

Original Article

Beyond abundance: How microenvironmental features and weather influence *Bartonella tribocorum* infection in wild Norway rats (*Rattus norvegicus*)

Jamie L. Rothenburger,^{1,2} Chelsea G. Himsworth,^{3,4} Nicole M. Nemeth,¹ David L. Pearl,⁵ and Claire M. Jardine^{1,2}

¹Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

²Canadian Wildlife Health Cooperative Ontario-Nunavut Region, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

³School of Population and Public Health, University of British Columbia, Vancouver, BC, Canada

⁴Animal Health Centre, British Columbia Ministry of Agriculture and Canadian Wildlife Health Cooperative, British Columbia Region, Abbotsford, BC, Canada

⁵Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Summary: Norway rats (*Rattus norvegicus*) inhabit cities worldwide and carry a number of zoonotic pathogens. Although many studies have investigated rat-level risk factors, there is limited research on the effects of weather and environment on zoonotic pathogen transmission ecology in rats. The objective of this study was to use a disease ecology approach to understand how abiotic (weather and urban microenvironmental features) and biotic (relative rat population abundance) factors affect *Bartonella tribocorum* prevalence in urban Norway rats from Vancouver, British Columbia, Canada. This potentially zoonotic pathogen is primarily transmitted by fleas and is common among rodents, including rats, around the world. During a systematic rat trap and removal study, city blocks were evaluated for 48 environmental variables related to waste, land/alley use and property condition, and rat abundance. We constructed 32 weather (temperature and precipitation) variables with time lags prior to the date we captured each rat. We fitted multivariable logistic regression models with rat pathogen status as the outcome. The odds of a rat testing positive for *B. tribocorum* were significantly lower for rats in city blocks with one or more low-rise apartment buildings compared to blocks with none (OR = 0.20; 95% CI: 0.04–0.80; $p = .02$). The reason for this association may be related to unmeasured factors that influence pathogen transmission and maintenance, as well as flea vector survival. *Bartonella tribocorum* infection in rats was positively associated with high minimum temperatures for several time periods prior to rat capture. This finding suggests that a baseline minimum temperature may be necessary for flea vector survival and *B. tribocorum* transmission among rats. There was no significant association with rat abundance, suggesting a lack of density-dependent pathogen transmission. This study is an important first step to understanding how environment and weather impacts rat infections including zoonotic pathogen ecology in urban ecosystems.

Keywords: *Bartonella*, environment, epidemiology, infectious disease, wild life, zoonoses

Correspondence to: Jamie L. Rothenburger, e-mail: jamie.rothenburger@ucalgary.ca

Rothenburger et al. Zoonoses Public Health 2018, 65(3): 339-351, which has been published in final form at <https://onlinelibrary.wiley.com/doi/full/10.1111/zph.12440>. This article may be used for non-commercial purposes in accordance with the Wiley Self-Archiving Policy [<https://authorservices.wiley.com/author-resources/Journal-Authors/licensing-open-access/open-access/self-archiving.html>].

Impacts

- Norway rats carry many bacteria, including *Bartonella* spp., which may be potentially transmitted to people, resulting in illness.
- We investigated weather, environmental and rat abundance factors associated with *Bartonella tribocorum* infection in rats. Rats from city blocks with low-rise apartment buildings were less likely to be infected, while rats from time periods with high minimum temperatures were more likely to be infected. The density of rats is not related to infection.
- Urban environments are complex, which may partially explain the existence of pockets of infected rats. Uncovering these associations is an important step in predicting where infected rats occur and designing ways to reduce the spread of potentially zoonotic pathogens.

1. INTRODUCTION

Cities are drastically altered environments that support certain wildlife species. These so-called urban exploiter species capitalize on abundant food resources and human-built environmental niches (McKinney, 2006). Among the globally invasive urban exploiter species, Norway and black rats (*Rattus norvegicus* and *R. rattus*, respectively) are the most harmful to humans. In cities worldwide, rats create several issues including social stigma, infrastructure damage and food contamination (Feng & Himsworth, 2014). Rats are also the source of zoonotic bacterial pathogens responsible for disease in people (Himsworth, Parsons, Jardine, & Patrick, 2013; Meerburg, Singleton, & Kijlstra, 2009). These include plague (*Yersinia pestis*) and leptospirosis (*Leptospira* spp.). As a consequence of the unprecedented rate of global urbanization, the impacts of zoonotic diseases associated with rats are expected to increase (Himsworth et al., 2013).

Among the many pathogens carried by rats are several species of *Bartonella* (Heller et al., 1998). Rat-associated *Bartonella* spp. likely originated in Southeast Asia, and then dispersed globally with invasion activities (Hayman, McDonald, & Kosoy, 2013). These Gram-negative bacteria adhere to erythrocytes in a variety of mammalian hosts without causing clinical signs (Meerburg et al., 2009; Schulein et al., 2001). A chronically infected primary location (likely endothelial cells) periodically releases bacteria (Schulein et al., 2001). Persistently infected, circulating erythrocytes provide ample opportunity for fleas and other arthropod vectors to ingest infected blood meals and transmit the bacterium to more hosts (Gutiérrez et al., 2015; Schulein et al., 2001).

There is growing evidence that rat-associated *Bartonella* spp. are zoonotic, causing lymphadenopathy, neuroretinitis, endocarditis, myocarditis, acute febrile illness, anaemia and chronic fatigue in people (Buffet, Kosoy, & Vayssier-Taussat, 2013; Kandelaki et al., 2016; Kosoy et al., 2010; Vayssier-Taussat et al., 2016). This association has prompted researchers to investigate individual-level factors associated with *Bartonella* spp. carriage in rodents that include age, body mass, sex, sexual maturity and flea abundance (Gutiérrez et al., 2015; Himsworth et al., 2015; Jardine, Waldner, Wobeser, & Leighton, 2006).

The prevalence of *Bartonella* spp. varies among geographical locations, even at small scales (Himsworth et al., 2015; Rothenburger, Himsworth, Nemeth, Pearl, & Jardine, 2017). The reason(s) for this variability is not understood, although it may be related to weather and features of the microenvironment (Ayrál et al., 2015; Rothenburger et al., 2017). The microenvironment is the small-scale habitat in which an organism lives and is part of the larger environment. Further, the microenvironment includes features influenced by home range size, which in the context of rats within cities, is generally less than 30 m in diameter (Davis, Emlen, & Stokes, 1948) and is typically a city block (Feng & Himsworth, 2014). Examples of microenvironmental features include land use type (e.g. building type, green space), anthropogenic activities (e.g. human use of the area, rat control activities, property maintenance) and waste disposal (e.g. recycling, garbage, hygiene). Some researchers have investigated associations between microenvironmental features and rat abundance. Muñoz-Zanzi, Mason, Encina, Gonzalez and Berg (2014) analysed rodent counts (including rats), finding positive associations with rainfall and signs of rodent infestation, and negative associations with the number of household cats. Himsworth et al. (2014) found that rat presence and abundance were associated with specific types of land use, amount of human refuse and building conditions. Yet, few studies

have analysed specific environmental features for associations with pathogen status in rats (Rothenburger et al., 2017).

Weather (i.e. precipitation and temperature) is another key environmental feature that may influence pathogen ecology in rats (Rothenburger et al., 2017). The prevalence of *Bartonella* spp. in many rodent species is highest in summer and fall compared to cooler seasons (Gutiérrez et al., 2015). Despite the pervasiveness of urban rats throughout the world and the potentially dangerous pathogens they carry, the influence of the microenvironment and weather on pathogen prevalence has not been well studied (Rothenburger et al., 2017). Furthermore, the exclusion of environmental characteristics in many studies of host-pathogen systems has prompted some researchers to call for increased integration of environmental factors in the application of One Health problem solving (Barrett & Bouley, 2015). This is important because information about the kinds of environments and weather patterns that support infected rats could be used to develop targeted surveillance and interventions for both people and rats, which may also reduce the risk of rat-associated zoonoses in people.

The objective of this study was to understand how abiotic (weather and environmental features) and biotic (rat abundance) factors affect *B. tribocorum* prevalence in urban Norway rats based on a causal diagram framework (Figure 1). Specifically, we tested the following questions: (1) Do impermanent environmental variables (e.g. human loitering) impact *B. tribocorum* in rats when controlled for confounding variables such as weather, season and permanent environmental variables? (2) Do permanent environmental variables (e.g. land use) impact *B. tribocorum* infection in rats when season is controlled for as a confounding variable? (3) Do weather variables for various time lags prior to rat capture (i.e. temperature and precipitation) impact *B. tribocorum* infection in rats when season is controlled for as a potential confounding variable? (4) Does rat abundance impact *B. tribocorum* infection when controlled for confounding variables such as impermanent and permanent environmental variables, season and weather?

