods

2.1. Ethical approval

The Committee for Experimental Operational Guidance and Animal Welfare at Bangladesh Agricultural University reviewed and approved the research protocol (Approved permit number XF2014-18). Prior to data and sample collection, informed consent was obtained from each pet owner , and samples were collected following an authorized protocol.

2.2. Study population, area, and duration

This cross-sectional study was conducted between June 2018 and June 2020. A total of 304 cats were randomly selected from three different regions of Bangladesh: Dhaka, Mymensingh, and Rajshahi districts (Fig. 1). Specifically, client-owned cats with access to beds and those brought to Veterinary teaching hospitals of various Universities, Government hospitals, and Private pet clinics for routine examinations, vaccinations, or addressing health issues, were included in the randomized sampling process.

2.3. Data and sample collection

A pre-structured questionnaire was administered to gather relevant data, including location, age, sex, breed, and overall health status, the presence of fleas, outdoor access, and regular use of anti-fleacidal drugs

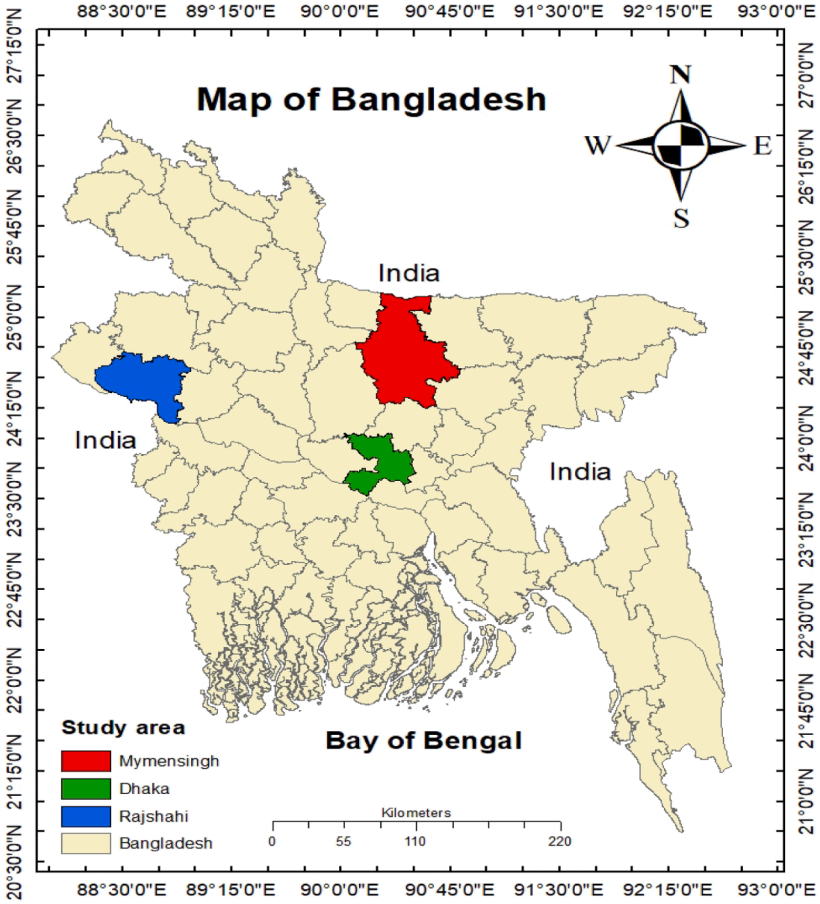

Fig. 1. The map of Bangladesh shows the sampling areas for the bartonellosis study in cats.

during the sample collection process. A total of 304 oral swabs were collected using a sterile cotton applicator, and then carefully placed in sterile eppendrop tubes. The collected samples were promptly transferred to the Department of Medicine, Faculty of veterinary science, Bangladesh Agricultural University maintaining the cool chain.

