

Culling of Urban Norway Rats and Carriage of *Bartonella* spp. Bacteria, Vancouver, British Columbia, Canada

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We investigated the effects of culling on *Bartonella* spp. bacteria carriage among urban rats in Canada. We found that the odds of *Bartonella* spp. carriage increased across city blocks except those in which culling occurred. Removing rats may have prevented an increase in *Bartonella* spp. prevalence, potentially lowering human health risks.

Urban Norway rats (*Rattus norvegicus*) carry *Bartonella* spp., which are bacteria transmitted among rats and to humans through vectors including fleas (1). Infection in humans can result in fever, fatigue, myalgia, and endocarditis (2). In Vancouver, British Columbia, Canada, a serosurvey of residents of an underresourced neighborhood found that 3% of participants had been exposed to *B. tribocorum* (3), a species found in rats in this neighborhood (4), suggesting that rats may be an exposure source for humans in this area.

Although aimed at decreasing disease risks, culling methods (i.e., lethal removal) may increase zoonotic pathogen prevalence by altering normal behaviors that modify pathogen transmission (5,6). We sought to determine whether culling rats altered *Bartonella* spp. prevalence in rats and their fleas in

the Downtown Eastside neighborhood of Vancouver. The University of British Columbia's Animal Care Committee (A14-0265) approved study procedures.

The Study

We trapped rats in 12 study sites (5 intervention, 7 control), each comprising 3 contiguous city blocks (36 total blocks) (Figure, panel A) during June 2016–January 2017 (Appendix, <https://wwwnc.cdc.gov/EID/article/28/8/21-1164-App1.pdf>). We placed 10 live traps (Tomahawk Live Traps, <https://www.livetraps.com>) in the alley of each block. We conducted the experiment in 3 trapping phases: before, during, and after the intervention (Figure, panel B). Before and after the intervention, we captured rats, gave each a numbered ear tag, and released it to its capture site. In the center block of intervention sites culling occurred during the second trapping phase. In flanking blocks (those adjacent to the intervention block) and control blocks, no culling occurred (Figure, panel A).

We collected blood from all rats via jugular puncture under isoflurane anesthesia. We collected fleas by brushing the coat.

We identified fleas to species (7), and pooled ≤ 5 fleas per rat. We extracted DNA from rat blood and fleas using the DNEasy Blood and Tissue Kit (QIAGEN, <https://www.qiagen.com>). We tested DNA extracts for *Bartonella* spp. by real-time PCR. For rat blood, we used primers to detect a 380-bp segment of the citrate synthase gene (*gltA*) (8). For fleas, we used a probe-based real-time PCR assay to detect a 302-bp fragment of the *ssrA* gene (9). We conducted our analysis as described in Himsworth et al. (10).

We used generalized linear mixed models to assess the relationship between the intervention and *Barton-*