2. MATERIALS AND METHODS

2.1 Study Design

Data were collected for this study as part of the Vancouver Rat Project (www.vancouverratproject.com), a trap and removal cross sectional study of zoonotic pathogens in rats from Vancouver, British Columbia, Canada. Himswoth et al. (2014) describe details of the study design and trapping protocol. Briefly, rats were trapped in the back alleys of 43 city blocks that were randomly allocated to a 2-week trapping period between September 2011 and August 2012. We completed systematic autopsy and tissue collection for each of the euthanized rats. Blood clot samples from 393 rats were cultured for *Bartonella* spp. and the species identity was confirmed as *B. tribocorum* by PCR (Himswoth et al., 2015). The University of British Columbia's Animal Care Committee (A11-0087) approved this study.

2.2 Environmental and weather characteristics

During the trapping interval, the research team used a systematic environmental observation tool to collect information about the city block as previously described (Himswoth et al., 2014). The tool consisted of 58 components (hereafter referred to as “environmental variables”) in the following categories: land use, green space and alley surface characteristics, alley use by people, property condition and waste. The observers examined the block street-front, alleyway and aerial photographs to score each environmental variable. Of the 58 variables, we eliminated 14 from analysis that were redundant (i.e. other variables included the same information), less informative compared to other variables and/or lacked variability (i.e. more than 95% of the observations had the same score). Therefore, we considered 44 environmental variables for statistical modelling (Table S1).

We acquired historical weather data (temperature and precipitation) from Environment Canada (<http://climate.weather.gc.ca>) for the dates of interest from the Vancouver Harbour CS site, which is near the trapping locations. We filled in missing data from the next closest sites (i.e. North Vancouver Wharves, then Vancouver International Airport locations). We constructed 32 weather variables organized in three sets to represent several time lags prior to when rats were captured. The first set of weather variables consisted of the mean of mean daily temperatures (°C) in the 5, 10, 30, 60 and 90 days prior to capture. The second set consisted of

the total precipitation (mm) in each of the 5, 10, 30, 60 and 90 days prior to capture. The third set was calculated by taking the mean of a week's weather values for 7, 14, 30, 60 and 90 days prior to capture. This set included minimum, maximum and mean daily temperatures (°C), as well as mean daily precipitation (mm). An example of this type of variable is the mean of total precipitation on days 54–60 prior to capture. We used relative trap success during the study period as a proxy for rat abundance by city block as described by Himsforth et al. (2014). In this previous study, rat abundance was calculated using the total trap effort (i.e. number of traps set multiplied by the number of trapping nights) with an adjustment to account for sprung traps (i.e. total trap effort minus 1/2 unit for each trap sprung by any cause) to calculate relative trap success.

2.3 Statistical analysis

We fitted multilevel univariable logistic regression models with rat *B. tribocorum* infection status (positive or negative) as the outcome and environmental, weather, season and rat abundance as predictor variables. We included random effects to account for autocorrelation (i.e. clustering) among rats collected from the same city block (multiple rats were captured in each city block).

2.4 Variable assessment and construction

For data that were collected as categorical variables, we collapsed categories with fewer than 40 observations per category and/or those that only represented data from ≤ 2 blocks to construct either categorical or dichotomous variables. We individually assessed linearity between the log odds of *B. tribocorum* infection status against continuous independent variables (i.e. counted versus scored environmental and weather variables) using lowess curves (i.e. locally weighed regression). If the variable was nonlinear, we assessed the significance of a quadratic term and its main effect in a logistic regression model with a random intercept for block. If the quadratic term was significant in the model and a lowess curve revealed a quadratic relationship, then we used the quadratic term and its main effect for multivariable modelling. If it was not appropriate to model a quadratic relationship, we explored log transformation of the variable (i.e. natural log), replacing zero and negative numbers with half the value of the lowest positive observation, noting that few days were $< 0^{\circ}\text{C}$. If we could not linearize the relationship, we then categorized the variable by quartiles. We categorized season by spring (March–June), summer (June–August), fall (September–November) and winter (December–February).

2.5 Statistical modelling

Following any variable transformations, restructuring or the addition of a quadratic term, we fit each variable using multilevel logistic regression models with rat *B. tribocorum* infection status (positive or negative) as the outcome and city block as a random intercept. We considered variables with a statistically significant association with *B. tribocorum* for inclusion in two-variable models.

Due to the large number of constructed weather variables, we estimated the correlation between all weather variables that were significant with univariable modelling using Pearson's correlation coefficients. If the correlation between variables was high (i.e. $|\rho| > 0.8$), we preferentially analysed and presented the data of continuous vs. categorical variables for two-variable modelling. If both correlated variables were continuous, we selected the variable with the strongest univariable association for further analysis.

We constructed two-variable models to assess the impact of potential confounding variables. We first constructed individual causal diagrams for all significant and non-correlated variables based on the main causal diagram (Figure 1; Dohoo, Martin, & Stryhn, 2009). Using the diagrams, we fitted two-variable models for each potential confounding relationship and considered variables as confounders if they were non-intervening variables that resulted in $\geq 30\%$ change to the coefficients when added to the model. As *B. tribocorum* was significantly associated with sexual maturity and season in a previous study of this population (Himsforth et al., 2015), we also considered these variables in two-variable models as appropriate based on causal diagrams. We decided *a priori* to test for biologically plausible interactions (between temperature and precipitation variables within the same lag time frame and between sexual maturity and abundance). We used the lme4 and gmodels packages in R (R Development Core Team, Vienna, Austria) for all statistical analyses except for graphs, log transformations, correlation analyses and when

models failed to converge in R, which were fitted in Stata (Stata 14, College Station, TX, USA). We set the significance level at $\alpha = 0.05$.

3. RESULTS

The prevalence of *B. tribocorum* based on culture results was 25.7% (101/393; 95% Confidence Interval [CI]: 21.4%–30.3%). These rats were captured from 32 city blocks. Based on univariable analysis, significant variables included five environmental variables, 14 weather variables and rat abundance. Due to the large number of constructed weather variables that were collinear and captured similar information, we eliminated ten weather variables from further analyses, bringing the total number of variables considered to 10 (Tables 1 and 2).

3.1 Environmental variables

Four of the environmental risk factors identified with univariable analysis included features of the built environment, while only one related to human behaviour (Table 1). The proportion of the block occupied by low-rise apartments was the only environmental variable that was not affected by any confounding relationships. The odds of a rat testing positive for *B. tribocorum* were significantly lower for rats in city blocks with one or more low-rise apartment buildings compared to blocks with none (OR = 0.20; 95% CI: 0.04–0.80; $p = .02$; Table 3).

3.2 Weather and season variables

In univariable analyses, the odds of a rat being *B. tribocorum*-positive were significantly decreased in winter, spring and summer compared to fall (Himsworth et al., 2015). Significant weather variables related to minimum temperatures on the 0–7, 24–30 and 84–90 days prior to when the rats were captured (Table 2). Among these, the odds of a rat testing positive for *B. tribocorum* were significantly higher with increased mean minimum temperatures (°C) on days 24–30 and days 84–90 prior to capture. Neither relationship was affected by confounding variables, although the mean minimum temperatures (°C) on days 24–30 became non-significant when season was included in the model (Table 3). The odds of a rat testing positive for *B. tribocorum* were significantly decreased when the mean minimum temperatures on the 0–7 days prior to capture were between 6.1 and 10.1°C compared to when temperatures were <3.6°C. This association remained significant after controlling for the confounding effect of season (Table 3). When controlling for season, there was a significant difference between when temperatures ranged from 3.6–6.0°C compared to 6.1–10.1°C (OR = 9.11; 95% CI = 1.62–51.37; p value = .01) and 6.1–10.1°C compared to $\geq 10.2^\circ\text{C}$ (OR = 0.14; 95% CI = 0.03–0.64; p value = 0.01); there was no significant difference between 3.6–6.0°C compared to $\geq 10.2^\circ\text{C}$ (OR = 1.23; 95% CI = 0.28–5.40; p value = 0.78).

Only one precipitation variable was significant (mean of total precipitation on 0–7 days prior to capture; Table 2). The relationship was quadratic with an increased probability of a rat testing positive for *B. tribocorum* until precipitation reached approximately 5mm; the probability decreased when precipitation was >6 mm (Figure 2). However, this association was no longer significant after controlling for the confounding effect of season (Table 3). There was no significant interaction between minimum temperature and mean precipitation in the 7 days prior to capture ($p = .25$).

3.3 Rat abundance

There was a significant quadratic relationship between rat abundance (i.e. relative trap success) and testing positive for *B. tribocorum*. The probability of a rat being infected with *B. tribocorum* decreased until abundance reached approximately 0.4, and subsequently increased when abundance was more than 0.6 (Figure 3). This relationship was confounded by the proportion of the block occupied by housing over commercial buildings, human loitering, season and mean of minimum temperatures (°C) on days 84–90 prior to capture with each relationship becoming non-significant in the adjusted models. There was no significant interaction or confounding relationships between *B. tribocorum* infection status and sexual maturity or abundance (Table 3).

