THE IMPACTS OF KILL-TRAPPING ON CARRIAGE OF LEPTOSPIRA INTERROGANS AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS BY WILD RATS IN VANCOUVER’S DOWNTOWN EASTSIDE

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Abstract

The ecology of wild urban rats is an important determinant of the transmission and carriage of rat-associated zoonoses (RAZ). For example, previous research has shown that RAZ are clustered in rat populations at the level of the city-block, possibly because rats, which rarely move beyond a single city-block, live in colonies that do not interact with rats from other colonies. It may therefore be the case that rat colonies self-limit the spread of RAZ to within the infected colony. Because anthropogenic disturbances can disrupt animal ecology, I hypothesized that human disruptions of typical rat ecology, such as pest-control, can also disrupt the epidemiology of RAZ within the disturbed rat population. This dissertation tests the hypothesis that kill-trapping wild urban rat populations can lead to unpredictable changes in RAZ epidemiology, even leading to increases in the transmission and/or spread amongst rat populations. This work tested rats for Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Leptospira interrogans* both before and after enacting a kill-trapping intervention at the level of the city-block in Vancouver Canada’s Downtown Eastside (DTES). Study blocks were categorized as either intervention blocks (in which kill-trapping was enacted), non-kill flanking blocks (blocks immediately next to intervention blocks in which no kill-trapping was enacted), and control blocks (in which no kill-trapping was enacted). Rats caught after kill-trapping in intervention blocks had 9.55 (95% CI 1.75-78.31) times the odds of carrying *Leptospira interrogans* than did rats that were caught in any block type before the intervention. This effect was not observed in any other block type for *Leptospira interrogans* or for carriage of MRSA. These results indicate that kill-trapping may impact the odds that rats that survive the kill-trapping carry some RAZ.
Wild urban rats are known carriers of many different pathogens capable of causing disease in people. The factors that determine which rats carry these pathogens appear to be connected with specific rat behaviours. This dissertation tests the hypothesis that kill-trapping wild urban rats can disrupt these typical behaviours, leading to a novel set of rat-to-rat interactions. These novel interactions may, in turn, change how pathogens are transmitted between rats and lead to new or even increased levels of the pathogen amongst the rats that are not killed. Indeed, this work found that rats caught in city-blocks after kill-trapping had a higher likelihood of carrying a specific human pathogen. This has important implications for both the public and the pest-control community, because not only may kill-trapping be ineffective at reducing the risk of disease carriage in wild rats, it may even be increasing those risks.
Preface

The research carried out in this dissertation was a part of a wider body of research conducted by the Vancouver Rat Project (http://www.vancouerratproject.com/vancouver_rat_project/home). This project is led by Dr. Chelsea Himsworth and Dr. David Patrick, both of whom were my co-supervisors. Additionally, Dr. Craig Stephen, the executive director of the Canadian Wildlife Health Cooperative, served on my supervisory committee and provided valuable feedback, advice, and insight, especially during the analysis and writing phase of the work.

I, Michael Lee, designed this research project based upon earlier research performed by Dr. Himsworth when she initiated the Vancouver Rat Project. The entirety of the field work was conducted by myself and a PhD candidate, Kaylee Byers, who was simultaneously collecting data on the movements of wild rats. MRSA and *Leptospira interrogans* samples were tested by Erin Zabek and Julie Bidulka, respectively, at the Animal Health Centre in Abbotsford, British Columbia, Canada. I performed all data analysis and manuscript writing myself. Work with wild rats was approved by the University of British Columbia’s Animal Care Committee (A14-0265).
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List of Abbreviations

(CA-MRSA) – Community Acquired MRSA

(CI) – Confidence Interval

(DTES) – Downtown Eastside

(EBPM) – Ecological Based Pest Management

(ICC) – Intra-class Correlation Coefficient

(IPC) – Internal Positive Control

(MMLR) – Mixed Effects Multivariate Logistic Regression

(MRSA) - Methicillin-resistant *Staphylococcus aureus*

(OR) – Odds Ratio

(PCP) – Pest Control Professional

(PCR) - Polymerase Chain Reaction

(RAZ) – Rat-Associated Zoonoses

(RT-PCR) – Real-Time Polymerase Chain Reaction

(SNV) – Sin Nombre Virus

(SPHS) - Severe Pulmonary Hemorrhage Syndrome

(TB) - Tuberculosis

(TSB) – Trypticase Soy Broth

(VANDU) – Vancouver Area Network of Drug Users

(VRP) – Vancouver Rat Project
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Chapter 1: Introduction

Of all the mammalian genera, *Rattus* is the most diverse with 61 species that have succeeded in colonizing every major habitat-type on the planet (1,2). Rats, specifically black rats (*Rattus rattus*) and Norway rats (*Rattus norvegicus*) are found on all continents except for Antarctica (1). These two species spread around the globe historically using human transportation pathways such as ships (3). Today, these rats are closely associated with people and they are rarely found living in the wild away from habitats that have been anthropogenically altered (1,2).

Norway and black rats are particularly well adapted to living in urban centres (4). This is problematic because this promotes close contact between people and rats, thereby increasing the risk of zoonotic pathogen transmission (2). Indeed, rats are the source of a number of pathogens associated with significant human morbidity and mortality. Historically, rats are associated with the infamous bubonic plague outbreak in the 14th century that resulted in the deaths of 40% of the European population (2). Today, rat-associated zoonoses (RAZ) still regularly occur all around the world (2,3). For example, there are hundreds of cases of rat-associated plague that occur annually in Madagascar, with black rats as the most common reservoir (5,6). Furthermore, rats are known to carry at least 40 different zoonotic pathogens (4) and they are the most common reservoir for the most widespread zoonotic disease in the world, Leptospirosis (7). There are at least one million human cases of Leptospirosis each year and it is considered one of the most important zoonotic causes of morbidity globally (8). With over half of the world’s population currently living in urban centres and unprecedented rates of global urbanization, the disease risks associated with rats are likely to concomitantly increase (2,3,9).

Within urban centres, rat infestations are most common in impoverished areas, as factors associated with poverty, such as building dilapidation and neglect, high housing densities, increased numbers of abandoned buildings, and accumulation of exposed garbage, provide food and harborage for rats (4). Residents of these neighbourhoods are at increased risk of acquiring RAZ because of increased exposure to rats combined with a decreased hygiene and health status (3).
The ecology of rats within urban centres is intimately connected with their carriage of RAZ. Previous studies have found that, in the absence of sudden changes to the environment or to the rat populations themselves, urban rats have a small home range often limited to the confines of single city block and that roads act as barriers to their movements (10-13). Additionally, rats form family-groups, or colonies, that share a nest/burrow and have limited interactions with conspecifics of other colonies (14). Rat colonies are territorial and hierarchal, including dominant individuals that may attack and exclude foreign rats (10,14).

These features of rat ecology may be linked to RAZ dynamics in two distinctive ways. First, limited movements of and limited interactions between rats may limit the overall spread of pathogens within a given rat population. In their 2011-2012 sample from Vancouver, Canada, the Vancouver Rat Project (VRP) found that RAZ were clustered in rat populations, sometimes at the level of the city block (15,16). This suggested to Himsworth et al. (15) that rat colonies, with their limited interactions, may limit the spread of RAZ to within an infected colony. Spatial heterogeneity in the distribution of RAZ within rat populations has been similarly noted in several other studies (17-20). For example, Cueto et al. (18) found that the prevalence of hantavirus in rats varied from 0-26.1% in different sites across Buenos Aires, Argentina, indicating again that pathogen spread amongst rat populations may be limited due to limited interactions between colonies.

