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Five-striped Indian palm squirrel (*Funambulus pennanti*), a silent vector of human Bartonellosis in Bahawalpur, Pakistan

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Abstract

Bartonella species, known as stealth pathogens, are particularly challenging to detect and diagnose. In Pakistan, Bartonella species have been detected in various rat species indicating that they can vary in host specificity, with some infecting multiple host species. Despite the favorable conditions for disease transmission in Pakistan, no studies have been conducted on squirrel reservoirs within the country. In regions like Bahawalpur, where humans and squirrels often coexist, might create a significant health risk. We aimed to detect Bartonella in the blood of the Five-striped Indian Palm Squirrel (*F. pennanti*) to investigate its potential role as a reservoir and vector for human bartonellosis. We examined the blood smears of 18 Indian palm squirrels (*F. pennanti*) including **7** males and 11 females. Out of the 18 squirrels examined, 11 (61.1%) tested positive for Bartonella infection, revealing a notably high prevalence of this hemoparasite in the local squirrel population. This finding highlights the requirement for additional investigation and public health surveillance to evaluate the effect of Bartonella transmission from squirrels to people around here.

Introduction

Bartonellosis is a worldwide vector-borne zoonosis caused by Bartonella species [1] which are capable of infecting various mammals, including humans [2], with at least 22 species have been detected in rodents [3]. Humans typically contract Bartonella from mammals either through blood-sucking arthropods or via scratches or bites from an infected mammalian host [4], and remain sustained within a mammalian reservoir host [5]. The manifestations of bartonellosis in humans can vary widely, ranging from asymptomatic infections to subclinical and self-limiting conditions, and extending to severe, life-threatening diseases [6], and can evade the host's immune system within the host, leading to recurrent bacteremia [7]. It can result in symptoms such as fever, fatigue, muscle pain (myalgia), and endocarditis [8] infecting both erythrocytes and endothelial cells, establishing long-term infections in mammals, which the host immune system often struggles to clear quickly [9]. Bartonella species, known as stealth pathogens, are particularly challenging to detect and diagnose [10]. The most effective tissues for detecting Bartonella DNA were found to be spleen, ear, lung, and liver tissues [11].

Human-rodent interactions are common, as many rodent species inhabit areas close to human environments [6]. By 2050, an estimated 75% of the global human population will reside in urban areas, necessitating significant environmental changes. As a result, the frequency of human encounters with rodents and the diseases they carry is expected to rise [12] The Bartonella Cycle showing horizontal and vertical transmission of Bartonella is demonstrated in **Fig. 1**. While only a few Bartonella species have been identified as causative agents of human disease, it has been suggested that any Bartonella species found in animals may have the potential to infect humans [13]. Various species of the Sciuridae family have been identified as hosts of Bartonella spp. in countries worldwide [14]. *Bartonella washoensis* were detected in ground squirrels in California, serving as a vertebrate reservoir of zoonotic *B. washoensis* [15], and from Red squirrels and their ectoparasites in Lithuania [16]. In Pakistan Bartonella spp. has been detected in *R. rattus* and *R. norvegicus* from the Sahiwal area of Punjab [2], indicating that Bartonella species can vary in host specificity, with some infecting multiple host species, while others are highly specific to a single host. For instance, *B. bovis* typically infects only one species of ruminant and is rarely found in other animals [17].

Funambulus pennanti is an active, agile, and vocal species, mainly arboreal, but it frequently forages on the ground, moving quickly as it searches for food [18]. It is very easily recognized by its slender body with five white or whitish strips [19] and a bushy tail [20]. Body color is greyish brown to olive brown with five conspicuous stripes running along their bodies [20]; absence of mid ventral line on tail makes the distinctive characters of the species [19]. Northern India is home to just *F. pennantii*, which also occurs in Pakistan and Nepal [21].

People who have close contact with animals in homes, zoos, or nature are at risk for zoonotic diseases [9]. Squirrels (Sciuridae) are common city dwellers and typically live in larger densities in cities than in rural regions [22]. Squirrels interact with people directly because they dwell close to where people reside, are well-liked by urban populations, with people often feeding them [23], and they are carrier of pathogenic Bartonella species that evolved from gut symbionts in arthropods to primarily inhabit blood-feeding insects, gaining the ability to colonize the bloodstream of mammals [24]. Despite the favorable conditions for disease transmission in Pakistan, no studies have been conducted on Bartonella spp. and squirrel reservoirs within the country. We aim to detect Bartonella in the blood of Five-striped Indian Palm Squirrel (*F. pennanti*) to investigate its potential role as a vector for human bartonellosis. This is the first study to examine the presence of Bartonella infections in blood of Five-striped Indian palm squirrels in Bahawalpur, Pakistan.