4. DISCUSSION

In the present study, univariable analyses revealed that several physical features of the urban environment, specifically building type and layout (which may create rat corridors among adjacent buildings), were associated with *B. tribocorum* infection in rats. However, only the proportion of the block occupied by low-rise apartments remained

significant after controlling for season. When low-rise apartments were present in a given block, rats were significantly less likely to be infected with *B. tribocorum*. The reason(s) for this association is uncertain but may relate to unmeasured factors that influence pathogen transmission and maintenance, such as vector ecology. In particular, blocks lacking low-rise apartments may provide better habitats (e.g. rat burrows) that facilitate pathogen transmission by flea vectors. Although we did not analyse flea abundance in this study, there is evidence to suggest that ectoparasite abundance, including fleas, varies significantly between specific locations within the urban environment (Frye et al., 2015), which may have implications for *B. tribocorum* transmission among rats. For example, >90% of Oriental rat fleas (*Xenopsylla cheopis*) recovered in a study of Norway rats in Manhattan, New York, originated from one residential building (Frye et al., 2015). Perhaps specific areas sustain rat populations with higher *Bartonella* spp. prevalence because fleas may be able to maintain environmental transmission, even in the absence of rats (Gutiérrez et al., 2015).

We did not observe significant associations between *B. tribocorum* infection in rats and characteristics related to green space, garbage and property upkeep. Yet these factors may seem to be conducive to rat and flea survival. Higher *Bartonella* spp. prevalence among rodents, including *Rattus* sp., is associated with habitats containing abundant organic material (e.g. agricultural areas and fragmented green space) compared to human settlements (Hsieh et al., 2010; Jiyipong, Morand, Jittapalapong, & Rolain, 2015; Morand et al., 2015). It is possible that we measured the environmental variables examined in our study at too coarse of a scale to uncover meaningful associations with specific environmental features (Estrada-Peña, Ostfeld, Peterson, Poulin, & de la Fuente, 2014). The protective association with low-rise apartments requires further study but hints that physical features of the urban environment may contribute to infection status in rats.

We found an association between *B. tribocorum* infection and high minimum temperatures for several time periods (approximately 1 week, 1 month and 3 months) before we captured the rats. This finding suggests that a baseline minimum temperature may be necessary for flea vector survival and *B. tribocorum* transmission among rats. Other studies of *Bartonella* spp. in rodents corroborate this observation. Prevalence tends to be highest in the summer and fall, when minimum temperatures are expected to be higher in the Northern hemisphere (Gutiérrez et al., 2015). Our use of time lags prior to rat capture when considering the influence of weather is an important technique (Guan et al., 2009). Assuming chronic infections are the norm (Gutiérrez et al., 2015), it is more biologically meaningful to analyse time lags since the weather on the day we capture a rat is unlikely to reflect the weather when it became infected.

There were no significant associations with any precipitation variables in the present study. This contrasts with a study of rodents in a variety of habitats in Southeast Asia, in which a higher *Bartonella* sp. prevalence was observed in the wet versus dry season (Jiyipong et al., 2015). Precipitation may be less important for *Bartonella* sp. transmission in an urban ecosystem, possibly related to year-round food availability that maintains host populations and minimizes changes in vector ecology (Bradley & Altizer, 2007). There is limited information about the effects of weather on *Bartonella* spp. in rodent hosts. However, the effects of weather on flea-transmitted *Yersinia pestis*, the causative agent of plague, in rodent hosts are better understood. In general, the risk of plague is increased following periods of high precipitation, which stimulates rodent and flea populations to increase in number (Gage & Kosoy, 2005). In contrast to our finding that increased minimum temperatures are associated with *B. tribocorum* infection, hot temperatures (>27°C) decrease the risk of plague via its negative effects on flea survival and subsequent blockage of the flea gastrointestinal tract by *Y. pestis* (Gage & Kosoy, 2005). Hot temperatures are not expected to have as profound an effect on *Bartonella* spp. transmission because flea-faeces inoculation is the suspected mode of transmission rather than blocking within fleas, as occurs with *Y. pestis* (Eisen & Gage, 2012). In the context of Vancouver, British Columbia, temperatures rarely exceed 25°C, so hot temperatures in this city are unlikely to negatively affect flea survival.

Although season was significant in univariable analyses in terms of *B. tribocorum* infection in rats, the strength of this finding is limited based on a single-year study that included one replication of each season. Multiyear studies are needed to conclusively demonstrate seasonal effects. We included season as a potential confounding variable for weather because it may capture additional information including other types of weather variables (e.g. humidity, barometric pressure), photoperiod and potential fluctuations in resource availability (e.g. food gardens are not productive in the winter vs. summer months) and anthropogenic activities (e.g. alley wash up occurs in summer months only; people loiter less in winter versus other seasons); however, this may not have been appropriate as season, temperature and precipitation variables may capture similar information (i.e. winter is cooler than summer). Similarly, it was unexpected that season would have confounding relationships with permanent environmental variables like building type. The effect of season on the significance of the other two building-type variables (i.e. the proportion of the block occupied by grocery stores and housing over commercial buildings) likely resulted from the study design, in which blocks with these features were inadvertently sampled in particular seasons.

The collective influences of season, weather and climate are an important consideration in the ecology of zoonotic diseases. In particular, understanding weather patterns associated with infected hosts may be useful for predictive modelling (Fisman, 2007; Mills & Childs, 1998). Knowing when hosts are most likely to be infected may be especially pertinent for coastal cities, such as Vancouver, where many of the detrimental effects of global climate change, including rising temperatures and flooding, are likely to be intensified (Lau, Smythe, Craig, & Weinstein, 2010). With climate change, higher minimum temperatures are expected to increase the prevalence of infected rats in this system, assuming extremely high temperatures do not negatively impact the flea or the bacterium. Climate change may also increase the risk of flooding, which may force rats into new areas (Gubler et al., 2001). Urban planners should consider the effects of climate change on rats and the ecology of their associated zoonotic pathogens.

We identified a nonlinear relationship between abundance and infection status; but the association was not significant after controlling for confounding by building type, human use and weather variables, and season. Some studies suggest that host abundance is a key factor influencing *Bartonella* spp. prevalence (Telfer et al., 2006). Others observed a lack of association between host abundance and *Bartonella* spp. prevalence, which is consistent with the present study. For instance, an experimental field study in which fenced enclosures excluded large mammals revealed an increased *Bartonella* spp. abundance due to increased numbers of small hosts (e.g. rodents) and vectors rather than a change in prevalence (Young et al., 2014). It is likely that the influence of host abundance is species and ecosystem-specific. This relationship requires further research in urban ecosystems because it is hard to compare natural sites to the highly modified urban environment.

The present study has several limitations. The sample size was small (i.e. low replication number of city blocks with similar features) as environmental variables were initially collected to assess associations with rat abundance (Himsworth et al., 2014). Small sample size together with a large number of variables and confounding relationships prevented us from fitting a single multivariable model. We overcame this limitation by fitting two-variable models based on causal diagrams. As this study contained a single year and city, generalizability may be limited to elsewhere in the world. Future studies should examine rats and associated environment and weather characteristics over many years and in multiple locations.

In the absence of similar previous research, we undertook an exploratory analysis. Considering numerous variables, we investigated the impact of abiotic and biotic factors on *B. tribocorum* prevalence in rats. Given the logistical challenges of wildlife research, this study is a step towards uncovering environmental characteristics associated with infections in rats. We observed increased odds of *B. tribocorum* infection in rats associated with higher minimum temperatures and decreased odds of infection associated with low-rise apartment buildings. This study analysed data across several levels of biological organization in an application of One Health problem solving (Barrett & Bouley, 2015; Estrada-Peña et al., 2014). Future studies could apply similar techniques to other pathogens in rats, as well as urban and non-urban wildlife species. Longitudinal and experimental studies that modify environmental features would be useful to elucidate causal relationships and mechanisms behind varying pathogen prevalence. Understanding how the environment and weather influences zoonotic pathogens in rats is important for creating active surveillance programmes in rats and people. If specific environmental features are consistently associated with high pathogen prevalence in rats, urban planning and maintenance efforts could modify the environment. It is possible that changing urban environments to reduce the prevalence of pathogens in rats may also reduce the risk of rat-associated zoonoses in people.

ACKNOWLEDGEMENTS

We wish to thank the following individuals: Kirbee Parsons and Alice Feng for their assistance with environmental data and rat collection, Victoria Chang and Heather Anholt for their assistance with sample collection, Michael Kosoy at the Bartonella & Rodent-Borne Diseases Laboratory, Centers for Disease Control and Prevention, Fort Collins, CO, for *Bartonella* diagnostic testing and Kate Bishop-Williams for informative discussions regarding analyses of time-lagged weather data. The field work was made possible by the assistance of the City of Vancouver (Murray Wightman and Stuart McMillan), the Urban Health Research Initiative, the Vancouver Injection Drug Users Study and the Vancouver Area Network of Drug Users. J. Rothenburger's research is supported by the following: Natural Sciences and Engineering Research Council Alexander Graham Bell Canada Graduate Scholarship- Doctoral, Canadian Federation of University Women Dr. Margaret McWilliams Pre-Doctoral Fellowship, Imperial Order Daughters of the Empire War Memorial Scholarship, Ontario Veterinary College Graduate Student Fellowship and the University of Guelph Dean's Tri-Council Scholarship. The Canadian Institutes of Health Research funded this study (MOP-119530).