2.4. Operational definitions: Regular use of antifleacide drugs

The regular use of antifleacide drugs was categorized as follows: "Yes," indicating that cat owners administer the medications upon observing fleas on their cat's body with veterinary consultation, and "No," denoting that owners do not use these drugs at the recommended time.

2.5. DNA extraction and polymerase chain reaction

According to the manufacturer's instructions, DNA extraction from oral swab samples for bartonellosis was conducted using the Purelink TM® Microbiome DNA Purification kit (Invitrogen, USA). The PCR amplification of *htrA* gene of *Bartonella* was accomplished with 414-bp amplicon size, using genus-specific primers CAT1 (5′- GATTCAATTGGTTTGAAGGAGGCT-3′) and CAT2 (5′- TCACATCACCAGGACGTATTC-3′). The PCR reaction mixture was 25 µl which comprised of extracted DNA: 5 µl, AMS buffer 5x: 2.5 µl, $MgCl_2$: 50 mM, dNTP: 100 µM, CAT1 and CAT2 each: 10 pmol, distilled water: 12.5 µl, and distilled water, and Taq DNA polymerase (Fermentas, Vilnius, Lithuania): 0.5 U. The PCR thermal condition was: single cycle of initial denaturation: 95 °C for 5 min, followed by 30 cycles of denaturation: 94 °C for 1 min, annealing: 57 °C for 1 min, and extension: 72 °C for 1.5 min, and finally, a single final extension: 72 °C for 7 min. Standard precautions were observed to prevent DNA sample contamination. Subsequently, The PCR results were run on an agarose gel with a concentration of 1.5 % and visualized using ethidium bromide (Oskouizadeh et al., 2010).

2.6. Sequencing and phylogenetic analysis

A single representative amplicon was chosen at random for partial sequencing. The PCR amplicons underwent purification using Favor-Prep™ GEL/PCR Purification Kit (FAVORGEN BiotechCorp., Taiwan) following the manufacturer's protocol. The purified amplicons were sequenced bidirectionally through the commercial services of DNA Solution Ltd, Dhaka, Bangladesh, employing an ABI 3500 Dx Genetic analyzer (Applied Biosystems, USA). Subsequently, the sequences were manually checked for potential sequencing errors using MEGA 11. These sequences were then used for querying the GenBank online database Nucleotide collection (nr/nt) using the Megablast algorithm, optimized for highly similar sequences, accessible via the BLAST online application (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Hossain et al., 2022). A neighbor-joining tree was constructed for phylogenetic analysis, and evolutionary distances were computed using the Jukes-Cantor method with a bootstrap value of 1000. The comprehensive evolutionary analyses were conducted within the MEGA 11.

2.7. Statistical analysis

The statistical package for social sciences (SPSS) 22 was used to input data and administration. A cat was considered *Bartonella* spp. infected if it was positive in PCR. Age was categorized as $\leq$ 1 year or $>$ 1 year. A Z-test for proportions was employed to determine statistically significant variations in prevalence among different districts (Chouhan et al., 2022). Initially, univariable logistic regression assessed the relationship between *Bartonella* infection and each explanatory variable. Subsequently, multivariable logistic regression was performed, incorporating only those explanatory variables with a univariable p-value of $\leq$ 0.2. The variation inflation factor was assessed for multicollinearity among explanatory variables, and variables with a VIF of $\leq$ 5 were considered acceptable before conducting multivariable logistic regression. The backward selection removed non-significant terms from the model ($P > 0.05$). All analyses were performed at a 95 % level of confidence, and $P \leq 0.05$ was used as the threshold for statistical significance. The Hosmer–Lemeshow test was conducted to determine the overall fit of the final model. The potential confounding and interactions were also assessed using the previously described method (Islam et al., 2020).

3. Results

3.1. Prevalence of bartonellosis

Out of the 304 oral swabs tested, 46 showed positive results in PCR amplification (Fig. 2) with a total bartonellosis prevalence of 15.1 % (Table 1). Mymensingh district (17.3 %) had a relatively higher prevalence of bartonellosis than the other two districts (Table 1). However, this difference was not statistically significant.