Materials and Methods

Study area

The study was conducted in Bahawalpur City, located in the southern part of the Punjab province of Pakistan. It lies along the south-eastern bank of Sutlej River on northern fringe of the Cholistan desert [25]. The study was carried out during December 2022 to May 2023.

Sample collection

Using rodent traps, squirrels were live captured in several locations throughout the city of Bahawalpur. 18 Indian palm squirrels (*F. pennanti*) were studied, including **7** males and 11 females (**Table 1**). They

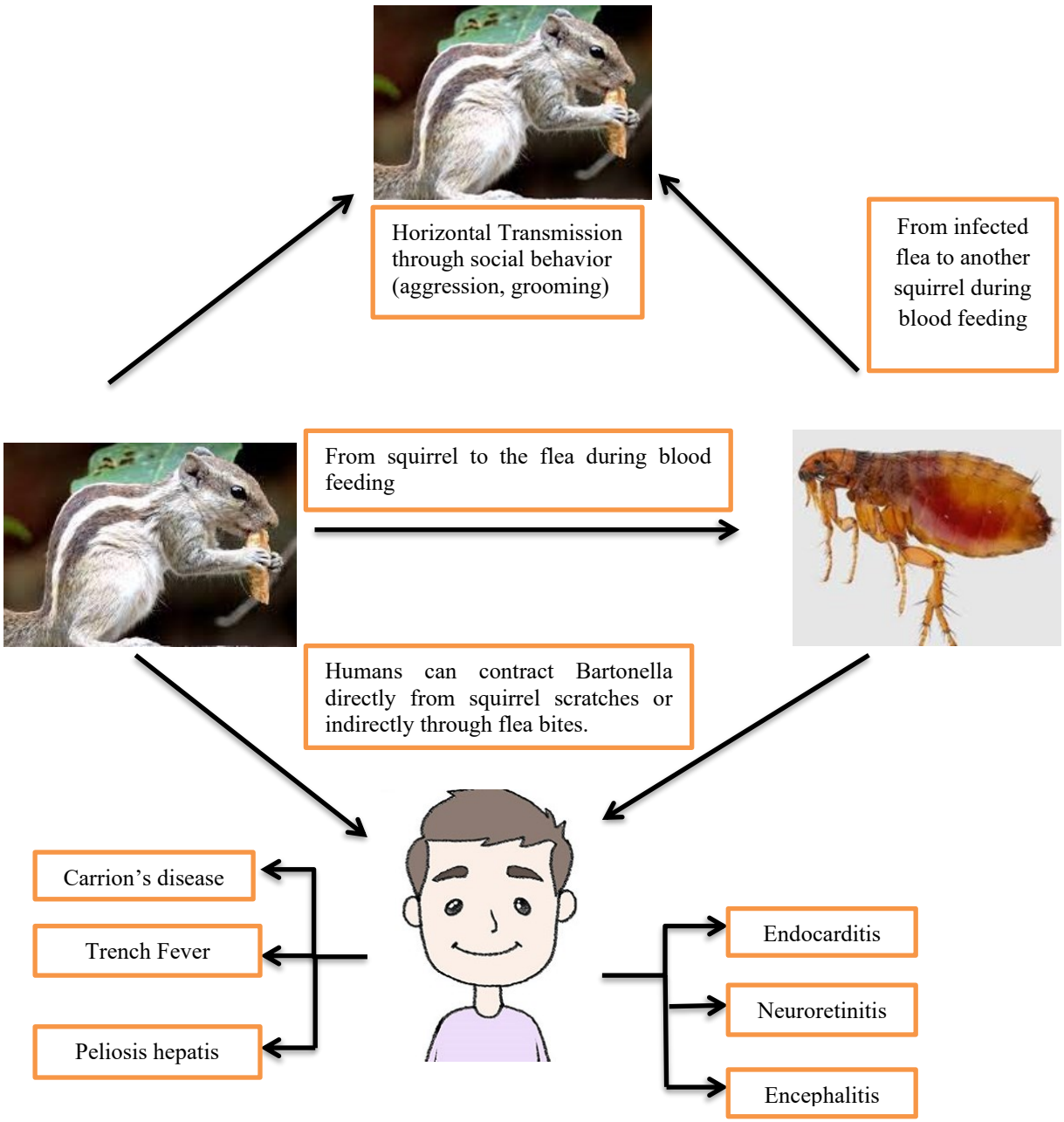

Fig. 1: Bartonella Cycle showing horizontal and vertical transmission of Bartonella