CONFLICT OF INTEREST

None.

REFERENCES

- Ayral, F., Artois, J., Zilber, A.-L., Widén, F., Pounder, K. C., Aubert, D., ... Artois, M. (2015). The relationship between socioeconomic indices and potentially zoonotic pathogens carried by wild Norway rats: A survey in Rhône, France (2010-2012). *Epidemiology and Infection*, *143*, 586–599. <https://doi.org/10.1017/S0950268814001137>
- Barrett, M. A., & Bouley, T. A. (2015). Need for enhanced environmental representation in the implementation of One Health. *EcoHealth*, *12*, 212–219. <https://doi.org/10.1007/s10393-014-0964-5>
- Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wild- life diseases. *Trends in Ecology & Evolution*, *22*, 95–102. <https://doi.org/10.1016/j.tree.2006.11.001>
- Buffet, J. P., Kosoy, M., & Vayssier-Taussat, M. (2013). Natural history of *Bartonella*-infecting rodents in light of new knowledge on genomics, diversity and evolution. *Future Microbiology*, *8*, 1117–1128. <https://doi.org/10.2217/fmb.13.77>
- Davis, D. E., Emlen, J. T., & Stokes, A. W. (1948). Studies on home range in the brown rat. *Journal of Mammalogy*, *29*, 207–225. <https://doi.org/10.2307/1375387>
- Dohoo, I. R., Martin, W., & Stryhn, H. E. (2009). *Veterinary Epidemiologic Research*, 2nd ed. Charlottetown, PEI, Canada: VER Inc.
- Eisen, R. J., & Gage, K. L. (2012). Transmission of flea-borne zoonotic agents. *Annual Review of Entomology*, *57*, 61–82. <https://doi.org/10.1146/annurev-ento-120710-100717>
- Estrada-Peña, A., Ostfeld, R. S., Peterson, A. T., Poulin, R., & de la Fuente, J. (2014). Effects of environmental change on zoonotic disease risk: An ecological primer. *Trends in Parasitology*, *30*, 205–214. <https://doi.org/10.1016/j.pt.2014.02.003>
- Feng, A. Y. T., & Himsworth, C. G. (2014). The secret life of the city rat: A review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). *Urban Ecosystems*, *17*, 149–162. <https://doi.org/10.1007/s11252-013-0305-4>

- Fisman, D. N. (2007). Seasonality of infectious diseases. *Annual Review of Public Health*, 28, 127–143. <https://doi.org/10.1146/annurev. publhealth.28.021406.144128>
- Frye, M. J., Firth, C., Bhat, M., Firth, M. A., Che, X., Lee, D., ... Lipkin, W. I. (2015). Preliminary survey of ectoparasites and associated pathogens from Norway rats in New York City. *Journal of Medical Entomology*, 52, 253–259. <https://doi.org/10.1093/jme/tjv014>
- Gage, K. L., & Kosoy, M. Y. (2005). Natural history of plague: Perspectives from more than a century of research. *Annual Review of Entomology*, 50, 505–528. <https://doi.org/10.1146/annurev.ento.50.071803.130337>
- Guan, P., Huang, D., He, M., Shen, T., Guo, J., & Zhou, B. (2009). Investigating the effects of climatic variables and reservoir on the incidence of hemorrhagic fever with renal syndrome in Huludao City, China: A 17- year data analysis based on structure equation model. *BMC Infectious Diseases*, 9, 109. <https://doi.org/10.1186/1471-2334-9-109>
- Gubler, D. J., Reiter, P., Ebi, K. L., Yap, W., Nasci, R., & Patz, J. A. (2001). Climate variability and change in the United States: Potential impacts on vector- and rodent-borne diseases. *Environmental Health Perspectives*, 109(Suppl 2), 223–233. <https://doi.org/10.2307/3435012>
- Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I. S., & Harrus, S. (2015). *Bartonella* infection in rodents and their flea ectoparasites: An overview. *Vector Borne and Zoonotic Diseases*, 15, 27–39. <https://doi.org/10.1089/vbz.2014.1606>
- Hayman, D. T. S., McDonald, K. D., & Kosoy, M. Y. (2013). Evolutionary history of rat-borne *Bartonella*: The importance of commensal rats in the dissemination of bacterial infections globally. *Ecology and Evolution*, 3, 3195–3203. <https://doi.org/10.1002/ece3.702>
- Heller, R., Riegel, P., Hansmann, Y., Delacour, G., Bermond, D., Dehio, C., ... Piémont, Y. (1998). *Bartonella tribocorum* sp. nov., a new *Bartonella* species isolated from the blood of wild rats. *International Journal of Systematic Bacteriology*, 48, 1333–1339. <https://doi.org/10.1099/00207713-48-4-1333>
- Himsworth, C. G., Bai, Y., Kosoy, M. Y., Wood, H., DiBernardo, A., Lindsay, R., ... Patrick, D. (2015). An investigation of *Bartonella* spp., *Rickettsia typhi*, and Seoul hantavirus in rats (*Rattus* spp.) from an inner-city neighborhood of Vancouver, Canada: Is pathogen presence a reflection of global and local rat population structure? *Vector Borne and Zoonotic Diseases*, 15, 21–26. <https://doi.org/10.1089/vbz.2014.1657>
- Himsworth, C. G., Parsons, K. L., Feng, A. Y. T., Kerr, T., Jardine, C. M., & Patrick, D. M. (2014). A mixed methods approach to exploring the relationship between Norway rat (*Rattus norvegicus*) abundance and features of the urban environment in an inner-city neighborhood of Vancouver, Canada. *PLoS ONE*, 9, e97776. <https://doi.org/10.1371/journal.pone.0097776>
- Himsworth, C. G., Parsons, K. L., Jardine, C. M., & Patrick, D. M. (2013). Rats, cities, people, and pathogens: A systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne and Zoonotic Diseases*, 6, 349–359. <https://doi.org/10.1089/vbz.2012.1195>
- Hsieh, J. W., Tung, K. C., Chen, W.-C., Lin, J.-W., Chien, L.-J., Hsu, Y. M., ... Chang, C. C. (2010). Epidemiology of *Bartonella* infection in rodents and shrews in Taiwan. *Zoonoses Public Health*, 57, 439–446. <https://doi.org/10.1111/j.1863-2378.2009.01234.x>
- Jardine, C., Waldner, C., Wobeser, G., & Leighton, F. A. (2006). Demographic features of *Bartonella* infections in Richardson's ground squirrels (*Spermophilus richardsonii*). *Journal of Wildlife Diseases*, 42, 739–749. <https://doi.org/10.7589/0090-3558-42.4.739>

Jiyipong, T., Morand, S., Jittapalapong, S., & Rolain, J. M. (2015). *Bartonella* spp. infections in rodents of Cambodia, Lao PDR, and Thailand: Identifying risky habitats. *Vector Borne and Zoonotic Diseases*, 15, 48–55. <https://doi.org/10.1089/vbz.2014.1621>

Kandelaki, G., Malania, L., Bai, Y., Chakvetadze, N., Katsitadze, G., Imnadze, P., ... Kosoy, M. Y. (2016). Human lymphadenopathy caused by ratborne *Bartonella*, Tbilisi, Georgia. *Emerging Infectious Diseases*, 22, 544–546. <https://doi.org/10.3201/eid2203.151823>

Kosoy, M., Bai, Y., Sheff, K., Morway, C., Baggett, H., Maloney, S. A., ... Lerdthusnee, K. (2010). Identification of *Bartonella* infections in febrile human patients from Thailand and their potential animal reservoirs. *American Journal of Tropical Medicine and Hygiene*, 82, 1140–1145. <https://doi.org/10.4269/ajtmh.2010.09-0778>

Lau, C. L., Smythe, L. D., Craig, S. B., & Weinstein, P. (2010). Climate change, flooding, urbanisation and leptospirosis: Fuelling the fire? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 104, 631–638. <https://doi.org/10.1016/j.trstmh.2010.07.002>

McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation*, 127, 247–260. <https://doi.org/10.1016/j.biocon.2005.09.005>

Meerburg, B. G., Singleton, G. R., & Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, 35, 221–270. <https://doi.org/10.1080/10408410902989837>

Mills, J. N., & Childs, J. E. (1998). Ecologic studies of rodent reservoirs: Their relevance for human health. *Emerging Infectious Diseases*, 4, 529–537. <https://doi.org/10.3201/eid0404.980403>

Morand, S., Bordes, F., Blasdel, K., Pilosof, S., Cornu, J. F., Chairsiri, K., ... Tran, A. (2015). Assessing the distribution of disease-bearing rodents in human-modified tropical landscapes. *Journal of Applied Ecology*, 52, 784–794. <https://doi.org/10.1111/1365-2664.12414>

Muñoz-Zanzi, C., Mason, M., Encina, C., Gonzalez, M., & Berg, S. (2014). Household characteristics associated with rodent presence and *Leptospira* infection in rural and urban communities from Southern Chile. *American Journal of Tropical Medicine and Hygiene*, 90, 497–506. <https://doi.org/10.4269/ajtmh.13-0334>

Rothenburger, J. L., Himsworth, C. G., Nemeth, N. M., Pearl, D. L., & Jardine, C. M. (2017). Environmental factors and zoonotic pathogen ecology in urban exploiter species. *EcoHealth*, 14, 630–641. <https://doi.org/10.1007/s10393-017-1258-5>.