Second, the transmission dynamics of some RAZ may be tied to social interactions within and between rat colonies. For example, De Oliveira et al. (21) identified the bacteria *Leptospira spp.* in the breast milk of naturally infected urban rats, suggesting that at least some transmission of the bacteria between rats may occur vertically. If transmission does occur in this way, it could explain some of the site-specific clustering of *Leptospira spp.* seen in rat populations across a city; for instance, a high level of vertical and a low level of horizontal transmission could lead to high prevalence within colonies, while nearby colonies remain unaffected. On the other hand, Himsworth et al. (15), found that carriage of the bacteria was closely associated with an increasing volume of internal fat and the number of bite wounds, characteristics associated with dominance in rat social structures. This suggested to Himsworth et al. (15) that the behaviours of dominant rats, such as increased exploratory behaviour (22) or heightened aggression (23), predisposed them to becoming colonized with *Leptospira interrogans*. Beyond *Leptospira*, other
RAZ have also been associated with specific social interactions, for example hantavirus transmission in rats has been tied to intraspecific aggression and biting (24).

These connections between rat ecology and RAZ (spatial heterogeneity of RAZ because of colony isolation and connections between RAZ transmission and rat social interactions) suggest that RAZ transmission patterns may be impacted by disturbances that interrupt typical rat ecology. For example, Davis (10,25) suggested that any sudden changes to a rat colony or its environment may alter either the typical movement patterns of rats and/or their social organization, both of which could influence RAZ transmission by altering the levels of transmission within and/or between colonies. While the impacts of anthropogenic disturbances on RAZ have not been directly tested, their impacts on zoonotic pathogens in other species have been the subject of several other studies (26-28). For instance, in the United Kingdom, badgers are reservoirs for bovine tuberculosis (TB) (27). Donnelly et al. (27) found that culling badgers increased the incidence of TB in cattle in the region surrounding the culled area by increasing the ranging behaviour of badgers, thereby increasing the probability of contact between badgers and cattle in these areas. Another study, conducted by Douglass et al. (26) found that removing deer mice from ranch buildings actually led to an increase in the numbers of mice in these buildings, at least in part due to immigration from outside the building. Further, they noted, on three occasions, that the removal of Sin Nombre Virus (SNV) negative rats led their replacement with immigrant SNV positive mice. Together, these two studies (26,27) indicate that killing animals in an attempt to control zoonotic disease could have unintended consequences. Given that one purpose of rat control in urban centres is to reduce risks to human health, and given that killing animals through trapping and poisoning are the most common methods of urban rat control (29,30), it is important to determine how these techniques impact RAZ prevalence in rat populations.

The degree and manner in which anthropogenic rat removal could alter RAZ likely depends upon the mode of transmission of the particular RAZ, such that the impacts of culling are likely different for different RAZ. For example, for pathogens that are spread through intra-specific transmission (e.g. *Leptospira interrogans* (15) and hantavirus (24)), culling may disrupt typical social structures within a rat population by removing key members of the established social hierarchies. For instance, trapping may preferentially remove dominant rats because they
are more exploratory and may investigate traps first (30,31). Removal of dominant rats could increase intraspecific aggression as the remaining rats establish a new hierarchy. This, in turn, could increase rat-rat pathogen transmission. In contrast, the impact of ecological disturbances on RAZ acquired primarily through environmental exposure (e.g. Methicillin-resistant *Staphylococcus aureus* (16) and *Clostridium difficile* (32)), is less clear. For example, rat culling may not impact pathogen prevalence, or it could increase the ranging behaviours of rats that are not killed, leading to increased or altered exposure to pathogens in the environment.

The objective of this study was to assess the effect of rat-culling on the prevalence of two different RAZ, *Leptospira interrogans* and Methicillin-resistant *Staphylococcus aureus* (MRSA). More specifically, this study tests the hypothesis that rat culling is associated with a change in the odds that rat carry *L. interrogans* and MRSA after culling has taken place. This work was carried out in the Downtown Eastside (DTES), an inner-city neighbourhood in Vancouver, British Columbia, Canada (Figure 1.1). This neighbourhood was chosen as a study site for two reasons. First, the DTES was the focus of study for the VRP, which previously identified both *L. interrogans* and MRSA in resident rat populations (15,16). Secondly, the DTES is one of the most impoverished areas of Canada, making it a priority for study because it is particularly vulnerable to rat infestations, rat-human contact, and zoonotic disease transmission (15,16,33).

Although reactive (deploying after complaints are received) rat culling through trapping and/or poisoning is known to be relatively ineffective at reducing, eliminating, or preventing rat infestations, particularly in the long term, this approach is popular because it is relatively inexpensive and easy to do (29). However, if the goal of a rat control campaign includes the reduction of rat-associated human health risks, then it may be counterproductive to employ these techniques. The results of this dissertation will help inform the public, pest control workers, health professionals, and municipalities about the potential unintended consequences of lethal rat control methods and may inform the development or implementation of more effective alternatives.
Red marker marks the location of Vancouver, Canada, where this study was carried out. Grey lines indicate within country borders; Yellow lines indicate borders between countries. Map was made in Google Earth Professional.
Chapter 2: Methodology and Experimental Design

2.1 Introduction

This study was designed to detect changes in RAZ carriage as a result of a rat culling intervention. As such, it required testing rats for the presence of *L. interrogans* and MRSA before and after an intervention. However, there were three main issues that had to be addressed when designing this study; 1) the best culling technique to use for the intervention, 2) the best experimental design to detect the aforementioned changes, and 3) the best method to sample rats before and after the intervention.

Considering issue one, there are two main approaches to rat control; reactive and proactive. Reactive methods, which rely on after-a-rat-complaint-is-received approach, are ineffective at controlling rat populations (in that they often do not remove the entire population), while proactive methods, which rely on prevention techniques such as environmental modification (removing rat-available resources), are far more efficacious and do not simply kill rats (29). However, reactive control remains the more popular form, with the rodenticides and kill-trapping being most common methods (29,30,34,35). The impacts of these reactive control methods on RAZ have not been studied.

As such, there were two options to choose from for our intervention; rodenticides or kill-trapping. Rodenticides can be observed in use all over Vancouver, but there are several caveats to using this method. First, using rat poison would not allow us to know if our intervention was successful (i.e., that we had killed any rats), because rats do not die inside the poison bait boxes. Second, because bait boxes are so common, they were already being used by pest control professionals (PCPs) in the alleys of the DTES. This meant that rats in the DTES may have already been accustomed to bait boxes and the addition of more in this study could have a negligible impact on what was already occurring. Finally, rat poison can be harmful to the environment by secondary consumption by predators and scavengers (36,37). Kill-trapping (killing, by various methods, animals that enter a trap), provided a solution to these issues, in that it would likely not be as ubiquitous as rodenticides, therefore it would be a novel disturbance, it
would allow us to know that we had killed rats during the intervention, and it would not be harmful to non-target species.