were primarily discovered from fruit trees. We placed food-filled traps close to certain trees. Due to the aroma of food, squirrels descended from the treetops. The squirrels were captured using café leftovers. After trapping the animal, it was brought to the laboratory and given 1-3mm anesthesia. The amount of anesthesia varies with animals. Blood samples were obtained through 3CC syringes from the ventral tail artery. Smears were made from freshly drawn blood. A little drop of blood was applied using a micropipette to a glass slide in order to prepare smears. A second glass slide was then used to spread the blood drop. The slide was then given time to dry. It was washed with ethanol after drying and left to air dry at ambient temperature. The slides were then stained using Field's solutions A and B and let air dry. The dried slides were then examined under a microscope for any hemoparasite presence. In order to prevent sample mixing, slides were labeled. The smears were examined under the microscope. We found an unusual structure inside the erythrocytes of some squirrels. After examining them thoroughly, these unusual

stains were found to be bartonella.

Table 1: Prevalence of Hemoparasites

		Count	Mean± SD
	Y	11	1.29±0.488
Hemoparasites	N	07	1.45±0.522

Results

In our study, we captured 18 Five-Striped Indian Palm Squirrels (Funambulus pennanti) from various urban and peri-urban locations in Bahawalpur, South Punjab, Pakistan, an area where this species thrives in close proximity to human populations. Out of the 18 squirrels examined, 11 (61.1%) tested positive for Bartonella infection, revealing a notably high prevalence of this hemoparasite in the local squirrel population (**Table 1**). Bartonella, a known agent of zoonotic diseases, was detected at the genus level through blood analysis. Microscopic examination of blood smears revealed numerous intra-erythrocytic bacteria, consistent with Bartonella infection pattern. These observations were corroborated by reference to studies such as Foucault, Rolain [26] and Rolain, Foucault [27], which also reported Bartonella residing within red blood cells (RBCs) in bone marrow smears. Furthermore, our findings align with Karbowiak, Rychlik [28], who identified similar haemoparasites in tail-tip blood smears. The bacterium's presence within red blood cells was confirmed using a Pixel Pro microscope, offering clear evidence of its intra-erythrocytic localization (**Fig. 2**).

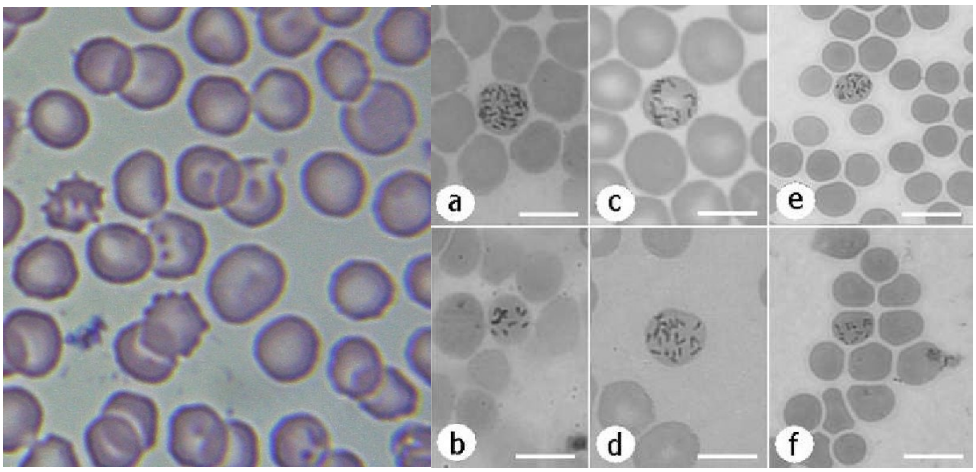

Fig. 2: Bartonella recognized in squirrel blood smears, lining up with results of Karbowiak, Rychlik [28]

Given the high rate of Bartonella infection among squirrels in this densely populated region, our results suggest that the Five-Striped Indian Palm Squirrel could act as a silent vector for human bartonellosis, potentially contributing to the transmission of this pathogen in the human population of South Punjab, Pakistan.