Schulein, R., Seubert, A., Gille, C., Lanz, C., Hansmann, Y., Piémont, Y., & Dehio, C. (2001). Invasion and persistent intracellular colonization of erythrocytes. A unique parasitic strategy of the emerging pathogen *Bartonella*. *Journal of Experimental Medicine*, 193, 1077–1086. <https://doi.org/10.1084/jem.193.9.1077>

Telfer, S., Begon, M., Bennett, M., Bown, K. J., Burthe, S., Lambin, X., ... Birtles, R. (2006). Contrasting dynamics of *Bartonella* spp. in cyclic field vole populations: The impact of vector and host dynamics. *Parasitology*, 134, 413–418. <https://doi.org/10.1017/S0031182006001624>

Vayssier-Taussat, M., Moutailler, S., Féménia, F., Raymond, P., Croce, O., La Scola, B., ... Raoult, D. (2016). Identification of novel zoonotic activity of *Bartonella* spp., France. *Emerging Infectious Diseases*, 22, 457–462. <https://doi.org/10.3201/eid2203.150269>

Young, H. S., Dirzo, R., Helgen, K. M., McCauley, D. J., Billeter, S. A., Kosoy, M. Y., ... Dittmar, K. (2014). Declines in large wildlife increase landscape-level prevalence of rodent-borne disease in Africa. *Proceedings of the*

National Academy of Sciences of the United States of America, 111, 7036– 7041.
<https://doi.org/10.1073/pnas.1404958111>

Figures

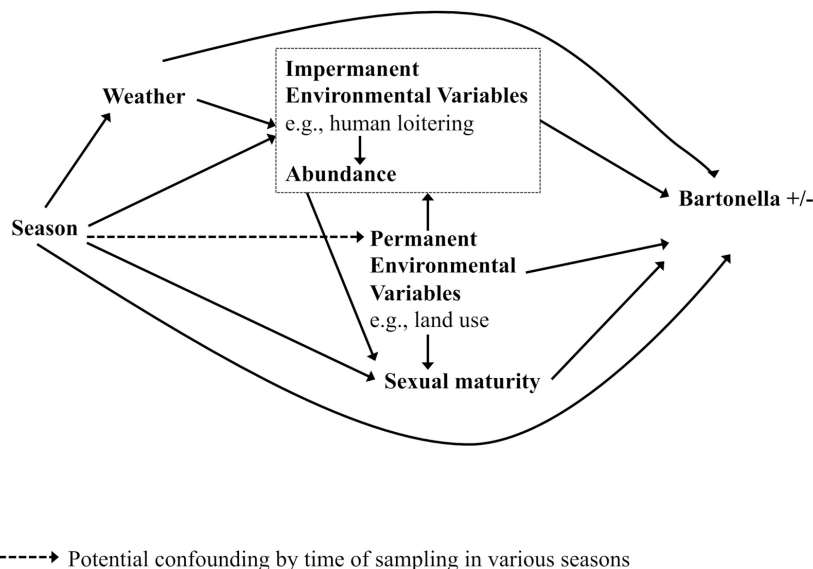


FIGURE 1 Causal diagram of sexual maturity, environmental and weather factors potentially affecting *Bartonella tribocorum* infection status in Norway rats. The dashed arrow indicates a potential confounding relationship based on time of sampling in various seasons

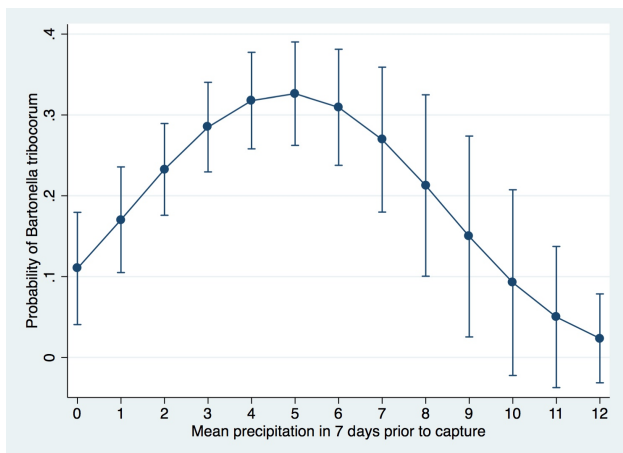


FIGURE 2 Predicted probability curve demonstrating a quadratic relationship between *Bartonella tribocorum* infection status in Norway rats and mean precipitation in the 7 days prior to rat capture with 95% confidence intervals. This association was no longer significant after controlling for the confounding effect of season.

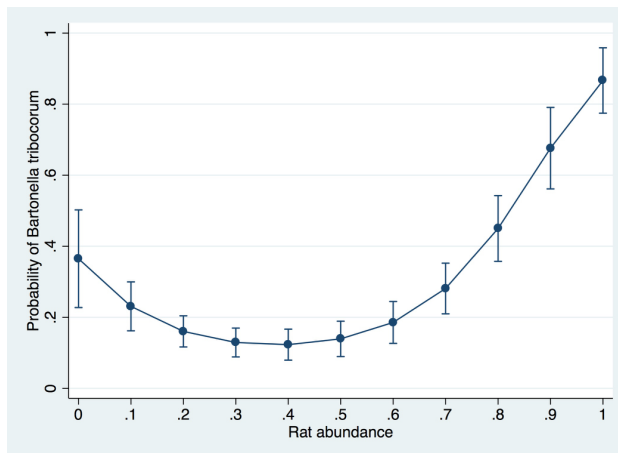


FIGURE 3 Predicted probability curve demonstrating a quadratic relationship between *Bartonella tribocorum* infection status in Norway rats and relative rat abundance with 95% confidence intervals. This association was no longer significant after controlling for the confounding effect of season.

Table 1. Characteristics and univariable associations of significant microenvironmental features among Norway rats (*Rattus norvegicus*) infected with *Bartonella tribocorum* in Vancouver, Canada.

Variable Description	Sub-category	Number of Rats	Number of Blocks	Number of <i>Bartonella tribocorum</i> positive rats within each category (%) (n=101)	Univariable Associations			
					Odds Ratio	95% CI	P	Overall P for Categorical Variables*
Proportion of block occupied by low-rise apartments	0	217	12	86 (39.6)	ref			
	> 0	176	20	15 (8.5)	0.20	0.04-0.80	0.02	
Proportion of block occupied by housing over commercial buildings**	< 0.25	150	18	37 (24.7)	ref			< 0.01
	0.25-0.50	160	11	13 (8.1)	0.12	0.05-1.07	0.06	
	> 0.5	83	3	51 (61.4)	12.61	1.88-156.35	0.01	
Proportion of block occupied by grocery stores	0	43	7	10 (23.3)	ref			0.03
	< 0.25	195	20	19 (9.7)	0.34	0.05-1.97	0.21	
	≥ 0.25	155	5	72 (46.5)	3.64	0.49-38.50	0.20	
Number of rat corridors at the alley face	0	249	21	58 (23.3)	ref			
	≥ 1	144	11	43 (29.9)	6.25	1.44-39.51	0.02	
Amount of human loitering	None to light	200	19	74 (37.0)	ref			
	Moderate to heavy	193	13	27 (14.0)	0.17	0.03-0.79	0.03	

* Calculated with likelihood-ratio test ** Refers to buildings in which the ground floor is dedicated to commercial businesses and upper floors are residential.

Table 2. Characteristics and univariable associations of significant season, abundance and weather variables among Norway rats (*Rattus norvegicus*) infected with *Bartonella tribocorum* in Vancouver, Canada.