For issue two, we needed to determine the scale and configuration of our study design. First, we determined the unit of analysis [the unit to which the intervention was applied] to be an entire city-block, because past research has shown that the home range of urban rats is small and is often limited to the extent of a block (11,12,38). Second, past research has also shown that urban rats do sometimes, infrequently, move between city blocks (11). We hypothesized that trapping and removal could elicit between block movements both as rats ‘flee’ the intervention block and as rats move into the intervention block because of the reduced population size there. As such, our study sites needed a zone around the intervention blocks to capture the possibility that trapping could induce changes in RAZ due to immigration/emigration into and out of the intervention block (the scale of this zone is described further in the methods section). Finally, we included control sites in which no kill-trapping was enacted to monitor and observe any changes in RAZ that occurred ‘naturally’ in the blocks in the absence of our intervention, but during the same time periods.

For the third issue, we needed to select a method of sampling rats before and after the intervention that met the following criteria; 1) rats needed to be captured live so they could be re-released with minimal ecological impact, because we needed our kill-trapping to be our only intervention, 2) rats needed to be accessible for collection of biological samples, and 3) the method needed to have minimal selection bias in terms of the proportion of the population that was captured (i.e., ideally, all members of the population would have an equal probability of entering traps). Essentially, the only option that allowed for these criteria to be met was live-trapping. These traps hold captured rats in a safe, well-fed environment while incurring no physical harm to the animal. This simultaneously satisfied criteria one and two because rats could be caught and released with minimal impact and they were immediately available for sampling. Trapping bias, however, was not specifically mitigated. For example, we did not design our statistical modelling approach to account for the assumption of equal catchability of the entire population that accompanies mark-recapture studies (39). However, because we were measuring the prevalence of RAZ in the same way before and after an intervention, biases, such that might result from assumptions like equal catchability, impacted both periods of
measurement with the same magnitude. As a result, any biases incurred by live-trapping were shared equally before and after the intervention so they would not differentially bias the measurements made in either trapping period. Therefore, we compared the change in RAZ in the same trappable proportion of the population both before and after the intervention. However, this design could not account for induced trap shyness. Past work has shown that rats have the capacity to develop a wariness of traps and may avoid them after a period of trapping (40). As such, we cannot account for the possibility that trapping in the periods after the interventions systematically missed rats that were newly shy to the traps.

This chapter outlines the methods and experimental design used to study the impacts of kill-trapping on urban rats in Vancouver’s DTES and describes our overall sample of rats. Specifically, this chapter discusses our original study design and changes made to it as early data was collected. It summarizes issues related to animal morbidity and mortality that are important from both an experimental and ethical point of view and it describes methodological tips that increased the feasibility of performing this research in the field.

2.2 Methods

2.2.1 Animal Ethics Statement

All procedures and protocols that were a part of this work were approved by the University of British Columbia’s Animal Care Committee (A14-0265). To reduce the number of animals used, this study was designed to test multiple hypothesis in multiple studies using the same animals.

2.2.2 Experimental Design

The original study design, shown in Figure 2.1, was constructed to satisfy the needs of two separate studies; 1) tracked changes [following a kill-trapping intervention] in RAZ carriage
and 2) tracked between block movements\(^1\) of rats. In this design, study sites consisted of nine city-blocks and were defined as control or intervention sites. In intervention sites, kill-trapping [rats were live-captured and later humanely euthanized as described in section 2.2.5] was carried out in the central of the nine blocks, while in control sites no kill-trapping occurred. The eight blocks surrounding the intervention block served as a zone to observe any between block movements of rats.

Following early data collection\(^2\), the experimental design was revised to better suit the needs of study two by reflecting the new data on DTES rat ecology. The revised study design, shown in Figure 2.2, differed from the original in that study sites consisted of three city-blocks with contiguous alleys separated by minor streets, rather than nine blocks (Figure 2.3). Blocks in different sites had parallel alleys that were separated by larger streets (Figure 2.3). Kill-trapping was enacted in the central of the three blocks in intervention sites [with the two flanking blocks serving as a zone to capture between block movements of rats] and no kill-trapping occurred in control sites. Flanking blocks in intervention sites are henceforth referred to as non-kill flanking blocks. In this design, study sites were randomly selected from the larger set of all eligible locations in the DTES, by recording eligible locations and using a random number generator to select sites.

### 2.2.3 Experimental Timeline

Trapping in each intervention site (in the revised study site design) was divided into three distinct two-week periods; the period before kill-trapping, the period during kill-trapping, and the period after kill-trapping (Figure 2.4). In the period before kill-trapping [in any given site] rats were caught, sampled (Section 2.2.5), and released in all three blocks (Figure 2.2) for two weeks. In the following two weeks (the period during kill-trapping) any rat that was caught in the central of the three blocks was euthanized, while capture-sample-and-release continued in the non-kill flanking blocks. Traps were then removed for at three to six weeks, after which time they were

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\(^1\) Movement data is not a part of this study – see PhD Dissertation Interdisciplinary Studies Graduate Program University of British Columbia

\(^2\) See PhD Dissertation Interdisciplinary Studies Graduate Program University of British Columbia
returned to their exact locations and capture-sample-and-release continued for two more weeks (the period after kill-trapping). Control sites were different from intervention sites only in that in the period during kill-trapping, capture-sample-and-release continued in all three blocks and no rats were euthanized.

2.2.4 Trapping

All trapping was carried out in 12 study sites (36 city-blocks) in Vancouver’s Downtown Eastside from June 2016 to January 2017. Ten Tomahawk Rigid Traps for rats (Tomahawk Live Traps, Hazelhurst, USA) were placed down the entire length of the alley that bisected each city block. This number of traps was chosen to balance the likelihood of capturing enough rats (based upon methods used in phase one of the VRP (41) and the advice of the lead researcher Dr. Himsworth) with the feasibility of carrying out this field work. To prevent destruction, theft, and harm to the captured animals, traps were fitted into stainless steel trap covers (Integrated Pest Supplies Ltd, New Westminster, Canada) and chained to immovable objects (Figure 2.5). Traps were baited with peanut butter mixed with oats. Hydrogel (ClearH2O, Westbrook, USA) was provided as a water source.

Three study sites were trapped at a time, such that 90 traps were deployed in nine city-blocks at any given point during this study (Figure 2.2 and 2.4). Trapping occurred in three stages; pre-baiting, active trapping, and kill-trapping (Figure 2.4). During the pre-baiting stage, traps were fixed open with zip-ties, and re-baited each day as needed. Pre-baiting served to accustom rats to traps. In the active and kill-trapping stages, traps were set and baited each evening by 1600 hours and checked each morning by 0700 hours, five days a week. On the sixth and seventh day, traps were fixed open and baited. Figure 2.4 describes the progression of these stages in each site.

At 0700 hours during each morning of the active and kill-trapping stages, all traps were checked for rats. Non-target animals (birds, skunks, and squirrels) were released immediately and to prevent catching more animals during the day, all traps that were still open were shut. Starting in a randomly selected block each day (using a random number generator) rats were transported into the back of a mobile lab-van, given extra bait and hydrogel to promote urination.
and their cages were covered with a blanket until sampling. When more animals were caught than could be sampled in a single day, excess rats were immediately released. Selection bias in releasing rats was prevented by the randomization of the block in which we started each morning, in that the rats that were released were in a random distribution of blocks each day. Captured rats that had been previously captured and sampled (termed recaptured) were completely re-sampled if they had not been sampled for at least seven days. If a recapture had been sampled within the past seven days it was released. Seven days was chosen as the re-sampling timeframe for two reasons: 1) experimentally infected rats begin shedding Leptospires as early six days post-infection (42), and 2) day-to-day time-effort-money constraints did not allow us to continually re-sample the same rats over and over again. Note, that because the epidemiology of MRSA in wild rats is not a well-characterized field (16,43-45), the time between transmission and detectability of MRSA in urban rats was unknown, and as such the seven day threshold was based upon *L. interrogans* epidemiology only.