Human activities are increasingly affecting the global environment, with rising temperatures intensifying the interactions between arthropods, humans, and other mammalian species [29]. In Bahawalpur, a region rich in agriculture and fruit-bearing trees, squirrels benefit from abundant food sources and often inhabit areas close to humans. Gardeners and fruit farm workers, in particular, have more frequent contact with these rodents than others. During our study, we observed that some squirrels displayed aggressive behavior when workers attempted to drive them away from ripened fruits, occasionally leading to physical attacks. These encounters resulted in scratches and injuries, which could serve as entry points for Bartonella bacteria into the human body. This highlights a potential risk of zoonotic transmission, especially given the high prevalence of Bartonella observed in the local squirrel population. Indirect transmission of Bartonella can occur through fleas that feed on the blood of infected squirrels and subsequently bite humans. These fleas act as vectors, transferring the bacteria as they move between hosts. In regions like Bahawalpur, where humans and squirrels often coexist, this creates a significant health risk. The close proximity of squirrels to human habitats, particularly in agricultural areas, increases the likelihood of flea-borne transmission, making this an important public health concern. Flea bites could serve as a hidden route for Bartonella infection, emphasizing the need for monitoring and control measures in such environments.

Out of the 18 squirrels examined, 11 tested positive for hemoparasites in their blood smears, while the remaining 7 did not show any signs of infection. This resulted in a positive rate of 61.1%, with the remaining 38.9% of samples testing negative, as shown in Table 1. These findings indicate a significant portion of the squirrel population was infected. The mean count of hemoparasites in the positive samples (Y) was 1.29 ± 0.488, while the mean count in negative samples (N) was 1.45 ± 0.522. Interestingly, the mean count of hemoparasites in positive samples (1.29 ± 0.488) was slightly lower than in negative samples (1.45 ± 0.522). While this appears counterintuitive, it is essential to note that the mean count and standard deviation refer to general measurements of parasite load and not absolute values of parasite presence. This suggests variability in the hemoparasite loads within both groups, highlighting the complexity of parasite-host interactions and the potential influence of factors such as immune response, host age, or environmental conditions.

Out of the 7 male squirrels captured, 5 were found to be positive for hemoparasites, resulting in a positivity ratio of 71.4%. Conversely, 2 male squirrels tested negative, accounting for 28.6% of the male group. Among the 11 female squirrels captured, 6 tested positive for hemoparasites (54.5%), while 5 were negative (45.5%). These findings, summarized in Table 2, show a noticeably higher prevalence of hemoparasite infection in male squirrels compared to females. Males demonstrated a significantly higher positivity rate (71.4%) compared to females (54.5%). This suggests that male squirrels are more likely to harbor hemoparasites than their female counterparts.

Table 2: Female to male positive ratio of hemoparasite

		Gender			
		Male		Female	
		Count	age%	Count	age%
Hemoparasite	Positive	5	71.4%	6	54.5%
	Negative	2	28.6%	5	45.5%

Discussion

Wild rodents are recognized as carriers of numerous pathogens transmissible to humans, yet there is limited information regarding the role of small mammals in spreading vector-borne blood parasites. Several rodent species across the globe have been found to host various Bartonella species and their variants in significant proportions [30]. Squirrels, in particular, are peri-urban rodents that frequently cohabit with humans, often scavenging food scraps and causing damage to farm crops. These behaviors may contribute to the transmission of zoonotic diseases [31].

Bartonella species are associated with several human diseases, including cat scratch disease (*Bartonella henselae*), Carrion's disease (*Bartonella bacilliformis*), and trench fever (*Bartonella quintana*). Bartonella infections can affect various organs and systems, causing skin conditions (bacillary angiomatosis), liver inflammation (peliosis hepatis), heart infections (endocarditis), eye inflammation (neuroretinitis), blood infections (bacteremia), and even brain inflammation (encephalitis). In many cases, infections with Bartonella are asymptomatic, with some studies reporting clinically healthy individuals testing seropositive for Bartonella despite showing no signs of illness. While the infection is typically mild and self-limiting in healthy individuals, it can result in severe and life-threatening conditions for immunocompromised patients, including those undergoing cancer treatments, organ transplants, or individuals with HIV/AIDS [32]. Bartonella resides both within erythrocytes and endothelial cells, which allow them to develop long-term, constant infections in their mammalian hosts. This approach helps the bacteria avoid the host's immune system, allowing the infection to last for extended periods without being cleared by the immune system [33].