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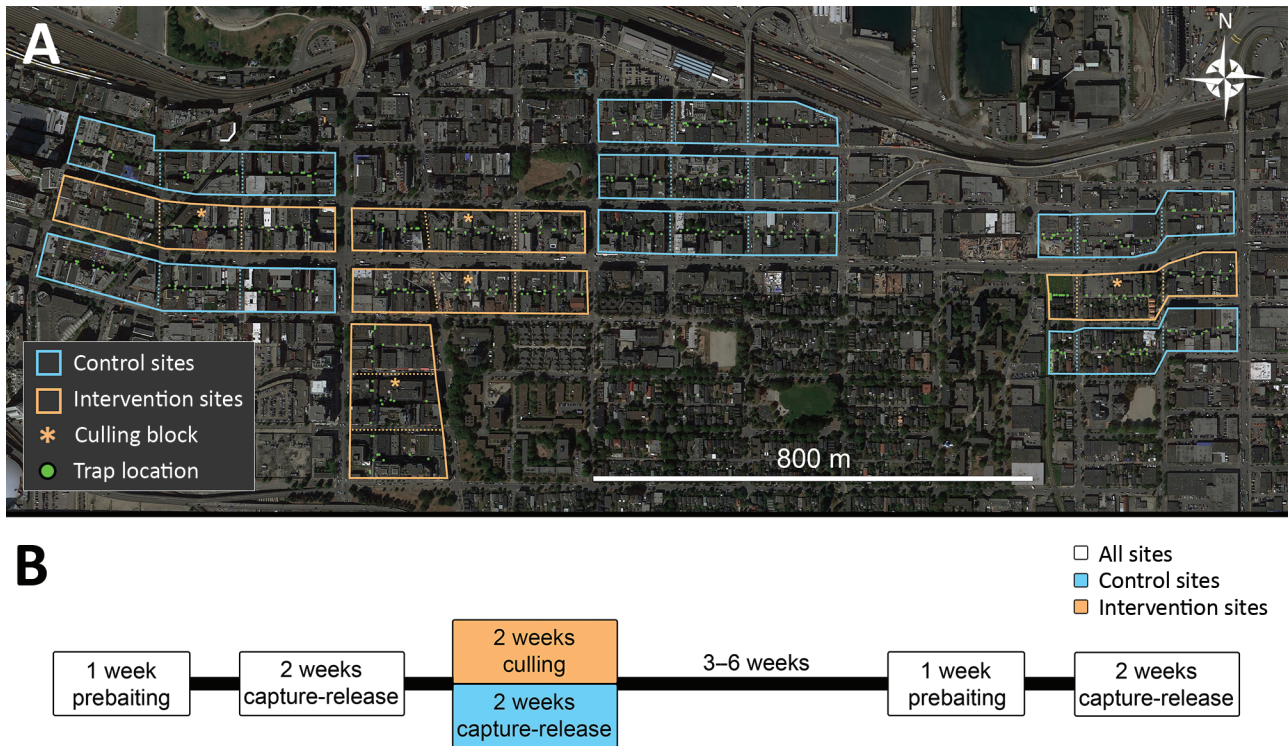


Figure. Trapping locations for Norway rats (*Rattus norvegicus*) caught in Vancouver, British Columbia, Canada. A) Trapping sites consisting of 3 contiguous city blocks. Each site was designated as a control or intervention site. Control sites did not involve culling (lethal animal removal); intervention sites included culling in the central block. B) Depiction of the study timeline. We first baited traps without capture to acclimatize rats to traps, then trapped and tagged rats with numbered ear tags and released the rats to their site of capture. After an intervention that involved culling rats in intervention sites, we resampled 3–6 weeks later to determine whether *Bartonella* spp. carriage differed between trapping periods before and after the intervention.

ella spp. carriage. We controlled for spatial clustering by city block as a random effect. We assessed positive or negative carriage by rats (model A) and fleas (model B) and the number of fleas per rat (model C). We analyzed carriage models A and B by logistic regression and model C by negative binomial regression. For all models, the intervention variable consisted of 4 categories indicating when rats or fleas were caught: before the intervention in all blocks; after the intervention in control blocks; after the intervention in flanking blocks; and after the intervention in intervention blocks.

We used a hypothesis-testing model building approach to estimate the effect of the intervention while accounting for covariates (Table). We retained covariates if they confounded the relationship between the intervention and the outcome (i.e., if they changed the effect of any level of the intervention by $\geq 10\%$ or if their association with the outcome and intervention had $p \leq 0.25$). We also kept independent predictors of the outcome if they significantly improved the model, as indicated by a likelihood ratio test result of $p \leq 0.05$; that test compared 2 nested models, each with the intervention variable and all confounders present, but

with and without the potential predictor variable.

We trapped 512 Norway rats; 206 (40.2%) of them had fleas. The median number of fleas per rat was 0 (range 0–58; mean 1.18). All fleas were *Nosopsyllus fasciatus*. We obtained blood from 454 rats; 90 (20%) tested positive for *Bartonella* spp. We tested 201 flea pools; 86 (42.8%) tested positive for *Bartonella* spp. (Table). In the final model A, which contained the variables season, presence of *Bartonella* spp.–positive fleas, and wound presence as covariates, the odds of *Bartonella* spp. carriage were significantly higher among rats caught after the intervention in control blocks (odds ratio [OR] 2.68; 95% CI 1.22–6.67) and flanking blocks (OR 7.26; 95% CI 1.56–38.17), but not in the intervention blocks (OR 2.03; 95% CI 0.22–15.41), when compared with the odds of carriage before the intervention in all block types (Table). We saw no association between the intervention and the number of fleas per rat or *Bartonella* spp. carriage by fleas.