### 2.2.5 Sample Collection

To prepare rats for sampling, they were transferred, using Kevlar rodent handling gloves (Tomahawk Live Traps, Hazelhurst, USA), into an inhalation induction chamber (Kent Scientific, Torrington, USA) and anesthetized with 5% isoflurane in oxygen using an isoflurane vaporizer (Associated Respiratory Veterinary Services, Lacombe, Canada). Once they reached a sufficient plane of anesthesia (determined by the absence of a righting reflex), rats were transferred onto a sanitized and covered surgical table where anesthesia was maintained using a nose cone (Kent Scientific, Torrington, USA) attached to the isoflurane vaporizer. All work was carried out in the back of the lab-van.

Sampling each rat took approximately 10-20 minutes. First, each rat was given a unique laser etched ear tag (Kent Scientific, Torrington, USA) for identification if that rat was recaptured. Additionally, ear punches were given in a systematic way such that punches marked the site and block number that a rat was captured in. To prevent dehydration and to promote urine production, rats were given up to 12cc of subcutaneous lactated ringers solution depending on their size. Using a sterile cotton tipped applicator, an over the counter ophthalmic gel was
applied to their eyes to prevent desiccation and the formation of ulcers. The oropharynx and rectum were swabbed separately to test for the presence of MRSA (Chapter four). Additionally, the weight (grams), total length (centimeters), sexual maturity (males with scrotal testes and females with a perforate vagina were considered mature), sex (male or female), and the number of cutaneous bite wounds were recorded for each rat. Any cutaneous wound present was considered a bite wound as per Himsworth et al. (46). Finally, bladders were manually expressed between the thumb and forefinger directly into a sterile collection tube (urine was tested for \textit{L. interrogans} - Chapter three). Once completely sampled, rats were placed back inside cages under the blanket. After fully recovering to a pre-anesthesia behavioural state (alert and moving around the cage), rats were released at the exact location of their capture.

When rats were caught during the two-week kill-trapping period in intervention blocks (Figure 2.2 and 2.4) they were anesthetized using isoflurane and euthanized via intracardiac injection of pentobarbital. These rats were sampled on site following the same procedures as rats that were released.

\textbf{2.2.6 Sanitation and Disinfection}

To prevent the transmission of RAZ as an incidental result of our study, care was taken to disinfect all tools, equipment, and cages that could serve as a common source of infection/colonization for rats. Cages and trap covers were scrubbed clean with 10% bleach (sodium hypochlorite) whenever they were moved between sites and after the capture of any animal. Additionally, all cages were wiped and sprayed with 10% bleach following any period of being fixed open (pre-baiting, weekends). Kevlar rodent handling gloves were sanitized using Clorox bleach wipes (The Clorox Company, Oakland, USA) between each rat. The surgery table was sprayed with 10% bleach and left to sit for 10 minutes before wiping and covering with a new bench pad. All sample collection tools (ear clip, ear tag applicator, forceps) were disinfected in 70% Ethanol. Finally, the weigh-scale was disinfected with 10% bleach. Both \textit{L. interrogans} and MRSA are susceptible to sodium hypochlorite and ethanol (47,48).
2.2.7 Dataset

To account for longitudinal data from multiple captures of the same individuals, rat morphological and demographic characteristics were averaged across each recapture. Any rat with missing data in any of the descriptive variables being considered were excluded. Rats were missing data because of difficulties and challenges of working in the field such as data entry errors and the weigh-scale being stolen.

2.3 Results and Discussion

2.3.1 Intervention and Experimental Design

This study only tested the impact of kill-trapping, while in practice, rodenticides are also a very common technique used for rat-elimination (30). Future studies should examine the impacts of rodenticides on RAZ, but should aim to do so in an environment where poisoning is not already in use.

This study optimized an experimental design for examining the impacts of an anthropogenic disturbance on urban rat populations. The change in design after early data collection was significant because it allowed us to increase the number of replicates of both the intervention and the controls.

2.3.2 The Sample

Eleven rats missing data were excluded. Forty black rats and 728 Norway rats were caught as a part of this study. Black rats may have a different ecology than Norway rats, for example they are more likely to nest off the ground as compared to Norway rats which often burrow into the ground (49) and as a result, the two species may respond differently to the kill-trapping intervention. Because of this and because of the small sample size, black rats were
excluded from any analysis in this dissertation. From this point on, any reference to rats is referring to Norway rats alone.

Overall, 728 rats were captured in 34 out of the 36 city-blocks that were trapped. Table 2.1 and Figure 2.6 show the distributions of sex, sexual maturity, the number of bite wounds, length, and weight of these rats. The number of rats euthanized and removed from each intervention block are given in Table 2.2.

We recaptured 131 rats. Of these 131 rats, 82 (62.6%) were recaptured once, 31 (23.7%) were recaptured twice, 10 (7.6%) were recaptured three times, 6 (4.6%) were recaptured four times, 1 (~1%) was recaptured six times, and 1 (~1%) was recaptured nine times (213 total recaptures). However, only 70 rats were re-sampled (rats were only re-sampled if they were recaptured at least seven days after their previous sampling), representing an average of less than six rats per site or two rats per block (70 rats / 12 sites = 5.8 rats per site or 70 rats / 36 blocks = 1.9 rats per block).

Initially, we had hoped that this study could detect changes in RAZ status over time in individual rats, as well as in the block level populations. Unfortunately, few rats were captured both before and after the intervention, precluding this sort of longitudinal analysis. Future studies seeking to track the pathogen status of individual rats over time should maximize the time they spend trapping in each site rather than increasing the number of sites.

Finally, this study does not specifically address the possibility that the trapping scheme selected for a specific proportion of the population, which is likely given that an equal probability of capture of any individual in the population is rare for small mammals (39). As such, we cannot determine whether this sample, shown in Table 2.1 and Figure 2.6, is representative of the overall population of rats or if it is a trappable subset. However, except for trap shyness, we assume that rats entered our cages with the roughly the same probability both before and after the intervention3.

3 Trapability– see PhD dissertation Interdisciplinary Studies Graduate Program University of British Columbia
2.3.3 Rat Morbidity and Mortality

Rat morbidity and mortality incurred during this study was important both because this study was designed such that its only impact on the rat population was supposed to be the kill-trapping intervention and because of animal welfare considerations. Overall, there were two major sources of rat morbidity and mortality in this study. The first was dehydration in traps, especially in the summer months. We originally used apple slices as a water source, but the rats completely ignored them. While they were similarly uninterested in hydrogel, we found that they consumed it if it was rolled into the peanut butter and oat mixture. For rats that were anesthetized, subcutaneous ringer’s solution was used to support a full recovery.

Second, human induced morbidity occurred in this study. Because loitering and alley-usage was heavy in many of our study areas, this research was highly visible to the public. As a result, captured rats occasionally caught the attention of alley users, some of whom treated the animals cruelly. For example, we caught people shaking cages, urinating on rats, and poking them with sticks and even needles.