Various studies revealed the presence of Bartonella species in different species of squirrels and other rodents as well. Kosoy, Murray [34] observed that *Bartonella washoensis* isolated from a California ground squirrel (*Spermophilus beecheyi*) was genetically identical to a human case isolate, indicating squirrels as a source of human infection. Similarly [35] found a high prevalence of Bartonella infections among lesser bandicoot rats (*Bandicota bengalensis*), black rats (*Rattus rattus*), and house shrews (*Suncus murinus*) in Dhaka, Bangladesh. Other studies conducted by Ziedins, Chomel [36] and Lipatova, Razanske [16] suggest that ground squirrels and their ectoparasites animals are significant reservoirs and vectors of the bacteria and confirmed Bartonella spp—infections in these animals. Further supporting this point, [13] identified *B. grahamii*, *B.*

melophagi, and *B. washoensis* in Eurasian red squirrels. Recently Xue, Chen [37] detected *Bartonella washoensis and B. grahamii* in Daurian ground squirrels, pathogens that are known to cause a public health risk, especially for people in close contact with infected animals, such as herders exposed to their ectoparasites.

In addition to Bartonella, squirrels harbor a wide range of other parasites. Jokelainen and Nylund [38] *Toxoplasma gondii* parasites belonging to endemic genotype II caused fatal infection in three (16%) of 19 Eurasian red squirrels (*Sciurus vulgaris*) sent for necropsy in Finland. Kumar, Melotti [39] further demonstrated the effect of *Toxoplasma gondii* on wild squirrel species near human settlements. Vincent, Ruder [40] found *Baylisascaris* larva migrans in fox squirrels (*Sciurus niger*). In another study Liu, Ge [41] collected 171 fecal samples from Pallas's squirrels (*Callosciurus erythraeus*) sold as pets to investigate the prevalence and genetic characteristics of Blastocystis and found 10 Blastocystis-positive samples (10/171, 5.9%) by PCR amplifcation and DNA sequencing of the barcode region of the SSU rRNA gene [42] reported 2 species of Eimerians from southern flying squirrels (*Glaucomys volans*) and the endemic Prince of Wales flying squirrel (*Glaucomys sabrinus griseifrons*).

Identifying haemoparasites in these squirrels is critical, as it proposes that this species might go about as a likely repository for these parasites. Repository species assume a basic part in sending various irresistible illnesses by filling in as a source from which parasites can spread to different hosts, including people. Rodents, specifically, have been distinguished as normal repositories for different Bartonella species. The connection among rodents and Bartonella is critical because of the rodents' capacity to hold onto relentless, subclinical bacteremia that can keep going for broadened periods. Besides, various rat species worldwide have been found to have high disease rates with various Bartonella species and their variations [30].

This study underlines the basic requirement for continuous reconnaissance and exploration on Bartonella diseases in natural life populaces. It gives an essential reference to future examinations, empowering correlations after some time and across various geographic locales. Such information is fundamental for grasping the examples of Bartonella diseases, their likely consequences for species, and the dangers of transmission to people. Concentration likewise features the worth of interdisciplinary cooperation among untamed life environmentalists, parasitologists, and general wellbeing specialists. By pooling their insight, these disciplines can develop the comprehension of haemoparasite the study of disease transmission, distinguish potential vectors, and think up viable methodologies for illness anticipation and control.

Conclusion

The squirrels' vicinity to the human population, especially in metropolitan regions and rural settings, expands the risk of zoonotic transmission, particularly through flea bites or direct contact with the animals. The study conducted in Bahawalpur, South Punjab, Pakistan, distinguished a high commonness of Bartonella contaminations in Five-Striped Indian Palm Squirrels (*Funambulus pennanti*), with 61.1% of the squirrels testing positive for the hemoparasite. This is the first report of Bartonella in Five-Striped Indian Palm squirrel (*F.pennanti*) in Bahawalpur, Pakistan, featuring its possible job as a quiet vector of human bartonellosis. This finding highlights the requirement for additional examination and general well-being observation to evaluate the effect of Bartonella transmission from squirrels to people around here.

Conflict of interest

The authors declare no conflict of interest.

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