Conclusions

The prevalence of *Bartonella* spp. bacteria among rats in this neighborhood has been shown to increase in

the fall (4). Our study suggests that culling rats may have prevented this increase within the blocks where culling occurred.

Removing rats may change how individual rats interact within colonies, which alters pathogen transmission. *Bartonella* spp. transmission via fleas (1) requires close contact among individual rats. Rats burrow communally, establishing a network of chambers with some shared nests (11). Those

nests promote close contact among rats and act as a source of fleas that spend time in the nest (12). Decreased rat population density may lessen nest sharing and behaviors such as social grooming, thereby reducing opportunities for fleas to transmit *Bartonella* spp. among individual rats. A reduction in *Bartonella* spp. prevalence may decrease exposure risk for humans, but the relationship between rodents, vectors, pathogens, and humans is

Table. Mixed effects logistic regression models of the effect of intervention on *Bartonella* spp. carriage by Norway rats (*Rattus norvegicus*), Vancouver, British Columbia, Canada*

Variable	<i>Bartonella</i> prevalence, no. positive/no. tested (%)	Bivariable models			Final model†	
		Unadjusted OR (95% CI)	p value in model	LRT p value‡	Adjusted OR (95% CI)	p value in model
Intervention						
Rats caught before the intervention in all blocks	58/267 (22)	Referent	Referent	Referent	Referent	Referent
Rats caught after the intervention in control blocks	24/109 (22)	1.26 (0.67–2.39)	0.47	<0.01	2.68 (1.22–6.67)	0.02
Rats caught after the intervention in flanking blocks	6/37 (16)	0.56 (0.18–1.46)	0.26	NA	7.26 (1.56–38.17)	0.01
Rats caught after the intervention in intervention blocks	2/41 (5)	0.12 (0.02–0.46)	<0.01	NA	2.03 (0.22–15.41)	0.50
Sex						
F	38/221 (17)	Referent	Referent	Referent	NA	NA
M	52/233 (22)	1.32 (0.82–2.14)	0.26	0.26	NA	NA
Sexual maturity						
Juvenile	34/177 (19)	Referent	Referent	Referent	NA	NA
Mature	56/277 (20)	0.98 (0.60–1.63)	0.95	0.95	NA	NA
Wound presence						
Absent	59/339 (17)	Referent	Referent	Referent	Referent	Referent
Present	31/115 (27)	1.67 (0.97–2.81)	0.06	0.06	1.49 (0.83–2.63)	0.17
Weight§						
	NA	1.04 (0.81–1.32)	0.75	0.75	NA	NA
Presence of fleas on rats						
Absent	46/261 (18)	Referent	Referent	Referent	NA	NA
Present	44/193 (23)	1.39 (0.86–2.25)	0.18	0.18	NA	NA
No. fleas on rat						
	NA	1.02 (0.95–1.09)	0.50	0.52	NA	NA
Flea index#						
	NA	1.13 (0.90–1.43)	0.31	0.32	NA	NA
Presence of positive fleas per rat						
Absent	67/376 (18)	Referent	Referent	Referent	Referent	Referent
Present	23/78 (30)	1.83 (1.00–3.25)	0.04	0.05	1.94 (1.00–3.69)	0.05
Season						
Summer, June–August	16/124 (13)	Referent	Referent	Referent	Referent	Referent
Fall, September–November	65/208 (31)	3.16 (1.59–6.73)	<0.01	<0.01	2.90 (1.32–6.31)	<0.01
Winter, December–March	9/122 (7)	0.50 (0.18–1.30)	0.15	NA	0.16 (0.03–0.68)	0.02

*OR refers to the odds of *Bartonella* spp. carriage among rats in each group relative to the reference group for that variable. Variables were included in the final model if they confounded the relationship between the intervention and the outcome (changed the effect of any level of the intervention by $\geq 10\%$ and/or were associated with the outcome and intervention; $p \leq 0.25$) or if they were independent predictors that improved the model as indicated by a significant ($p \leq 0.05$ likelihood ratio test with all confounders and intervention present). LRT, likelihood ratio test; NA, not applicable; OR, odds ratio.

