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# Flea Presence and Abundance Are Not Predictors of *Bartonella tribocorum* Carriage in Norway Rats (*Rattus norvegicus*) from an Underserved Neighborhood of Vancouver, Canada

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## Abstract

Urban Norway rats (*Rattus norvegicus*) carry pathogenic *Bartonella* spp. that are transmitted among rats and from rats to people through arthropod vectors, particularly fleas. There is marked temporospatial variation in *Bartonella* spp. carriage among Norway rats in Vancouver, Canada, and we investigated whether this variation is associated with flea presence or abundance. *Bartonella triborocum* was isolated from 96/370 (35%) rats and 211 (57%) rats had fleas with an average of one flea per rat. All fleas were identified as *Nosopsyllus fasciatus*. There was no significant relationship between *B. tribocorum* carriage and flea presence or abundance, suggesting that, in contrast to other rat-associated zoonoses transmitted by fleas (*e.g.*, *Yersinia pestis*) flea indices may not be informative for understanding the ecology of *Bartonella* spp. in rats, particularly for *N. fasciatus*.

### Keywords

*Bartonella* spp.; fleas; rats; *Rattus norvegicus* ; *Nosopsyllus fasciatus* ; urban

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## Introduction

Rats (*Rattus* spp.) are the reservoir for a variety of zoonotic pathogens (Himsworth et al. 2013), including *Bartonella* spp.—a genus of vector-borne intracellular bacteria that infect endothelial cells and erythrocytes (Kosoy and Bai 2019). At least 22 *Bartonella* spp. have been found in 98 rodent species, with *Bartonella tribocorum*, *Bartonella elizabethae*, *Bartonella rattimalssiliensis*, and *Bartonella queenslandensis* being associated with rats (Kosoy and Bai 2019). *Bartonella* spp. are transmitted among rats and from rats to people by hematophagous arthropods, particularly fleas (Gutierrez et al. 2015). In rats, *Bartonella* spp. cause a persistent asymptomatic bacteremia (Gutierrez et al. 2015). In people, infection with rat-associated *Bartonella* spp. can cause septicemia with a range of clinical presentations (Kosoy and Bai 2019).

Understanding the ecology of zoonotic pathogens in the reservoir host is critical for monitoring and mitigating the risk of spillover from rats to people. The ecology of *Bartonella* spp. in rodents is dependent on a complex set of interactions among the *Bartonella* spp., the rodent host, the environment, and the vector(s) (Gutierrez et al. 2015). In other rodent species, it is thought that flea abundance may be an important determinant of infection (Gutierrez et al. 2015), yet the role of fleas in the ecology of *Bartonella* spp. in urban rats is unclear.

A previous study of urban Norway rats (*Rattus norvegicus*) from Vancouver, Canada, found significant unexplained temporospatial variation in *B. tribocorum* carriage (Himsworth et al. 2015). The objective of this study is to determine whether this variation could be attributed to the presence and abundance of fleas infesting the rats under study.

## Materials and Methods

For the aforementioned previous study (Himsworth et al. 2015), rats were trapped in 43 contiguous city blocks over the course of 1 year. Blood was collected through intracardiac puncture under isoflurane anesthesia before pentobarbital euthanasia and, subsequently, fleas were collected by combing the fur. For each rat, the date and location of trapping, species, and maturity were recorded (animals were considered sexually mature if they had scrotal testes or a perforate vagina). Blood clots underwent *Bartonella* culture and isolates were identified by *gltA* sequencing, while fleas were stored at −80°C.

For this study, collected fleas were examined using a compound microscope (40 × ) for enumeration and identification to species (Holland 1985). Bivariate and multivariate generalized linear mixed models (GLMMs) controlling for clustering by city block of origin were used to assess whether flea indices are associated with is *Bartonella* spp. carriage in rats. The unit of analysis was the rat and the outcome variable was *Bartonella* spp. carriage (positive vs. negative based on culture and sequencing of blood clots). Explanatory variables included for consideration included flea presence (0 flea/rat vs. ≥ 1 flea/rat), flea abundance (number of fleas per rat), and block-level flea index (average number of fleas per rat for the city block in which the rat was trapped). Sexual maturity (immature vs. mature) and season (fall: September–November; winter: December–February; spring: March–May; summer:

June–August) were forced into the model as these variables had been identified as predictors of *Bartonella* spp. carriage in previous models of these data (Himsworth et al., 2015). Variables that were significantly associated with *Bartonella* spp. carriage at an alpha level of ≤ 0.10 in a bivariable GLMM were considered for inclusion in multivariable modelling. The Kruskal–Wallis test was used to assess seasonal differences in flea abundance and flea index. All statistical analyses were conducted using R (R Development Core Team, Vienna, Austria). The trap location and number of *B. tribocorum*-positive and -negative rats as well as the block-level flea index were mapped using ArcGIS 10.0 (ESRI, Redlands, USA).