Constant researcher engagement with the alley-user community alleviated many of these issues. We conversed with alley-users daily and partnered with a local group of current and former drug users dedicated to improving the lives of those who use illicit drugs (Vancouver Area Network of Drug Users or VANDU). Many people we alerted to the ongoing issues within our study were outraged and agreed to watch cages and protect the animals when researchers could not be there, forming an unofficial ‘community watch’. After the formation of this ‘watch’ many of the issues of animal cruelty were alleviated.

The success of community engagement may be tied to the close-knit nature of the relationships between people living in the DTES. For example, in light of the unprecedented fentanyl crisis (50) ongoing during our study, drug-users in the DTES were, anecdotally, on the constant lookout for others in need of assistance. This likely increased the success of our ‘community watch’ by helping to spread the word of our issues around the neighbourhood.
2.3.4 Methodological Tips to Increase Research Feasibility

There were several methodological issues that we faced in this study that decreased the feasibility of performing this research. For example, approximately 7% of recaptured rats in this study had their ear tags torn out. In these instances, the ear clips we gave rats to mark their site and block of capture often allowed us to identify which specific individual it was by comparing its morphological characteristics to all the rats captured within the block of interest. When a rat with a torn ear tag could not be identified, the ear clips told us whether that rat had moved from its original block of capture, providing vital data to the study assessing movement.

Cage bleaching did not have any obvious impacts on captures; we continually caught new and recaptured rats in cages before and after bleaching. This is important because equipment sanitation was vital in preventing the transmission of RAZ to rats as a non-intended result of our study. However, while it is impossible to know whether bleaching biased our sample towards a specific cohort of the overall rat population, because we did not include no-bleach controls, three studies (51-53) comparing different mechanisms of trap disinfection to water controls found that trap bleaching did not have a significant impact on the trapability of small mammals (rats were not included in these studies). Finally, rats often voided their bladders when being transferred into the induction anesthesia chamber, making subsequent manual expression impossible. Instead, rats in cages were placed over individual bleach sanitized trays until they urinated into the tray, from which it was collected directly using a sterile syringe (Figure 2.7).

2.3.5 Conclusions

In conclusion, this work presents a design and methodology to examine the impact of kill-trapping on urban rat populations. We optimize that design and point to methodological difficulties that can decrease the feasibility of performing this kind of work in the field. Further, it highlights some of the key challenges to undertaking such a study and some of the solutions we developed to cope with and alleviate these issues. In the end, this study should serve as a framework or a set of guidelines for avoiding potential pitfalls for researchers seeking to engage in similar work.
### Description of Sample of Norway Rats (n=728)

<table>
<thead>
<tr>
<th></th>
<th>348 (48%)</th>
<th>380 (52%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Female]</td>
<td>[Male]</td>
</tr>
<tr>
<td>Sex</td>
<td>334 (46%)</td>
<td>394 (54%)</td>
</tr>
<tr>
<td></td>
<td>[Juvenile]</td>
<td>[Mature]</td>
</tr>
<tr>
<td>Number of Bite Wounds</td>
<td>0 [Median]</td>
<td>0-22 [Range]</td>
</tr>
</tbody>
</table>

Table 2.1 Description of the overall sample of 728 Norway rats.
### Table 2.2: The number of rats that were killed and removed from each intervention block.

<table>
<thead>
<tr>
<th>Site Number</th>
<th>Number of Rats Removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

The site numbers correspond to the site numbers shown in Figure 2.2.
Figure 2.1 The original study design.

Each red dot is an individual trap location in the alley that bisects each city block. Sites consisted of nine adjacent blocks. In intervention sites, kill-trapping was enacted in the central of the nine blocks (demarcated by the boxed X), while active trapping occurred in all other blocks. In control sites, active trapping was carried out in all blocks, and no kill-trapping occurred. Map was made using the ggplot2 package in R (54).
Figure 2.2 All trapping locations in Vancouver DTES and the revised study design.

Each red dot is an individual trap location in the alley that bisects each city block. Blue boxes are intervention sites; yellow boxes are control sites. Kill-trapping blocks are marked by the boxed ‘X’. Sites consisted of three blocks with contiguous alleys; each site is separated by a major street parallel to trapped alleys (Figure 2.3). Intervention sites consisted of a central kill-trapping block and the two flanking blocks to observe any between block movements of rats. In intervention sites kill-trapping was enacted for two weeks in the central of the three blocks, while in control sites no rats were killed as part of this study, only trapping and release occurred. Intervention sites are labeled 1-5. Map was made using the ggplot2 package in R (54).
Figure 2.3 Parallel versus contiguous alleys.

Boxed regions are separate city blocks. Blocks boxed in the same color have alleys that are contiguous. In other words, the alleys open towards each other and are separated by a relatively minor street. Blocks boxed in different colors (red versus yellow) have parallel alleys separated by a major street. Map imagery downloaded from Google Earth Professional.
During pre-baiting, traps were fixed open and re-baited each day. During active trapping, traps were set each night and checked each morning. During kill-trapping, any rats captured in the central block in intervention sites were killed humanely and removed while active trapping continued in the surrounding two blocks.
Figure 2.5 Two traps inside stainless steel covers chained in an alley in the DTES.
Figure 2.6 Distribution of the weight and length of 728 Norway rats.

Red lines indicate the median values.
Figure 2.7 Rat urine collection.

Urine was collected directly from bleach sanitized trays placed individually below each caged rat. Note, rats were separated from one another by a blanket placed over the top and between each cage, this was removed for this photograph.
Chapter 3: Leptospira interrogans

3.1 Introduction

The genus *Leptospira* is comprised of gram-negative, spirochete bacteria that have been identified in more than 180 different species of animals globally (55). The pathogenic species, *Leptospira interrogans*, is the causative agent of the disease Leptospirosis, one of the most widespread zoonotic diseases in the world, with more than 500,000 severe cases reported each year (7,55,56). In humans, the disease commonly presents with subclinical or mild influenza-like symptoms, but if left untreated can progress to Weil’s syndrome and severe pulmonary hemorrhage syndrome (SPHS) characterized respectively by, renal failure and pulmonary hemorrhage (7,55). The mortality rate for Weil’s syndrome is approximately 10% while it can be as high as 50% for SPHS (57).

*L. interrogans* has adapted particularly well to certain maintenance hosts that carry the bacteria while experiencing little to no disease (55). Rats, which are common maintenance hosts (56), are capable of shedding large quantities of the infectious agent, termed leptospires, into the environment via their urine where it can survive for months to years, especially in regions that are moist (55). People are incidental hosts and most commonly become infected with *L. interrogans* though contact with leptospires through open wounds and mucous membranes (55).

Urban conditions promote both the survival and growth of rat populations and the transmission of leptospires to people (3,56). The greatest burden of urban Leptospirosis is often felt by impoverished populations living in areas with poor environmental hygiene and sanitation (3,56). To understand the epidemiology of the pathogen and factors that may increase the associated zoonotic risk in these urban environments, it is important to understand the epidemiology of this pathogen in its common urban maintenance host, rats.

In 2011-2012 phase one of the VRP (15) identified *L. interrogans* in Norway rats living in the DTES in Vancouver, Canada and began to characterize the bacteria’s epidemiology. The study found that the bacteria had a highly heterogeneous distribution across the neighborhood, with a marked variation in prevalence between city blocks (0-66.7%). They theorized that this
effect may have been the result of rat population structure in urban environments. Himsworth et al. (15) also found that carriage of *L. interrogans* was predicted by rat weight, volume of internal fat, and the number of bite wounds, three characteristics thought to be associated with colony social structures, such as dominance and hierarchies. This association, which has been noted by other researchers (17,58), indicates that *L. interrogans* transmission between rats may depend on specific social interactions, rather than random environmental exposure (15).