†Final multivariable model: *Bartonella* status ~ intervention + wound presence + presence of positive fleas per rat + season + (city.block).

‡Likelihood ratio test comparing the generalized linear mixed model with and without the indicated variable; $p \leq 0.05$ indicates that the variable significantly improved the model with all confounders and as such was a significant predictor and was retained in the final model.

§Scaled and centered around its mean.

#Average number of fleas per rat per city block.

complex (13). For example, although a previous study revealed that residents in this neighborhood had been exposed to *Bartonella* spp. (3), it is unclear whether this exposure was associated with rats and to what extent humans encounter fleas. Furthermore, for other fleaborne pathogens such as *Yersinia pestis* (agent of the plague), culling rats may increase disease transmission to humans as fleas seek new hosts (14). Understanding how rat abundance and rat removal impacts intraspecies and interspecies dynamics and pathogen prevalence is necessary to anticipate management impacts on pathogen transmission.

Whereas our intervention involved removing rats and their fleas, we did not observe a change in the number of fleas on rats. The steady number suggests that culling did not reduce flea abundance, perhaps because *N. fasciatus* fleas also reside in the burrows, such that the number of fleas per rat does not reflect the total number of fleas in a city block (12). It is possible that our intervention removed a negligible proportion of the flea population. In addition, we did not observe a change in the odds of *Bartonella* spp. carriage among fleas. A past study in this neighborhood showed that *Bartonella* spp. carriage among rats was not related to flea presence or abundance; therefore, the role of *N. fasciatus* fleas in the ecology of *Bartonella* spp. in this ecosystem remains enigmatic (15).

Our findings counter a study of *Leptospira interrogans* using the same experimental design, in which culling was associated with an increased odds of infection among rats (5). This difference is likely attributable to differences in transmission; *L. interrogans* is spread via urine (13) and *Bartonella* spp. via fleas (1). Culling may alter a variety of social interactions (e.g., fighting, nest-sharing, grooming) which affect the spread of these pathogens differently. Together, these studies illustrate the complexity of managing rat-associated zoonoses; the intervention may have opposite effects on different pathogens. Indeed, past literature has shown that culling wildlife to control zoonoses can have unpredictable consequences (6) and that ecosystem-based approaches that manage the human-wildlife interface may be more effective.

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References

- Gutiérrez R, Krasnov B, Morick D, Gottlieb Y, Khokhlova IS, Harrus S. *Bartonella* infection in rodents and their flea ectoparasites: an overview. *Vector Borne Zoonotic Dis.* 2015;15:27–39. <https://doi.org/10.1089/vbz.2014.1606>
- Kosoy M, Bai Y, Sheff K, Morway C, Baggett H, Maloney SA, et al. Identification of *Bartonella* infections in febrile human patients from Thailand and their potential animal reservoirs. *Am J Trop Med Hyg.* 2010;82:1140–5. <https://doi.org/10.4269/ajtmh.2010.09-0778>
- McVea DA, Himsforth CG, Patrick DM, Lindsay LR, Kosoy M, Kerr T. Exposure to rats and rat-associated *Leptospira* and *Bartonella* species among people who use drugs in an impoverished, inner-city neighborhood of Vancouver, Canada. *Vector Borne Zoonotic Dis.* 2018;18:82–8. <https://doi.org/10.1089/vbz.2017.2179>
- Himsforth CG, Bai Y, Kosoy MY, Wood H, DiBernardo A, Lindsay R, et al. 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 Zoonotic Dis.* 2015;15:21–6. <https://doi.org/10.1089/vbz.2014.1657>
- Lee MJ, Byers KA, Donovan CM, Bidulka JJ, Stephen C, Patrick DM, et al. Effects of culling on *Leptospira interrogans* carriage by rats. *Emerg Infect Dis.* 2018;24:356–60. <https://doi.org/10.3201/eid2402.171371>
- Donnelly CA, Woodroffe R, Cox DR, Bourne FJ, Cheeseman CL, Clifton-Hadley RS, et al. Positive and negative effects of widespread badger culling on tuberculosis in cattle. *Nature.* 2006;439:843–6. <https://doi.org/10.1038/nature04454>
- Holland GP. The fleas of Canada, Alaska, and Greenland (Siphonaptera). *Memoirs of the Entomological Society of Canada.* 1985;117(S130):3–632. <https://doi.org/10.4039/entm117130fv>
- Hornok S, Beck R, Farkas R, Grima A, Orranto D, Kontschán J, et al. High mitochondrial sequence divergence in synanthropic flea species (Insecta: Siphonaptera) from Europe and the Mediterranean. *Parasit Vectors.* 2018;11:221. <https://doi.org/10.1186/s13071-018-2798-4>
- Diaz MH, Bai Y, Malania L, Winchell JM, Kosoy MY. Development of a novel genus-specific real-time PCR assay for detection and differentiation of *Bartonella* species and genotypes. *J Clin Microbiol.* 2012;50:1645–9. <https://doi.org/10.1128/JCM.06621-11>