This study was approved by the University of British Columbia's Animal Care Committee (A11–0087).

## Results

A total of 370 Norway rats were included in this study of which 96 (35%) were positive for *B. tribocorum*. Fleas were present on 211 (57%) of rats with a median of one flea per rat (range 0–14). All fleas were identified as *Nosopsyllus fasciatus.* The median flea index for a city block was 0.93 (range 0–5.6). Both flea abundance and flea index demonstrated significant ( $p < 0.01$) seasonal variation—being highest in the spring, lowest in the winter, and intermediate in the summer and fall. There was no significant relationship between *B. tribocorum* carriage and flea presence, flea abundance, or flea index (Table 1). Mapping also showed that block-level flea index was not clearly correlated with the number of rats carrying *B. tribocorum* in that block (Fig. 1). The odds of *B. tribocorum* carriage were increased in mature animals and decreased for rats trapped in the spring, summer, and winter compared with the fall (Table 1), consistent with previous models of these data (Himsworth et al. 2015).

## Discussion

Although many studies have examined the presence and diversity of *Bartonella* spp. in urban rats and their fleas (Kosoy and Bai 2019), far fewer have sought to determine how flea ecology correlates with pathogen prevalence and distribution in the rat host. Peterson et al. (2017) found that Norway rats that had fleas (*Xenopsylla cheopis*) were more likely to be infected with *Bartonella* spp. than those that did not. However, we did not identify an association between *B. tribocorum* carriage in Norway rats and flea presence, flea abundance, or block-level flea index.

Given that *Bartonella* spp. can cause a persistent bacteremia the fleas present on a rat at the time of trapping are unlikely to be those present at the time of infection. In addition, for *N. fasciatus*, a species that spends more time in the rat's nest compared with *X. cheopis*, the number of fleas on the rat at any given time may not reflect the number of fleas to which that rat has been exposed (Bitam et al. 2010). There has only been a single study of *N. fasciatus* as a vector for *Bartonella* spp. in rodents (specifically, *Rattus surifer* in Thailand (Parola et al. 2003). This paucity of information is unexpected as *N. fasciatus* is reported to be common on commensal Norway rats in temperate regions (Bitam et al. 2010).

It is interesting to note that rat-level flea abundance and block-level flea index were highest in the spring, whereas *Bartonella* spp. was most prevalent in the fall. *Bartonella* spp. carriage and flea abundance/index could, therefore, be linked, but temporally offset in such a manner that would not be revealed in a GLMM. Alternatively, it may be the case that there are seasonal variations in vector activity/competence and/or host–vector interactions. A limitation of this study is the fact that the fleas were not tested for *Bartonella* spp. and future studies should seek to clarify the relationship between *Bartonella* spp. carriage in rats and the fleas that infest them.

## Conclusion

This study suggests that, in contrast to other rat-associated zoonoses transmitted by fleas, such as *Yersinia pestis* (Dennis et al. 1999), flea indices may not be informative for understanding the ecology of *Bartonella* spp. in rats, particularly for *N. fasciatus*.