3.2. Risk factors

Five variables associated with bartonellosis at p-values of $\leq$ 0.2 were included in the multivariable analysis (Table 2). The feline bartonellosis was significantly associated with four variables in the final multivariable logistic regression model (Table 3). Specifically, the odds of bartonellosis were 3.37 times higher (95 % CI: 1.05–10.81) in local breed cats compared to the Persian breed. Cats aged $\geq$ 1 year had 3.23-fold increased odds (95 % CI: 1.38–24.40) of bartonellosis compared to cats aged $\leq$ 1 year. Cats with irregular use of antifleacidal drugs had 6.74-fold higher odds of bartonellosis (95 % CI: 3.17–14.31) than those with regular use. Cats with outdoor access were 2.54 times more likely (95 % CI: 1.16–5.57) to contract bartonellosis compared to indoor-raised cats. The presence of fleas on a cat's body resulted in a 2.33-fold increase (95 % CI: 1.93–13.45) in the odds of bartonellosis compared to cats without fleas.

3.3. Sequence and phylogenetic analysis

The nucleotide sequences of the selected amplicons have been deposited in the GenBank database with the accession number OQ698786.1. Through BLASTn analysis, a complete alignment of 100 % homology was observed between our sequence and previously documented *Bartonella henselae* sequences, confirming the species of our isolate. Subsequently, we constructed a Neighbor-Joining (NJ) tree (Fig. 3) based on a 397 bp fragment of the *Bartonella* serine protease (*htrA*) gene. Our isolate was observed to cluster alongside previously reported *Bartonella henselae* isolates from Brazil (KU179420.1) and Saint Kitts and Nevis (MT048283.1), exhibiting robust nodal support at 94.1 %.

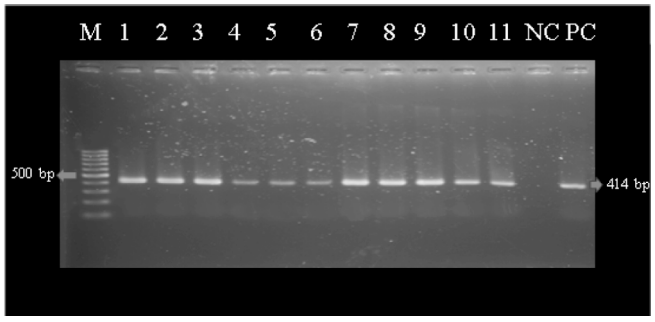

Fig. 2. This figure represents the PCR amplification of DNA of *Bartonella* spp. Lane M- 100 bp DNA ladder Lane 1–11: Complies with the samples of *Bartonella* spp showing approximately 414 bp. NC-Negative control, PC: Positive control (Houston-1; ATCC 49882).

Table 1
Overall prevalence of bartonellosis in cat.

District	No. of sample tested by PCR	PCR positive	Prevalence (%)
Dhaka	110	16	14.5[a]
Mymensingh	104	18	17.3[a]
Rajshahi	90	12	13.3[a]
Overall	304	46	15.1

[a] Values with the same superscripts within the same column do not differ significantly ($p > 0.05$).

Table 2
Univariable association between bartonellosis and various explanatory variables among apparently healthy cats in Bangladesh.

Risk factors	Category level	OR	95 % CI	P-Value
Breed*	Persian	Ref.	–	–
	Cross	1.57	0.47–5.26	0.466
	Local	2.51	0.93–6.73	0.068
Sex	Female	Ref.	–	–
	Male	1.13	0.60–2.13	0.704
Age*	≤ 1 year	Ref.	–	–
	> 1 Year	3.05	1.61–5.79	0.001
Presence of fleas on cat body	No	Ref.	–	–
	Yes	2.93	1.54–5.59	0.001
Health status	Clinically Healthy	Ref.	–	–
	Sick	1.15	0.50–2.64	0.747
Outdoor access*	No	Ref.	–	–
	Yes	2.67	1.40–5.07	0.003
Regular use of antifleacidal drugs*	Yes	Ref.	–	–
	No	7.43	3.77–14.64	<0.001

OR: Odds ratio; CI: 95% confidence interval.
* candidate variables for multivariable analysis.