Variable	Variable Format	Rat <i>Bartonella tribocorum</i> status		Univariable Associations			
		Number positive (%) or Median (IQR)	Number negative (%) or Median (IQR)	Odds Ratio	95% CI	P	Overall P for Categorical Variables
Mean of minimum temperatures on 7 days prior to capture (categorical by quartiles)	< 3.6°C	22 (22.0%)	78 (26.7%)	ref			0.01
	3.6-6.0°C	42 (41.6%)	48 (16.4%)	1.11	0.44-2.67	0.82	
	6.1-10.1°C	7 (7.0%)	93 (31.8%)	0.09	0.01-0.52	0.01	
	≥ 10.2°C	30 (29.7%)	73 (25.0%)	0.84	0.12-4.17	0.84	
Mean of minimum temperatures on days 24-30 prior to capture	continuous	10.61°C (7.98-12.66)	7.11°C (3.23-11.36)	1.23	1.08-1.44	< 0.01	
Mean of minimum temperatures on days 84-90 prior to capture	continuous	13.97°C (12.76-14.20)	5.03°C (3.11-13.36)	1.28	1.14-1.48	< 0.01	
Mean of total precipitation (mm) on 7 days prior to capture	main effect	4.00°C (3.01-5.29)	3.49°C (2.03-5.27)	1.72	1.11-2.80	0.02	
	quadratic term			0.94	0.89-0.99	0.03	
Season	fall	75 (74.3%)	83 (28.4%)	ref			0.02
	winter	9 (8.9%)	80 (27.4%)	0.26	0.08-0.91	0.03	
	spring	12 (11.9%)	93 (31.8%)	0.13	0.02-0.54	< 0.00	
	summer	5 (5.0%)	36 (12.3%)	0.10	0.01-0.63	0.03	
Rat abundance	main effect	0.55* (0.21-0.86)	0.39* (0.18-0.54)	1.6x10 ⁻⁵	3.1x10 ⁻¹⁰ -0.13	0.02	
	quadratic term			2.0x10 ⁶	25.40-2.02 x10 ¹²	0.01	

Table 3. Results from two-variable models assessing confounding based on causal diagrams among Norway rats (*Rattus norvegicus*) infected with *Bartonella tribocorum* in Vancouver, Canada. Adjusted values in bold indicated a confounding relationship (>30% change in coefficients when confounder was added to the model).

	Confounders (bold) and independent variable(s)	OR	Unadjusted		OR	Adjusted			
			95% CI	P		95% CI	P		
Environmental Variables	Proportion of block occupied by housing over commercial								
		Human loitering	0.17	0.03-0.79	0.030	0.53	0.10-2.44	0.407	
		Rat abundance							
			main effect	< 0.01	< 0.01-0.14	0.017	< 0.01-9.59	0.858	
			quadratic term	2.0 x10 ⁶	25-2.02x10 ¹²	0.012	< 0.01-5.85x10⁶	0.959	
		Number of rat corridors	6.25	1.44-39.51	0.019	16.39	3.05-24.39	< 0.001	
		Sexual maturity	5.18	1.66-22.83	0.011	4.84	1.56-21.28	0.014	
		Proportion of block occupied by grocery stores							
		Human loitering	0.17	0.03-0.79	0.030	0.21	0.04-0.78	0.003	
		Rat abundance							
			main effect	< 0.01	< 0.01-0.14	0.017	< 0.01	< 0.01-0.03	0.004
			quadratic term	2.0 x10 ⁶	25-2.02x10 ¹²	0.012	2.0 x10 ⁶	29-1.10x10 ¹⁰	0.007
		Number of rat corridors	6.25	1.44-39.51	0.019	3.60	0.95-17.89	0.063	
		Sexual maturity	5.18	1.66-22.83	0.011	5.15	1.66, 22.60	0.011	
		Proportion of block occupied by low-rise apartments							
	Human loitering	0.17	0.03-0.79	0.030	0.16	0.04-0.48	0.001		
	Rat abundance								
		main effect	< 0.01	< 0.01-0.14	0.017	< 0.01	< 0.01-0.02	0.002	
		quadratic term	2.0 x10 ⁶	25-2.02x10 ¹²	0.012	2.1x10 ⁵	71-7.56 x10 ⁹	0.003	
	Number of rat corridors	6.25	1.44-39.51	0.019	3.46	0.75-23.84	0.129		
	Sexual maturity	5.18	1.66-22.83	0.011	5.26	1.70-23.07	0.010		
	Number of rat corridors								

	Confounders (bold) and independent variable(s)	OR	Unadjusted			Adjusted		
			95% CI	P	OR	95% CI	P	
Season & Weather	Rat abundance	main effect	< 0.01	< 0.01-0.14	0.017	< 0.01	< 0.01-< 0.01	< 0.001
		quadratic term	2.0 x10 ⁶	25-2.02x10 ¹²	0.012	2.01x10 ⁷	7478-6.5110 ¹¹	< 0.001
	Sexual maturity		5.18	1.66-22.83	0.011	5.12	1.65-22.54	0.012
	Loitering							
	Rat abundance	main effect	0.17	0.03-0.79	0.030	0.31	0.05-1.46	0.131
	Quadratic term		2.0 x10 ⁶	25-2.02x10 ¹²	0.012	5.9x10⁴	0.32-5.48 x10¹⁰	0.065
	Rat abundance							
	Sexual maturity		5.18	1.66-22.83	0.011	5.20	1.67-22.90	0.011
	Season							
	Mean of minimum temperatures (°C) on 7 days prior to capture (categorical)				0.015*			0.042*
	< 3.6	ref						
	3.6-6.0		1.11	0.44-2.67	0.818	0.59**	0.19-1.65	0.330
	6.1-10.1		0.09	0.01-0.52	0.013	0.09	0.01-0.54	0.014
	≥ 10.2		0.84	0.12-4.17	0.836	0.56	0.09-3.20	0.505
	Mean of minimum temperatures (°C) on days 24-30 prior to capture		1.23	1.08-1.44	0.003	1.22	1.00-1.54	0.062
Mean of minimum temperatures (°C) on days 84-90 prior to capture		1.28	1.34-1.48	< 0.001	1.27	0.16-2.69	0.029	
Mean of total precipitation (mm) on 7 days prior to capture		1.72	1.11-2.80	0.021	1.38	0.82-2.55	0.258	
	quadratic term	0.94	0.89-0.99	0.029	0.96	0.90-1.02	0.202	
Rat abundance	main effect	< 0.01	< 0.01-0.14	0.017	< 0.01	< 0.01-5.03	0.115	
	quadratic term	2.13x10 ⁶	23.05-1.97x10 ¹¹	0.012	4932	0.21-1.15x10⁸	0.097	
Human loitering		0.17	0.03-0.79	0.030	0.20	0.05-0.62	0.007	
Sexual maturity		5.18	1.66-22.83	0.011	5.20	1.67-22.97	0.011	
Proportion of block occupied by housing over commercial ^{***}								

Confounders (bold) and independent variable(s)	OR	Unadjusted			Adjusted		
		95% CI	P	OR	95% CI	P	
	< 0.25	ref					
	0.25-0.50	0.12	0.05-1.07	0.062	0.33	0.08-1.26	0.090
	> 0.5	12.61	1.88-156.35	0.014	4.89	0.89-48.89	0.081
Proportion of block occupied by grocery stores							
	none	ref					
	< 0.25	0.34	0.05-1.97	0.208	0.39	0.08-1.84	0.206
	≥ 0.25	3.64	0.49-38.55	0.200	2.44	0.42-22.74	0.337
Proportion of block occupied by low-rise apartments							
	none	ref					
	> 0	0.20	0.04-0.80	0.017	0.31	0.07-1.13	0.069
Mean of minimum temperatures (°C) on days 84-90 prior to capture							
Rat abundance	main effect	< 0.01	< 0.01-0.14	0.017	0.01	< 0.01-11.55	0.164
	quadratic term	2.13x10 ⁶	23.05-1.97x10 ¹¹	0.012	807.79	0.05-3.49x10⁷	0.145
Human loitering		0.17	0.03-0.79	0.030	0.30	0.08-0.92	0.040
Mean of total precipitation (mm) on 7 days prior to capture		1.72	1.11-2.80	0.021	1.52	1.01-2.41	0.056
	quadratic term	0.94	0.89-0.99	0.029	0.96	0.91-1.00	0.075
Sexual maturity		5.18	1.66-22.83	0.011	5.19	1.68-22.78	0.011
Mean of minimum temperatures (°C) on days 24-30 prior to capture							
Rat abundance	main effect	< 0.01	< 0.01-0.14	0.017	< 0.01	< 0.01-0.05	0.006
	quadratic term	2.13x10 ⁶	23.05-1.97x10 ¹¹	0.012	357990	53.93-2.90x10 ¹⁰	0.004
Mean of total precipitation (mm) on 7 days prior to capture		1.72	1.11-2.80	0.021	1.41	0.89-2.31	0.153
	quadratic term	0.94	0.89-0.99	0.029	0.96	0.91-1.01	0.150
Human loitering		0.17	0.03-0.79	0.030	0.18	1.09-1.42	0.016
Sexual maturity		5.18	1.66-22.83	0.011	5.31	1.71-23.31	0.010

Confounders (bold) and independent variable(s)	OR	Unadjusted			Adjusted		
		95% CI	P	OR	95% CI	P	
Mean of minimum temperatures (°C) on 7 days prior to capture							
Human loitering****	0.17	0.04-0.85	0.030	0.31	0.64-1.49	0.145	
Rat abundance	main effect	< 0.01	< 0.01-0.14	0.017	< 0.01	< 0.01-0.07	0.007
	quadratic term	2.13x10 ⁶	23.05-1.97x10 ¹¹	0.012	3.6 x10 ⁵	42.27-3.36 x10 ¹¹	0.005
Mean of total precipitation (mm) on 7 days prior to capture		1.72	1.11-2.80	0.021	1.78	1.07-3.14	0.033
	quadratic term	0.94	0.89-0.99	0.029	0.94	0.88-0.99	0.031
Mean of total precipitation (mm) on 7 days prior to capture							
Rat abundance	main effect	< 0.01	< 0.01-0.14	0.017	< 0.01	< 0.01-0.65	0.038
	quadratic term	2.13x10 ⁶	23.05-1.97x10 ¹¹	0.012	7.2x10 ⁵	3.27-2.18 x10 ¹²	0.030
Human loitering	0.17	0.03-0.79	0.030	0.13	0.02-0.38	0.010	

* Significant variable that was affected by a confounding relationship and overall p-value for categorical variable.