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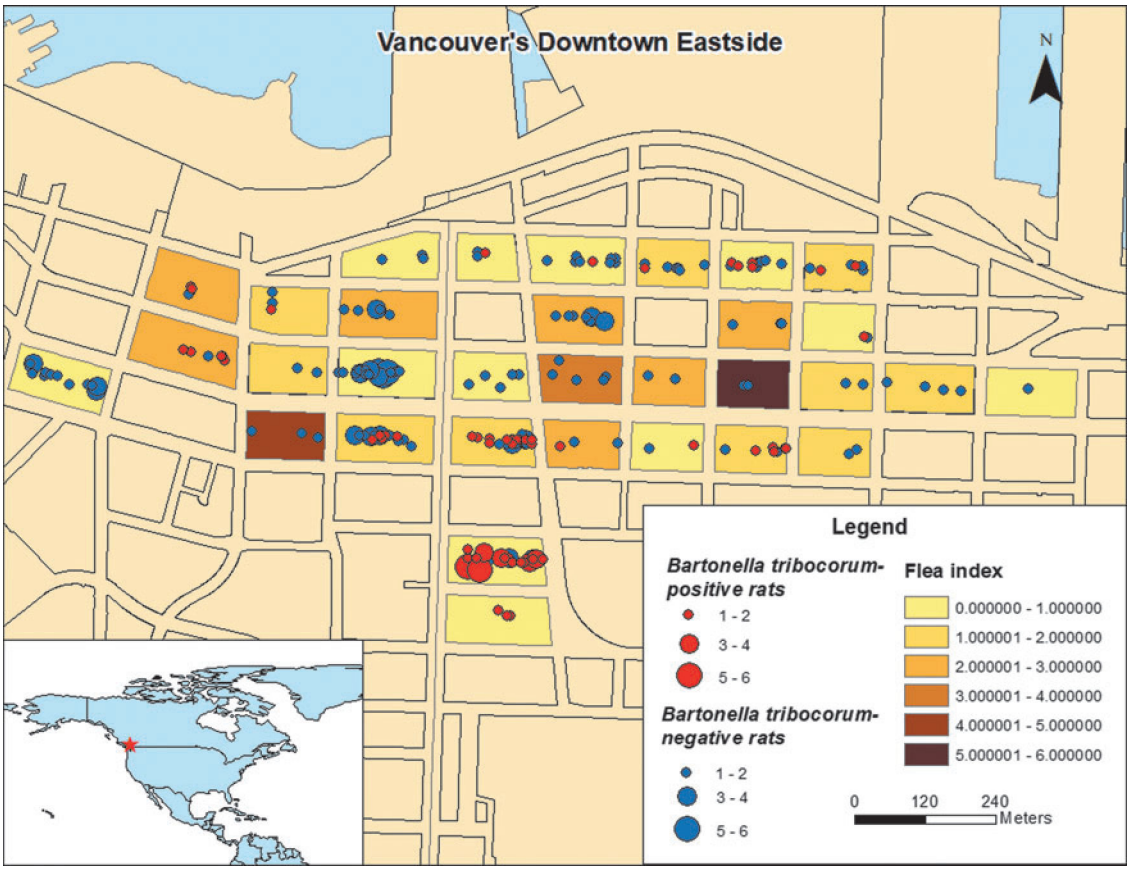


**FIG. 1.**

Distribution of *Bartonella tribocorum*-positive Norway rats (*Rattus norvegicus*) and flea index by city block. Color images are available online.

**Table 1.**

Distribution of Explanatory Variables and Associations with *Bartonella tribocorum* Status Among Norway Rats

| | | | ***Bartonella tribocorum* status** | | **Bivariable GLMM[a]** | |
|---|---|---|---|---|---|---|
| ***Category*** | ***Subcategory*** | ***Total (%)* (n = *370*)** | ***Positive (%)* (n = *96*)** | ***Negative (%)* (n = *274*)** | ***OR (95% CI)*** | **p** |
| Season | Fall | 150 (40.5) | 74 (77.1) | 76 (27.7) | Ref | |
| | Winter | 77 (20.8) | 5 (6.5) | 72 (26.3) | 0.08 (0.02–0.45) | <0.001 |
| | Spring | 103 (27.8) | 12 (12.5) | 91 (33.2) | 0.09 (0.02–0.38) | <0.01 |
| | Summer | 40 (10.8) | 5 (5.2) | 35 (12.8) | 0.07 (0.01–0.59) | 0.01 |
| Maturity | Immature | 89 (24.1) | 9 (9.4) | 80 (29.2) | Ref | |
| | Mature | 281 (75.9) | 87 (90.6) | 194 (70.8) | 2.45 (1.07–5.64) | 0.03 |
| Flea presence | No | 159 (43.0) | 35 (36.5) | 124 (45.3) | Ref | |
| | Yes | 211 (57.0) | 61 (63.5) | 150 (54.7) | 1.17 (0.65–2.11) | 0.59 |
| Flea abundance | 0 | 159 (43.0) | 35 (36.5) | 124 (45.3) | Ref | |
| | 1–3 | 167 (45.1) | 45 (46.9) | 122 (44.5) | 1.10 (0.60–2.04) | 0.76 |
| | >3 | 44 (11.9) | 16 (16.7) | 28 (10.2) | 1.48 (0.61–3.61) | 0.39 |
| Flea index | ≤ 0.65 | 151 (40.8) | 53 (55.2) | 98 (35.8) | Ref | |
| | 0.65–1.3 | 107 (28.9) | 26 (27.1) | 81 (29.6) | 0.39 (0.05–3.25) | 0.39 |
| | >1.3 | 112 (30.3) | 17 (17.7) | 95 (34.7) | 0.21 (0.03–1.47) | 0.12 |

[a]GLMM controlling for clustering by city block in which the rat was trapped.

CI, confidence interval; GLMM, generalized linear mixed model; OR, odds ratio.