Table 3
Risk factors for feline bartonellosis that were identified through multivariable analysis.

Risk factors	Category level	OR	95 % CI	P-Value
Breed	Persian	Ref.	–	–
	Cross	2.03	0.52–7.97	0.309
	Local	3.37	1.05–10.81	0.041
Age	≤ 1 year	Ref.	–	–
	> 1 year	3.23	1.54—6.80	0.002
Presence of fleas on cat body	No	Ref.	–	–
	Yes	2.33	1.07–5.08	0.033
Regular use of antifleacidal drugs	Yes	Ref.	–	–
	No	6.74	3.17—14.31	<0.001
Outdoor access	No	Ref.	–	–
	Yes	2.54	1.16–5.57	0.020

OR: Odds ratio; CI: 95% confidence interval.

4. Discussion

We estimated the prevalence and identified risk factors for feline bartonellosis for the first time in Bangladesh. The findings highlight that approximately one in every six apparently healthy cats in the study areas carried bartonellosis. High-risk groups, especially young and local breed cats, deserve focused attention for future surveillance and preventive strategies. Effective and consistent flea control measures are recommended to mitigate its transmission among Bangladeshi cats. Furthermore, implementing measures to limit outdoor exposure or adopting preventive measures for outdoor cats can help decrease the impact of the disease. The potential human health risk can be substantially reduced by proactively managing this disease within the feline population.

The prevalence observed in this study was 15.1 %, remarkably lower than the prevalence reported in China (38.61 %), Russia (60–83 %), Canada (48–90 %), and the Netherlands (72 %) (Gutiérrez et al., 2015; Kokkinaki et al., 2022). While previous studies primarily aimed to detect *Bartonella* from blood samples, oral swabs may yield a lower prevalence due to the necessity of gum bleeding for *Bartonella* to be present in saliva (Chomel et al., 1995). Due to resource constraints and limited owner participation, our study could not utilize blood samples for *Bartonella* detection. Notably, our focus was on client-owned pet cats, whereas other studies covered both stray and pet cats, which could potentially contribute to the observed lower prevalence. Stray animals are more susceptible to ectoparasite infestations, a significant risk factor for bartonellosis (Angioni et al., 2020).

Bartonellosis was significantly higher in local breed cats compared to the Persian breed. This might be because owners of local cat types are less likely to regularly groom their pets, which can lead to a higher risk of flea infestations (Gutiérrez et al., 2015). Grooming helps keep cats clean and less likely to have fleas, so the risk of getting *Bartonella* infection increases when it's not done regularly.

Cats older than 1 year have a higher chance of getting bartonellosis than cats younger than 1 year (26.1 % vs. 10.4 %). This difference is significant and supported by a previous study (Kokkinaki et al., 2022). Older cats spend more time outside, which increases their risk of getting *Bartonella* infection (Kokkinaki et al., 2022). This shows that age affects how likely a cat is to have bartonellosis.

Having fleas on a cat's body was significantly associated with *Bartonella* infection. Fleas act as vectors of this disease, and a previous study has reported the presence of this bacteria in fleas (Gutiérrez et al., 2015). So, when cats have fleas, it raises their risk of getting *Bartonella* infection. This suggests that fleas play a role in spreading this disease among cats.

Moreover, cats that use anti-fleacide drugs for prevention are better protected against flea infestations than those that don't. This helps reduce the likelihood of getting a *Bartonella* infection. This finding is consistent with other studies (Zhang et al., 2021; Kokkinaki et al., 2022). Using these preventive drugs makes it less likely for cats to have fleas, lowering the risk of *Bartonella* infection.