** Coefficient changed from a positive to negative value

*** Refers to buildings in which the ground floor is dedicated to commercial businesses and upper floors are residential.

**** There are slightly different values for human loitering between potential confounding variables due to model non-convergence in R for mean of minimum temperatures (°C) on 7 days prior to capture. Models that did not converge in R were run in Stata.

Supplemental Table 1. Characteristics of environmental risk factor variables and results of univariable mixed logistic regression models with random effect for block assessing associations with *Bartonella tribocorum* in Norway rats (*Rattus norvegicus*) captured in Vancouver Canada.

Category	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
Proportion of block occupied by commercial parcels	< 0.25	ref			0.08
	0.25-0.50	0.38	0.05, 2.49	0.30	
	0.5-0.75	1.51	0.15, 14.02	0.70	
	> 0.75	6.45	0.96, 66.02	0.06	
Proportion of block occupied by industrial parcels	none	ref			
	> 0	1.37	0.23, 8.88	0.71	
Proportion of block occupied by green space parcels	none	ref			
	> 0	0.32	0.03, 2.48	0.28	
Proportion of block occupied by vacant parcels	none	ref			
	> 0	1.57	0.13, 23.11	0.70	
Proportion of block occupied by parcels under construction	none	ref			
	> 0	2.41	0.03, 3.48	0.41	
Proportion of block occupied by abandoned parcels	none	ref			
	> 0	2.20	0.08, 2.30	0.31	
Proportion of block occupied by open parcels	none	ref			
	> 0	1.09	0.20, 6.34	0.91	
Proportion of block occupied by single family houses	none	ref			
	> 0	0.87	0.13, 5.25	0.87	
Proportion of block occupied by low-rise apartments	none	ref			
	> 0	0.20	0.04, 0.80	0.02	
Proportion of block occupied by mid-rise apartments	none	ref			
	> 0	0.69	0.12, 3.94	0.65	
Proportion of block occupied by housing over commercial buildings*	< 0.25	ref			< 0.001
	0.25-0.50	0.12	0.05, 1.07	0.06	
	> 0.5	12.61	1.88, 156.35	0.01	
Proportion of block occupied by buildings not associated with food	< 0.25	ref			

Category	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables	
Proportion of block occupied by restaurants	≥ 0.25	0.31	0.04, 1.79	0.19	0.03	
	none	ref				
Proportion of block occupied by grocery stores	> 0	1.84	0.32, 13.66	0.49		
	none	ref				
Proportion of block occupied by industrial food establishments	< 0.25	0.34	0.05, 1.97	0.21		
	≥ 0.25	3.64	0.49, 38.55	0.20		
Proportion of block occupied by other food establishments	none	ref				
	> 0	0.73	0.09, 5.22	0.75		
Proportion of block occupied by buildings in extremely poor condition	none	ref				
	> 0	1.53	0.28, 8.62	0.60		
Proportion of block occupied by buildings in fair condition	none	ref				0.74
	< 0.25	1.11	0.16, 7.85	0.91		
	0.25-0.50	2.37	0.20, 31.35	0.46		
Proportion of block occupied by buildings in good condition	≥ 0.25	ref				
	> 0.25	0.64	0.10, 3.73	0.61		
Proportion of block occupied by buildings in excellent condition	none	ref				0.70
	< 0.25	1.84	0.28, 14.43	0.51		
Proportion of block occupied by grounds in extremely poor condition	≥ 0.25	0.85	0.09, 7.54	0.87		
	none	ref				
Proportion of block occupied by grounds in poor condition	> 0	1.36	0.22, 10.84	0.74		
	< 0.25	ref				
Proportion of block occupied by grounds in fair condition	0.25-0.50	2.90	0.41, 26.37	0.26	0.39	
	< 0.25	ref				
Proportion of block occupied by grounds in good condition	0.25-0.50	0.30	0.04, 2.25	0.21		
	0.50-0.85	0.19	0.01, 3.12	0.22		
Proportion of block occupied by grounds in excellent condition	< 0.25	ref				
	≥ 0.25	0.72	0.12, 3.76	0.69		
Proportion of block occupied by grounds in excellent condition	none	ref				

Category	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables	
Proportion of block occupied by green space	> 0	0.45	0.06, 3.14	0.39	0.77	
	none	ref				
Proportion of block occupied by unkempt green space	> 0	0.27	0.03, 1.76	0.16		
	none	ref				
Proportion of block occupied by well-kempt green space	> 0	0.26	0.04, 1.27	0.08		
	none	ref				
Proportion of block occupied by food gardens	> 0	0.69	0.10, 3.69	0.65		
	none	ref				
Proportion of alley face in poor condition	> 0	0.16	0.01, 1.28	0.10		
	< 0.25	ref				
Proportion of alley face in fair condition	≥ 0.25	0.44	0.03, 5.99			
	< 0.25	ref				
	0.25-0.5	0.41	0.03, 5.35	0.49		
Proportion of alley face in good condition	0.5-0.75	0.64	0.09, 3.96	0.62		
	< 0.25	ref				
	≥ 0.25	1.09	0.19, 6.58	0.92		
Proportion of alley bordered by non-paved surface	> 0	2.21	0.27, 21.29	0.44		
	none	ref				
Number of rat holes at the alley face	< 2	ref				0.44
	2-3	0.29	0.04, 1.91	0.18		
	4-9	0.68	0.08, 6.80	0.71		
	10-26	2.14	0.17, 27.7	0.52		
Number of rat corridors at the alley face	0	ref				
	≥ 1	6.25	1.44, 39.51	0.02		
Amount of garbage/trash/junk/litter	A little	ref			0.20	
	Some	0.35	0.07-1.92	0.17		
	A lot	1.91	0.25-19.63	0.52		
Amount of overflowing garbage receptacles	A little	ref				
	Some to a lot	2.89	0.50, 24.05	0.52		
Number of commercial garbage receptacles	0-9	ref			0.08	

Category	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
Number of private garbage receptacles** (median [IQR])	10-11	0.37	0.04, 3.03	0.32	0.22
	12-18	1.36	0.18, 13.26	0.76	
	19-20	10.46	1.26, 209.15	0.05	
	0 (0-1)	0.86	0.61, 1.15	0.34	
	< 2	ref			
Number of commercial recycling receptacles	2-3	0.26	0.02, 2.46	0.22	0.40
	4-7	1.58	0.28, 13.07	0.61	
	0-2	ref			
	3-4	3.21	0.52, 26.80	0.20	
Presence of strong odors	5-9	1.16	0.11, 0.54	0.89	0.61
	yes	ref			
	no	0.65	0.10, 4.00	0.61	
Amount of loitering	None to light	ref			0.03
	Moderate to heavy	0.17	0.03, 0.79	0.03	
Amount of transport	Light	ref			0.80
	Moderate	0.72	0.09, 5.73	0.73	
	Heavy	1.36	0.14, 17.24	0.78	

* Refers to buildings in which the ground floor is dedicated to commercial businesses and upper floors are residential.

** Analyzed as a continuous variable

Supplemental Table 2. Characteristics of weather and abundance risk factor variables and results of univariable mixed logistic regression models with random effect for block assessing associations with *Bartonella tribocorum* in Norway rats (*Rattus norvegicus*) captured in Vancouver Canada.