10. Himsforth CG, Byers KA, Fernando C, Speerin L, Lee MJ, Hill JE. When the sum of the parts tells you more than the whole: The advantage of using metagenomics to characterize *Bartonella* spp. infections in Norway rats (*Rattus norvegicus*) and their fleas. *Front Vet Sci*. 2020;7:584724. <https://doi.org/10.3389/fvets.2020.584724>
11. Calhoun JB. The ecology and sociology of the Norway rat. Bethesda, MD: US Department of Health, Education, and Welfare, Public Health Service; 1963.
12. Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D. Fleas and flea-borne diseases. *Int J Infect Dis*. 2010;14:e667–76. <https://doi.org/10.1016/j.ijid.2009.11.011>
13. Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. *Crit Rev Microbiol*. 2009;35:221–70. <https://doi.org/10.1080/10408410902989837>
14. Keeling MJ, Gilligan CA. Metapopulation dynamics of bubonic plague. *Nature*. 2000;407:903–6. <https://doi.org/10.1038/35038073>
15. Himsforth CG, Byers KA, Whelan T, Bai Y, Kosoy MY. Flea presence and abundance are not predictors of *Bartonella tribocorum* carriage in Norway rats (*Rattus norvegicus*) from an underserved neighborhood of Vancouver, Canada. *Vector-Borne and Zoonotic Diseases*. 2021;21:121–24. <https://doi.org/10.1089/vbz.2020.2665>

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etymologia

***Dermatophilus congolensis* [dur''mə-tof'ĭ-ləs con-gō-len'sis]**

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D*ermatophilus congolensis* From the Greek *derma* (skin) + *philos* (loving), *Dermatophilus congolensis* is a Gram-positive, aerobic actinomycete, and facultatively anaerobic bacteria. *D. congolensis* infects the epidermis and produces exudative dermatitis termed dermatophilosis that was previously known as rain rot, rain scald, streptotrichosis, and mycotic dermatitis.

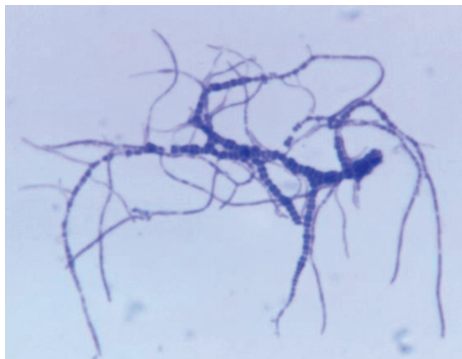


Figure 1. Photomicrograph of *Dermatophilus congolensis*, showing a Giemsa-stained, gram-positive bacteria. Source: Dr. Jerrold Kaplan, Centers for Disease Control, 1965.