If transmission between rats is closely associated with rat social structures, then the dynamics of *L. interrogans* in rat colonies may be susceptible to changes in these structures. Given that pest control strategies can be disruptive of typical animal ecology⁴, it may be the case that rat control techniques, such as kill-trapping, influence change in rat colonies and interrupt the normal patterns of *L. interrogans* transmission. This study was designed to examine the impacts of rodent control on the levels of *L. interrogans* in rat populations. Specifically, we sought to establish whether kill-trapping, a common rodent control technique, influenced the odds that urban Norway rats carried *L. interrogans*.

### 3.2 Methods

#### 3.2.1 Sampling and Urine Collection

The trapping and sample collection protocols are described in detail in Chapter two of this dissertation. Urine was obtained from rats by individually placing their cage directly above a bleach sanitized plastic tray⁵. Rats were given hydrogel, their cages were covered in a blanket, and were left undisturbed until they urinated into the tray below their cage. Rats remained in cages over trays for up to one hour. The urine was collected directly from the tray using a sterile syringe. Urine was stored at -80°C until testing for *Leptospira interrogans*.

⁴ See chapter two of this dissertation and the overall introduction for a full discussion of the impacts of pest control on animal ecology
⁵ See chapter two of this dissertation for a photograph of the urine collection tray
3.2.2 Leptospira interrogans Polymerase Chain Reaction

3.2.2.1 DNA Extraction of Rat Urine

Nucleic acid extraction from rat urine was done with a 96 well magnetic particle processor using the MagMAX Pathogen RNA/DNA kit. Extractions were performed following the procedure outlined, by the manufacturer, for low-cell content samples. Starting urine volumes ranged between 20 – 200 µL depending on the sample. All samples were volume corrected to 200 µL prior to extraction using sterile, 1X PBS buffer, pH 7.4.

3.2.2.2 Real-Time PCR

Nucleic acid extracts were then amplified using a real-time PCR (RT-PCR) assay which targets the LipL32 gene of pathogenic Leptospira species. The target gene encodes an outer membrane lipoprotein virulence factor (59). Real-time PCR was performed using the Agpath-ID™ One-Step RT-PCR Kit. A Taqman exogenous internal positive control (IPC) was also run to ensure that there was no PCR inhibition due to the inhibitory nature of urine samples.

Each 25 µl reaction contained 2X RT-PCR buffer, 25X RT-PCR enzyme, 800 nM each of forward primer (5'- AAG CAT TAC CGC TTG TGG TG -3') and reverse primer (5'- GAA CTC CCA TTT CAG CGA TT -3'), 200 nM probe (5'- FAM/AAA GCC AGG ACA AGC GCC G/BHQ1-3'), 10X Exo IPC Mix, 500X Exo IPC DNA, nuclease-free water and 5 µl of DNA template. The reaction was incubated at 50°C for 2 minutes, 95°C for 10 minutes, and then amplified for 45 cycles at 95°C for 15 seconds, 58°C for 1 minute. Samples were run on an ABI7500 Fast PCR system and analyzed using the SDS software version 1.4.

Extraction, PCR and IPC kits were purchased from Thermofisher (Burlington, Canada). The 50X Exo IPC DNA was diluted 10-fold prior to use. Leptospira spp. primers and probe were made by Integrated DNA Technologies (San Diego, USA). A negative extraction control, negative template control and two positive amplification controls were used per real-time PCR run. The positive control was Leptospira interrogans, serovar copenhageni, (provided by Tim Witchell, University of Victoria).
This assay had 100% published (59) accuracy in detecting *L. interrogans* in positive and negative samples of human blood and urine. Furthermore, Himsworth et al. (15) validated this RT-PCR in rat kidneys (100% specific) and a validation study to detect the same lipL32 gene, (albeit using a different assay) found 100% sensitivity and specificity in rat kidneys using RT-PCR (60).

### 3.2.3 Statistical Analysis

Individual rats that were caught in the period before the intervention and were recaptured in the period after the intervention were excluded from statistical analysis to prevent double counting some rats and not others (note that the final model was rerun including these animals to assess how they impacted the results). For PCR positive rats that were caught multiple times in the same period (either before or after the intervention), continuous variables (weight, length, bite wounds) were averaged across each positive recapture. For rats that were captured more than once in the same period (either before or after the intervention), but never tested positive for *L. interrogans*, continuous variables were averaged across each recapture. No juvenile rats recaptured in the same trapping period reached maturity in this timeframe. Any animal with missing data in any of the potential confounding variables being considered in the analysis were excluded from the dataset.

Mixed effects multivariate logistic regression (MMLR) was used to estimate the effect of the kill-trapping intervention on the odds that rats carried *L. interrogans*, while controlling for clustering by city block. The outcome was the *L. interrogans* status of individual rats (negative or positive). The intervention variable categorized rats as to whether they were caught before or after the intervention in control blocks, in non-kill blocks flanking intervention blocks, or in intervention blocks

6. This variable had four levels; 0 – if a rat was captured in the period before the intervention in any block type (reference group), 1 – if a rat was captured in the period after the intervention in a control block, 2 – if a rat was caught in the period after the intervention in a block flanking the intervention block, and 3 – if a rat was caught in the period after the

---

6 See chapter two of this dissertation for a description of block-types
intervention in an intervention block. As such, this variable compared the odds of *L. interrogans* carriage after the intervention period to the odds of carriage before any intervention separately for intervention blocks, control blocks, and non-kill blocks flanking intervention blocks.

A hypothesis-testing model building approach was used to estimate the effect of the intervention. Potential confounders considered for inclusion in the model included: sex (male or female); sexual maturity (juvenile or mature); dichotomous weight around the median (0; < 122, 1; ≥ 122g); length (centimeters); cutaneous bite wounds (number); and bite wound presence (0; no wounds, 1; at least one wound present). Variables were considered confounders and kept in the model if they changed the estimate of the effect of the intervention variable in intervention blocks by at least 10%. Collinear variables (length and weight [spearman’s ρ = 0.946, p<10⁻¹⁵]; and the number of bite wounds and wound presence) were considered in separate, competing models. Of the collinear variables, the one which had the largest impact on the effect of the intervention was kept in the model. Note that continuous variables were dichotomized around their medians if they did not satisfy model assumptions.

The proportion of variance associated with the random effect of the city block (termed intra-class correlation coefficient or ICC) was calculated using: \[ ICC = \frac{\text{variance}^{\text{block}}}{\text{variance}^{\text{block}} + \pi^2/3}, \]

as previously described by (61). All statistics were carried out in the open source software RStudio (Version 1.0.136, 2009-2016) using the lme4 package for regression (62).