Category	Variable Format	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
Mean Temperature in 5 days prior to capture (°C)	Categorized by quartiles	< 6.14	ref			0.59
		≥ 6.14-8.47	1.41	0.63, 3.16	0.40	
		≥ 8.48-12.45	0.66	0.20, 2.16	0.48	
		≥ 12.46	0.64	0.15, 2.50	0.52	
Mean Temperature in 10 days prior to capture (°C)	Categorized by quartiles	< 6.58	ref			0.79
		≥ 6.58-9.15	1.29	0.41, 3.87	0.65	
		≥ 9.16-12.74	0.92	0.27, 3.27	0.90	
		≥ 12.75	0.66	0.15, 2.67	0.56	
Mean Temperature in 15 days prior to capture (°C)	Categorized by quartiles	< 6.55	ref			0.77
		≥ 6.55-9.55	1.00	0.32, 2.97	0.99	
		≥ 9.56 – 12.74	1.28	0.37, 4.74	0.69	
		≥ 12.75	0.81	0.19, 3.41	0.77	
Mean Temperature in 30 days prior to capture (°C)	Categorized by quartiles	< 5.41	ref			0.35
		≥ 5.41-10.62	1.00	0.29, 3.01	1.00	
		≥ 10.64-14.24	0.86	0.21, 3.38	0.83	
		≥ 14.25	3.01	0.56, 17.77	0.19	
Mean Temperature in 60 days prior to capture (°C)	Continuous	NA	1.16	1.02, 1.34	0.02	
Mean Temperature in 90 days prior to capture (°C)	Continuous	NA	1.21	1.07, 1.39	< 0.001	
Total precipitation in 5 days prior to capture (mm)	Categorized by quartiles	< 7.60	ref			0.02
		≥ 7.60-17.99	1.41	0.47, 4.36	0.54	

Category	Variable Format	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
		≥ 18.00-30.59	1.80	0.61, 5.56	0.29	
		≥ 30.60	4.44	1.48, 14.57	0.01	
Total precipitation in 10 days prior to capture (mm)	Categorized by quartiles	< 26.50	ref			0.19
		≥ 25.50-35.19	0.42	0.16, 1.06	0.07	
		≥ 35.20-50.99	0.84	0.33, 2.08	0.70	
		≥ 51.00	0.63	0.25, 1.57	0.32	
Total precipitation in 15 days prior to capture (mm)	Categorized by quartiles	< 48.70	ref			0.19
		≥ 48.70-58.59	1.62	0.41, 6.11	0.48	
		≥ 58.60-72.59	2.20	0.58, 7.71	0.22	
		≥ 72.60	0.91	0.22, 3.48	0.88	
Total precipitation in 30 days prior to capture (mm)	Categorized by quartiles	< 107.3	ref			0.71
		≥ 107.30-119.39	1.33	0.52, 3.40	0.55	
		≥ 119.40-150.19	1.57	0.55, 4.67	0.40	
		≥ 150.20	0.82	0.23, 2.96	0.76	
Total precipitation in 60 days prior to capture (mm)	Categorized by quartiles	< 191.00	ref			0.83
		≥ 191.00-216.79	0.56	0.09, 3.99	0.52	
		≥ 216.80-310.79	0.48	0.08, 3.42	0.42	
		≥ 310.80	0.40	0.05, 3.97	0.39	
Total precipitation in 90 days prior to capture (mm)	Categorized by quartiles	< 227.50	ref			0.02
		≥ 227.50-358.99	0.76	0.10, 4.86	0.76	
		≥ 359.00-484.39	0.23	0.03, 1.43	0.09	
		≥ 484.40	0.09	0.01, 0.63	0.02	

Category	Variable Format	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
Mean of minimum temperatures on 7 days prior to capture (°C)	Categorized by quartiles	< 3.61	ref			0.01
		≥ 3.61-6.08	1.11	0.44, 2.67	0.82	
		≥ 6.09-10.19	0.09	0.01, ≥0.52	0.01	
		≥ 10.20	0.84	0.12, 4.17	0.84	
Mean of minimum temperatures on days 8-14 prior to capture (°C)	Categorized by quartiles	< 4.06	ref			0.18
		≥ 4.06-7.75	0.35	0.07, 1.39	0.16	
		≥ 7.76-10.28	0.49	0.09, 2.05	0.35	
		≥ 10.29	1.53	0.23, 9.40	0.64	
Mean of minimum temperatures on days 24-30 prior to capture (°C)	Continuous	NA	1.23	1.08, 1.44	< 0.001	
Mean of minimum temperatures on days 54-60 prior to capture (°C)	Categorized by quartiles	< 3.54	ref			0.05
		≥ 3.54-8.19	1.09	0.31, 3.75	0.90	
		≥ 8.20-13.30	6.60	1.58, 28.86	0.01	
		≥ 13.31	5.18	1.21, 22.54	0.02	
Mean of minimum temperatures on days 84-90 prior to capture (°C)	Continuous	NA	1.28	1.14, 1.48	< 0.001	
Mean of maximum temperatures on 7 days prior to capture (°C)	Categorized by quartiles	< 8.96	ref			0.94
		≥ 8.96-10.83	0.97	0.31, 2.82	0.95	
		≥ 10.84-15.15	0.71	0.20, 2.56	0.60	
		≥ 15.16	0.74	0.13, 3.81	0.71	
Mean of maximum temperatures on days 8-14 prior to capture (°C)	Categorized by quartiles	< 8.51	ref			0.33
		≥ 8.51-13.28	1.31	0.34, 4.59	0.68	
		≥ 13.29-16.01	0.92	0.23, 3.72	0.91	

Category	Variable Format	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
		≥ 16.01	2.04	0.46, 9.08	0.34	
Mean of maximum temperatures on days 24-30 prior to capture (°C)	Continuous with log transformation	NA	1.31	1.16, 21.5	0.03	
Mean of maximum temperatures on days 54-60 prior to capture (°C)	Continuous	NA	1.14	1.05, 1.23	< 0.001	
Mean of maximum temperatures on days 84-90 prior to capture (°C)	Categorized by quartiles	< 7.47	ref			0.01
		≥ 7.47-13.06	0.94	0.26, 3.37	0.93	
		≥ 13.07-21.02	2.61	0.56, 12.12	0.20	
		≥ 21.03	11.22	2.48, 51.23	< 0.001	
Mean of mean temperatures on 7 days prior to capture (°C)	Categorized by quartiles	< 6.44	ref			0.72
		≥ 6.44-8.33	1.38	0.47, 3.92	0.54	
		≥ 8.34-12.40	0.99	0.29, 3.46	0.98	
		≥ 12.41	0.68	0.16, 2.74	0.58	
Mean of mean temperatures on days 8-14 prior to capture (°C)	Categorized by quartiles	< 6.20	ref			0.38
		≥ 6.20-10.68	1.27	0.21, 6.40	0.78	
		≥ 10.69-12.73	1.08	0.17, 6.08	0.93	
		≥ 12.74	2.96	0.45, 18.51	0.23	
Mean of mean temperatures on days 24-30 prior to capture (°C)	Categorized by quartiles	< 5.69	ref			0.21
		≥ 5.69-10.85	0.97	0.20, 4.83	0.97	
		≥ 10.86-14.16	2.17	0.41, 11.32	0.34	
		≥ 14.17	1.75	0.21, 11.46	0.57	

Category	Variable Format	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
Mean of mean temperatures on days 54-60 prior to capture (°C)	Continuous	NA	1.18	1.08, 1.31	< 0.001	
Mean of mean temperatures on days 84-90 prior to capture (°C)	Categorized by quartiles	< 5.54	ref			< 0.001
		≥ 5.54-10.16	0.67	0.18, 2.53	0.55	
		≥ 10.17-17.30	4.17	1.06, 16.80	0.03	
		≥ 17.31	9.50	2.14, 42.89	< 0.001	
Mean of total precipitation on 7 days prior to capture (°C)	Main effect	NA	1.72	1.11, 2.80	0.02	
		Quadratic term	NA	0.94	0.89, 0.99	
Mean of total precipitation on 8-14 days prior to capture (°C)	Categorized by quartiles	< 2.46	ref			0.13
		≥ 2.46-4.06	0.54	0.21, 1.34	0.19	
		≥ 4.07-5.85	0.77	0.32, 1.80	0.56	
		≥ 5.86	0.27	0.08, 0.86	0.03	
Mean of total precipitation on 24-30 days prior to capture (°C)	Categorized by quartiles	< 2.64	ref			0.65
		≥ 2.64-3.79	1.69	0.72, 4.09	0.24	
		≥ 3.80-5.42	1.27	0.47, 3.38	0.64	
		≥ 5.43	1.22	0.42, 3.69	0.72	
Mean of total precipitation on 54-60 days prior to capture (°C)	Main effect	NA	0.76	0.56, 1.12	0.06	
		Quadratic term	NA	1.03	1.01, 1.06	
Mean of total precipitation on 84-90 days prior to capture (°C)	Categorized by quartiles	< 1.01	ref			0.51
		≥ 1.01-4.36	1.29	0.24, 13.83	0.80	
		≥ 4.37-6.99	0.67	0.11, 7.74	0.70	
		≥ 7.00	1.22	0.15, 26.46	0.88	

Category	Variable Format	Sub-category	Odds Ratio	95% CI	P	Overall P for Categorical Variables
Rat abundance	Main effect	NA	1.6 X10 ⁻⁵	3.1 X10 ⁻¹⁰ , 0.13	0.02	
	Quadratic term	NA	1.9X10 ⁶	25.40, 2.0X10 ¹²	0.01	