In 1915, René Van Saceghem, a Belgian military veterinarian stationed at a veterinary laboratory in the former Belgian Congo (thus, the species name *congolensis*), reported *D. congolensis* from exudative dermatitis in cattle. Local breeders and veterinarians had observed the disease since 1910, but the causal agent was not identified.

Dermatophilosis affects animals, mainly cattle, and more rarely humans. Outbreaks of *D. congolensis* infection have severe economic implications in the livestock and leather industries.



Figure 2. René Van Saceghem (1884–1965). Source: Mortelmans J. *Veterinary medicine in Belgian Congo and Ruanda-Urundi from 1885 to 1962* [in French]. *Vlaams Diergeneeskundig Tijdschrift*. 2003;72:83–95. Courtesy of the Institute of Tropical Medicine (Antwerp). <https://vdt.ugent.be/?q=nl/content/72-2-83-95>

Sources

1. Amor A, Enríquez A, Corcuera MT, Toro C, Herrero D, Baquero M. Is infection by *Dermatophilus congolensis* underdiagnosed? *J Clin Microbiol*. 2011;49:449–51. <https://doi.org/10.1128/JCM.01117-10>
2. Branford I, Johnson S, Chapwanya A, Zayas S, Boyen F, Mielcarska MB, et al. Comprehensive molecular dissection of *Dermatophilus congolensis* genome and first observation of *tet(z)* tetracycline resistance. *Int J Mol Sci*. 2021;22:7128. <https://doi.org/10.3390/ijms22137128>
3. *Dorland's illustrated medical dictionary*. 32nd ed. Philadelphia: Elsevier Saunders; 2012.
4. Van Saceghem R. Contagious skin disease (contagious impetigo) [in French]. *Bull Soc Pathol Exot*. 1915;8:354–9.

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Appendix

Methodology

Trapping

Trapping was conducted in the Downtown Eastside neighborhood of Vancouver, Canada from June 2016–January 2017. The study included 12 study sites, each consisting of 3 city blocks (total = 36); 5 were designated as intervention sites and 7 were designated as controls. In intervention sites, lethal trapping occurred in the central block, while the 2 adjacent blocks were considered non-lethal flanking blocks. This designation was meant to account for potential rat movement between contiguous alleyways that might occur in response to the intervention. In control sites, no lethal trapping occurred.

To capture rats, we placed 10 Tomahawk rigid traps (Tomahawk Live Traps, Hazelhurst, WI, USA) in the alley of each city block. Traps were covered with stainless steel covers (Integrated Pest Supplies Ltd, New Westminster, BC, Canada) to prevent vandalism and to minimize contact between captured rats and humans. Traps were chained to immovable objects in alleyways to prevent removal.

Three study sites were trapped at a time, including a total of 9 city blocks and 90 active traps. Prior to active trapping, we prebaited traps for 1 week by baiting and fixing traps open to acclimatize rats to traps and to increase the likelihood of rats entering traps during the active trapping period (1,2). Traps were baited with a peanut butter–oat mixture and Hydrogel (ClearH2O, Westbrook, ME, USA) to provide a water source.

During active trapping periods, traps were set at 4 PM and checked every morning before 7 AM. Traps were active 5 days a week and on the sixth and seventh days, traps were fixed open

and baited to reacclimatize rats to traps (2). To minimize the potential of pathogen spread among rats due to contamination, all traps were sanitized in 10% bleach after coming into contact with any rat and after any prebaiting period (3).

Active trapping occurred for a total of 6 weeks, divided into 2-week trapping periods. These trapping periods were designated as before; during; or after the intervention. During the before and after periods, all rats that were caught were released at their site of capture after sampling (detailed below). During the intervention period, rats caught in intervention blocks were euthanized by intracardiac injection with pentobarbital after sampling.

Rat Sampling

Trapped rats were transported to a mobile laboratory-van where they were covered with a blanket to minimize stress before sampling. Each rat was transferred into an inhalation induction chamber (Kent Scientific, Torrington, CT, USA) and anesthetized with 5% isoflurane in oxygen using an isoflurane vaporizer (Associated Respiratory Veterinary Services, Lacombe, AB, Canada). Anesthesia was maintained throughout sampling through a nose cone administering the anesthetic.