### 3.3 Results

#### 3.3.1 Sample Description

64 out of 430 (14.9%, 95% confidence interval (CI) 11.7%-18.7%) rats included in this analysis were PCR positive for *L. interrogans*. The distributions of weight, sex, sexual maturity, length, and the number of bite wounds are presented in Table 3.1 and Figure 3.1.
3.3.2 Statistical Analysis

One rat was excluded because it was missing values of potential confounders and seven were excluded because they were caught both in the period before the intervention and recaptured in the period after the intervention. The final model (shown in Table 3.2) included dichotomous weight and wound presence as confounders. Holding these confounding variables constant, a rat caught in an intervention block after the intervention had 9.55 times higher odds (95% CI 1.75-78.31) of carrying *L. interrogans* than did a rat caught before the intervention. The odds of carriage after the intervention in control blocks (adjusted odds ratio (OR\textsubscript{adj})=0.77, 95% CI 0.22-2.58) and in blocks flanking intervention blocks (OR\textsubscript{adj}=2.22, 95% CI 0.65-7.47) were not significantly different from the baseline odds. In this final model, the random effect of the block had a variance of 3.656, therefore 52.6% of the total model variance was due to the random effect of the block. Rerunning the final model including animals that were caught both before and after the intervention did not substantially impact the results: the effect of the intervention in intervention blocks was (OR\textsubscript{adj}=8.88, 95% CI 1.68-68.08).

3.4 Discussion

This study suggests that kill-trapping, a rodent control technique, may increase the odds that rats carry *L. interrogans*. Specifically, this study found that three to six weeks after a kill-trapping intervention, rats caught in city-blocks subjected to kill-trapping had 9.55 times the odds of carrying *L. interrogans* than rats caught in blocks before kill-trapping occurred, while holding weight and the presence/absence of bite wounds constant. Importantly, this effect was not observed in either the control blocks or the non-kill blocks flanking the intervention blocks.

The effect of the intervention was confounded by both weight and the presence/absence of bite wounds. These characteristics are linked with dominance and social structures within rat colonies, supporting previous research suggesting that *L. interrogans* is likely acquired through specific social interactions (15,17,58). Taken together, these findings suggest that within intervention blocks, kill-trapping could have disrupted established rat social structures and altered intraspecific interactions in a manner that promoted *L. interrogans* transmission, for
example, by increasing biting and other aggressive interactions. This is supported by the fact that bite wound presence was a stronger confounder than was the continuous number of bite wounds. For instance, if the number of bite wounds was more important, it might suggest that biting is a mode of transmission of the bacteria and the probability of infection was additive with each extra bite. On the other hand, the association with wound presence suggests that wounding is indicative of other rat characteristics or behaviours that are associated with transmission/carriage, such as dominance in social hierarchies.

Alternatively, the increase in the odds of carriage may have been due to a survivorship bias, in that *L. interrogans* positive rats may have been less likely than other rats to be removed by the intervention, and as such the overall prevalence within the block increased. For instance, some have suggested that dominant rats, which may be more likely to carry the bacteria (15), may also be more likely to avoid traps (30). If this was the case, then trapping was not having the desired effect because it preferentially missed rats that posed the greatest risk of carrying *L. interrogans*.

Initially, we hypothesized that a disruption in the intervention blocks might cause rats to move to adjacent blocks to escape the disruption or cause rats from adjacent blocks to be attracted to the intervention block because of increased resource availability secondary to decreases in the resident population size. This movement, in turn, could have caused disruptions in colonies within adjacent blocks. However, the effect of the intervention on *L. interrogans* carriage was limited to the blocks in which the intervention actually occurred. This is likely attributable to the fact that urban rats have a small home range with minimal movement and colonies in different blocks have limited interactions. Thus, a marked disruption in one colony may have little impact on adjacent colonies. Alternatively, our study may not have been long enough to observe any changes induced by potential between block movements.

These results have important implications from both a human health perspective and for the pest control community. This study shows that rodent control methods can potentially increase the odds that rats that were not killed carry *L. interrogans*. As pest control activities often do not eliminate the entire population of rats (29) it may be the case that these activities are increasing the risk that any given rat left in the population carries *L. interrogans*. This means that
after rodent control activities, the risk posed by any given rat left in the population may be increased.
Description of Sample of Norway Rats (n=430)

<table>
<thead>
<tr>
<th>L. interrogans Status</th>
<th>Positive (n=64)</th>
<th>Negative (n=366)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>29(45%) Female</td>
<td>35(55%) Male</td>
</tr>
<tr>
<td></td>
<td></td>
<td>176(48%) Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>190(52%) Male</td>
</tr>
<tr>
<td>Sexual Maturity</td>
<td>5(8%) Juvenile</td>
<td>59(92%) Mature</td>
</tr>
<tr>
<td></td>
<td></td>
<td>173(47%) Juvenile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>193(53%) Mature</td>
</tr>
<tr>
<td>Number of Bite Wounds</td>
<td>1 Median</td>
<td>0 Median</td>
</tr>
<tr>
<td></td>
<td>0-13 Range</td>
<td>0-9 Range</td>
</tr>
</tbody>
</table>

Table 3.1 Description of the sample of 430 Norway rats included in the statistical analysis.
<table>
<thead>
<tr>
<th>Confounders</th>
<th>Levels</th>
<th>Estimate</th>
<th>SE_{estimate}</th>
<th>OR_{adj}</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>&lt;122g</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
</tr>
<tr>
<td></td>
<td>≥122g</td>
<td>2.3007</td>
<td>0.5405</td>
<td>9.981167</td>
<td>&lt;10-4</td>
<td>3.70-31.74</td>
</tr>
<tr>
<td>Wound Presence</td>
<td>No</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.3528</td>
<td>0.4219</td>
<td>3.868241</td>
<td>0.00134</td>
<td>1.73-9.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The Intervention</th>
<th>Variable Levels</th>
<th>Estimate</th>
<th>SE_{estimate}</th>
<th>OR_{adj}</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>Before Intervention, all Block Types (n=261)</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
<td>Ref$^a$</td>
</tr>
<tr>
<td></td>
<td>After Intervention, Control Blocks (n=97)</td>
<td>-0.2553</td>
<td>0.6202</td>
<td>0.7746841</td>
<td>0.68061</td>
<td>0.22-2.58</td>
</tr>
<tr>
<td></td>
<td>After Intervention, Non-Kill Flanking Blocks (n=33)</td>
<td>0.7991</td>
<td>0.6158</td>
<td>2.223539</td>
<td>0.19444</td>
<td>0.65-7.47</td>
</tr>
<tr>
<td></td>
<td>After Intervention, Intervention Blocks (n=39)</td>
<td>2.2566</td>
<td>0.9363</td>
<td>9.550562</td>
<td>0.01594</td>
<td>1.75-78.31</td>
</tr>
</tbody>
</table>

a – Reference Group

**Table 3.2 Summary of the final model of the effect of the kill-trapping intervention on the odds that individual rats carry *L. interrogans*.**

Levels of the intervention variable refer to the trapping period (before or after the intervention) and the block-type (control, non-kill flanking, or intervention) each rat was caught in.
Figure 3.1 Distributions of the weight and length of the 430 rats included in the statistical analysis.

Red lines mark the median value.
Chapter 4: Methicillin-resistant Staphylococcus aureus

4.1 Introduction

*Staphylococcus aureus* is an opportunistic pathogen that colonizes the epithelia, often in the nasopharynx, of an estimated 30-50% of the human population (63,64). This bacterium is a leading cause of skin, soft tissue, bloodstream, and respiratory infections globally, including in Canada and the United States of America (63,65,66).