Our study found a significant association between cats that have outdoor access for various reasons and *Bartonella* infection. This connection is likely because outdoor access increases the likelihood of cats getting fleas (Gutiérrez et al., 2015). When cats roam outside, they can come into contact with fleas more efficiently, which raises the risk of getting a *Bartonella* infection.

4.1. Study limitations

Due to limited funding, we were able to sequence only one sample. However, the sequencing results confirmed that our isolate is *Bartonella henselae*. We also observed a strong similarity with sequences previously reported from Brazil, Saint Kitts, and Nevis. However, a more comprehensive investigation is needed, involving additional sequenced isolates, to determine the prevailing *Bartonella* strain circulating in Bangladesh. This detailed study will provide a clearer understanding of the specific *Bartonella* variants present in our region and their potential consequences for public health.

5. Conclusions and recommendations

One out of every six apparently healthy cats in the study areas was found to be carrying bartonellosis. This highlights the widespread nature of the disease among feline populations in the region. To address this, it is crucial to focus on high-risk groups, particularly young and local breed cats, by implementing targeted surveillance and preventive strategies. We strongly recommend the consistent and appropriate use of flea control measures, as fleas play a significant role in transmitting the disease among cats. Furthermore, limiting outdoor exposure or introducing preventive measures for cats that spend time outdoors can

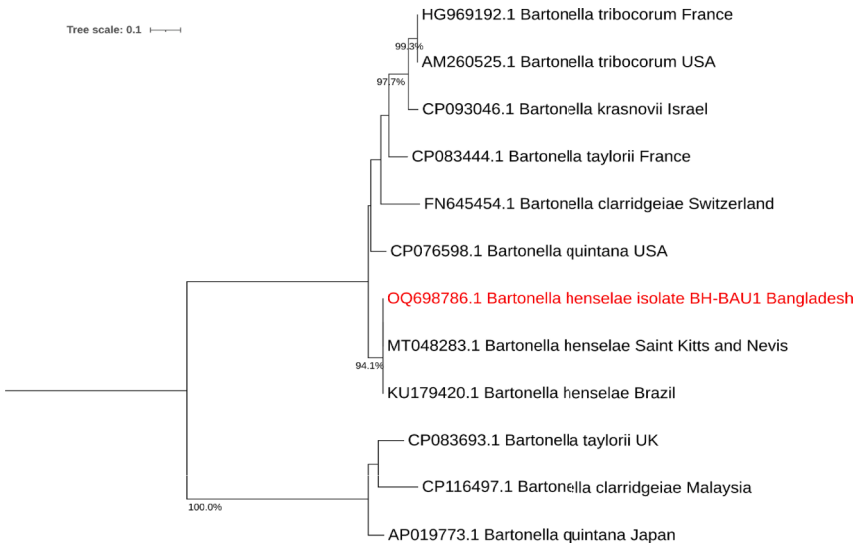

Fig. 3. The Neighbor-Joining tree based on a 414 bp segment of the *htrA* gene of *Bartonella* spp. The optimal tree is shown with the percentage of replicate trees from the bootstrap test (1000 replicates), indicating how often the associated taxa clustered together. The tree is drawn to scale, with branch lengths reflecting the evolutionary distances used to establish the phylogenetic relationships. These distances are calculated using the Jukes-Cantor method and are measured units of base substitutions per site. A gamma distribution (shape parameter = 5) accounted for rate variation among sites. This analysis involved 12 nucleotide sequences, considering all codon positions (1st, 2nd, and 3rd). Gaps and missing data were excluded (complete deletion option), resulting in a final dataset of 388 positions. These evolutionary analyses were conducted using MEGA11 software.

significantly contribute to reducing the overall disease burden. The associated human health risks can be reduced by effectively managing and controlling bartonellosis within the cat population. This emphasizes the importance of a proactive and comprehensive approach to tackling this neglected zoonotic disease and ensuring the well-being of pets and their owners.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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