To identify rats which had been previously caught, each rat was given a unique laser-etched ear-tag (Kent Scientific). The following demographic characteristics were recorded for each rat: bodyweight (grams); sexual maturity (maturity was determined as males with scrotal testes and females with a perforate vagina); sex (male or female); and the presence/absence of bite wounds. Bite wounds were considered an important characteristic in this study because they might indicate close contact among individuals (which would allow for fleas to spread among rats) and because *Bartonella* spp. is spread in flea feces and the bacteria is introduced through openings in the skin (4).

Blood was collected from each rat via the jugular and then stored in heparin-coated microtainers (BC, Mississauga, Canada). Fleas were collected from rats in 2 ways. First, the majority of fleas vacated the rat while in the induction chamber and were collected from the induction chamber using tweezers. Second, the coat of each rat was brushed thoroughly over a collection bowl to dislodge any remaining fleas.

Prior to release at their location of capture, rats were allowed to fully recover from the anesthesia (\approx 15–30 minutes). Individuals that were recaptured were resampled if they were

caught more than 7 days after their prior sampling. This period was informed by guidelines from the University of British Columbia's Animal Care Committee to ensure that rats had sufficient time to recover between blood collection periods.

DNA Extraction

Rat blood and fleas were stored at -80°C before DNA extraction. Fleas were identified to species by observing them under a compound microscope at $40\times$ (5). To extract DNA from fleas, we pooled up to 5 fleas per rat. Flea pools were surface-sterilized in 10% bleach and then rinsed in nuclease-free water and twice in 100% ethanol to remove bacteria from the external body. Flea pools were then crushed using a sterile scalpel. DNA from rat blood and fleas was extracted using the QIAgen DNEasy Blood and Tissues Kit following the manufacturer's protocol.

Detailed methods for *Bartonella* spp. testing are outlined in Himsworth et al. (6).

Statistical Analysis

Model A The impact of the intervention on *Bartonella* spp. carriage among rats.

To assess the impact of the intervention on *Bartonella* spp. carriage among rats we built mixed effects logistic regression models, otherwise known as generalized linear mixed models, or GLMMs. The outcome for Model A was the *Bartonella* spp. status of individual rats (positive or negative). The intervention was expressed statistically as a categorical variable consisting of four levels and was an indicator of whether rats were captured: 1) in the 2 weeks before the intervention in any block type (the reference category); 2) in the 2-week period after the intervention in control blocks; 3) in the 2-week period after the intervention in flanking blocks; or 4) in the 2-week period after the intervention in intervention blocks. Although we did not perform the intervention in control and flanking blocks, we considered the 2-week trapping periods after the intervention in control and flanking blocks independently to detect temporal changes in prevalence not related to the intervention. Finally, we included an indicator of the city-block as a random effect in all steps of the modeling process to control for heterogenous prevalence of *Bartonella* spp. among blocks (7).

For each GLMM we took a hypothesis testing model building approach to estimate the effect of the intervention on the *Bartonella* spp. status of each rat while controlling for important covariates. Potential covariates included sex (male or female), sexual maturity (juvenile or mature), weight (g), presence of fleas (present or absent), presence of bite wounds (present or

absent), number of fleas (count), flea index (average number of fleas per rat in a city-block), presence of positive fleas (present or absent), and season (summer, fall, winter). Summer was designated as June–August, fall was September–November, winter was December–March.

We included covariates in the model that were either confounders of the relationship between the intervention variable and the outcome or that were independent significant predictors of the outcome. In step one, we assessed whether covariates were confounders. To do this, we first examined the relationships between each potential confounder and the outcome in separate GLMMs containing only that variable and the random effect of the city block. Variables associated with the outcome ($p \leq 0.25$) were then assessed for their impact on the intervention variable. To do this, we evaluated whether the inclusion of each covariate, that was associated with the outcome ($p \leq 0.25$), in a GLMM containing that variable and the intervention variable, changed the effect estimate of any level of the intervention (i.e., rats caught before the intervention in any block type, rats caught after the intervention in control blocks, etc.) by $\geq 10\%$ relative to its effect estimate in a GLMM containing only the intervention variable. If the variable met both of these criteria, they were considered confounders. In step two, variables that did not meet the confounder criteria, but were still significantly ($p \leq 0.05$) associated with the outcome were considered as independent significant predictors. Each of these variables were entered into the model containing the intervention and identified confounders. Variables were retained in the model if they substantially decreased the standard error of any level of the intervention variable effect estimates and/or if they significantly improved the model as evidenced by a significant likelihood ratio test ($p \leq 0.05$) that compared the overall model with and without that variable. The final model contained all confounders and significant predictors that met these criteria.

Thirty-three animals were recaptured; 13 were recaptured in the 2 weeks before the intervention and 20 were recaptured after. For rats that were recaptured within the same period (before or after) rat characteristics were either averaged (e.g., average weight) or for characteristics that were categories (i.e., juvenile vs. mature) the value for the latest capture was used. Further, if any of these recaptured rats tested positive once, then they were considered to be *Bartonella* spp. positive. The resulting dataset included 454 rats.

Model B The impact of the intervention on *Bartonella* spp. carriage among fleas.

The impact of the intervention on *Bartonella* spp. carriage by fleas was modeled following the same GLMM building process as for rats. However, in this model the outcome was the *Bartonella* spp. status of flea pools (positive or negative), and the intervention variable categorized whether fleas were taken from rats before the intervention in any block type, after the intervention in control blocks, after the intervention in flanking blocks, or after the intervention in intervention blocks.

Among 33 recaptured rats, 7 had fleas that were tested. If these rats were captured in the same 2-week trapping period, the average number of fleas collected across the captures was used and the fleas were considered positive from that rat if they tested positive in ≥ 1 recapture in that 2-week period. The dataset included 201 rats from which fleas were collected.

Model C The impact of the intervention on the number of fleas on rats.

To assess whether the intervention influenced the number of fleas counted on each rat, we used a negative binomial GLMM to account for overdispersion in the outcome. The outcome was the number of fleas counted on each rat. This model was built following the same procedure as for Models A and B. This analysis used the same 454 rats as Model A.

References:

1. Barnett SA. The rat: a study in behaviour. Chicago, IL: Aldine Publishing Co. 1963.
2. Byers KA, Lee MJ, Bidulka JJ, Patrick DM, Himsworth CG. Rat in a cage: trappability of urban Norway rats (*Rattus norvegicus*). *Front Ecol Evol.* 2019;7:68.
<https://doi.org/10.3389/fevo.2019.00068>
3. Health Canada, Public Health Agency of Canada. Pathogen safety data sheets: infectious substances – *Leptospira interrogans*. P. 1–3. 2011 [cited 2022 Feb 6]. <http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds95e-eng.php>
4. Gutiérrez R, Krasnov B, Morick D, Gottlieb Y, Khokhlova IS, Harrus S. *Bartonella* infection in rodents and their flea ectoparasites: an overview. *Vector Borne Zoonotic Dis.* 2015;15:27–39.
[PubMed https://doi.org/10.1089/vbz.2014.1606](https://doi.org/10.1089/vbz.2014.1606)
5. Holland GP. The fleas of Canada, Alaska, and Greenland (Siphonaptera). *Mem Entomol Soc Can.* 1985;117(S130):3–632. <https://doi.org/10.4039/entm117130fv>

6. Himsworth CG, Byers KA, Fernando C, Speerin L, Lee MJ, Hill JE. When the sum of the parts tells you more than the whole: the advantage of using metagenomics to characterize *Bartonella* spp. infections in Norway rats (*Rattus norvegicus*) and their fleas. *Front Vet Sci.* 2020;7:584724. [PubMed https://doi.org/10.3389/fvets.2020.584724](https://doi.org/10.3389/fvets.2020.584724)
7. Himsworth CG, Bai Y, Kosoy MY, Wood H, DiBernardo A, Lindsay R, et al. 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 Zoonotic Dis.* 2015;15:21–6. [PubMed https://doi.org/10.1089/vbz.2014.1657](https://doi.org/10.1089/vbz.2014.1657)