Methicillin-resistant *Staphylococcus aureus* was identified as a problem nosocomial infection in the 1960s, but has now emerged globally, likely via many separate events distinct from the healthcare setting, in both the community (CA-MRSA) and domestic animals (67). Causing 11,000 estimated deaths per year in the USA alone (66), invasive MRSA infections are associated with a 64% higher risk of death than invasive infections with non-resistant *S. aureus* (68). Furthermore, in 2004 the estimated health care costs of MRSA in Canada were $82 million, double the costs in the early 2000s (63). Recent studies have indicated that CA-MRSA strains, which are predicted to displace the healthcare associated strains, have added virulence factors which may explain an increasing spectrum of invasive disease and a heightened morbidity (67,69).

The zoonotic potential of MRSA has been increasingly studied over the past decade with at least pigs, cattle, horses, poultry, rats, mice, and companion animals (such as dogs, cats, and hamsters) identified as carriers (44,45,70-72). Transmission from domestic animals to people has been shown, especially in those having close contact with colonized animals (70,71). Further, the reverse also appears to be true with mounting evidence that MRSA can move in the other direction, from people to animals (16,71). The bi-directionality of MRSA transfer may help ensure the continued colonization of both people and animals, especially when the two live in close contact with one another (71,73).

There have been few studies of MRSA in urban rats. Van de Giessen (45) identified MRSA via oropharyngeal swabs of wild black rats living on pig farms in the Netherlands, while Khalaf (43) identified MRSA in the urine of wild urban black rats living in garbage in Baghdad,
In 2011-2012, phase one of the Vancouver Rat Project identified oropharynx MRSA in wild urban Norway rats in Vancouver, Canada’s Downtown Eastside (16).

The VRP, through whole genome sequencing of bacteria, found that rats carried strains of MRSA that were suggestive of human to rat transmission (16). For example, the most common strain identified in the rats was genetically indistinguishable from the most prevalent strain in the DTES human population (16). The DTES is characterized by improperly disposed of needles/syringes, exposed garbage, public defecation, and heavy alley usage by both homeless and injection drug using populations (74). Because MRSA can be transferred horizontally on fomites and/or feces (75,76), and can survive in the environment for days to months depending on the type of surface, environmental conditions, and the bacterial strain (77,78), local wild rats may acquire MRSA through contact with colonized/infected people and with what they leave behind in and around rat habitat in alleys and buildings (16).

Given that rats live in highly social colonies that share a nest (10,23), the overall low prevalence of MRSA in Himsworth et al. (16) suggests that the overall rate of rat-to-rat transmission may be low. Furthermore, Himsworth et al. (16) found that MRSA carriage was associated with dominance characteristics in rat populations. It may be the case that dominant rats have an increased probability of exposure to MRSA in the environment because they exhibit more exploratory behaviour than subordinates (22). Because disturbances could increase the ranging behaviour of rats that are not killed as a part of the intervention, kill-trapping could increase their level of exposure to environmentally acquired pathogens. This suggests that human induced disturbances, such as pest control, may influence the levels of MRSA within a rat population. As such, this study was designed to assess the impacts of rodent control on the levels of MRSA in rat populations. Specifically, we seek to establish whether kill-trapping, a common rodent control technique, influenced the odds that urban Norway rats carry MRSA in Vancouver’s DTES.

7 See chapter one and two of this dissertation
4.2 Methods

4.2.1 Sampling and MRSA Swab Collection

The trapping and sample collection protocols are described in detail in Chapter two of this dissertation. Transport swabs containing Amies Agar Gel (Copan, Brescia, Italy) were stored at 4°C and were tested for MRSA within the same week of collection.

4.2.2 MRSA Culture

Using aseptic technique, transport swabs were placed in Trypticase Soy Broth (TSB) containing 6.5% NaCl (Becton, Dickinson Canada, Mississauga, MD, USA) as per industry standards (79), vortexed and then incubated at 35°C +/- 2°C under aerobic conditions for 18-24 hours. After incubation, an approximately 10ul aliquot was inoculated onto BBL CHROMagar MRSA II (Becton, Dickinson and Company, Sparks, MD, USA) and streaked for isolation (analytical specificity and sensitivity of this test: 97.3% and 92.7%, respectively (80)). Agar plates were then incubated 35°C +/- 2°C under aerobic conditions for 18-24 hours. All plates were checked for MRSA suspect colonies (mauve coloured colonies), any plates negative for MRSA-like colonies at 18-24 hours were incubated a further 18-24 hours and re-checked before being designated as negative. To rule out cross-reactivity of the agar with other bacteria, up to 3 MRSA like colonies from each specimen observed on BBL CHROMagar MRSA II agar were sub-cultured to Columbia Blood agar with 5% sheep blood (Oxoid, Canada). Sub-cultured isolates were confirmed as *Staphylococcus aureus* based on colony morphology, gram stain, catalase reaction (positive) and tube coagulase reaction (positive). All isolates confirmed as *S.aureus* were then tested for resistance to the industry standard antibiotic, Cefoxitin (30ug) (79). All isolates showing resistance were deemed suspect MRSA isolates and were stored in cryovials at -80°C for further testing and confirmation.
4.2.3 Statistical Analysis

A rat was considered MRSA-positive if MRSA was isolated using the preceding protocol from either the oropharynx or rectum during either the period before the intervention or the period after the intervention. Any rats that were captured in the period before the intervention and were recaptured in the period after the intervention were excluded from the analysis (note that the final statistical model was rerun including these animals to determine whether their inclusion had an impact on the results). For positive rats captured more than once in the same period (either before or after the intervention), continuous variables (weight, length, bite wounds), were averaged across each positive recapture. For rats that were captured more than once in the same period, but never tested positive for MRSA, continuous variables were averaged across each recapture. No juvenile rats recaptured in the same trapping period reached maturity in this timeframe. Any animal with missing data in any of the potential confounding variables being considered were excluded from the dataset.

MMLR was used to estimate the effect of the kill-trapping intervention on the odds that rats carried MRSA, while controlling for clustering by city-block. The outcome was MRSA status of individual rats (negative or positive). The intervention variable categorized rats as to whether they were caught before or after the intervention in control blocks, non-kill blocks flanking intervention blocks, or in intervention blocks. This variable had four levels; 0 – if a rat was captured in the period before the intervention in any block type (reference group), 1 – if a rat was captured in the period after the intervention in a control block, 2 – if a rat was caught in the period after the intervention in a block flanking the intervention block, and 3 – if a rat was caught in the period after the intervention in an intervention block. This variable compared the odds of MRSA carriage in the period after the intervention to the odds of carriage before any intervention separately for intervention blocks, control blocks, and non-kill blocks flanking intervention blocks.

A hypothesis-testing model building approach was used to estimate the effect of the intervention. Potential confounders considered for inclusion in the model included: sex (male or female); sexual maturity (juvenile or mature); dichotomous weight (0; < 121.5, 1; ≥ 121.5g); dichotomous length (0; < 31.35cm, 1; ≥ 31.35); cutaneous bite wounds (number); and wound
presence (0; no wounds, 1; at least one wound present). Variables were considered confounders and kept in the model if they changed the estimate of the effect of the intervention variable in intervention blocks by at least 10%. Of the collinear variables (length and weight [spearman’s $\rho = 0.912, p<10^{-15}$]; and the number of bite wounds and wound presence), the one which had the largest impact on the effect of the intervention was used in the model building process. Note that continuous variables were dichotomized around their medians if they did not satisfy model assumptions.

The proportion of variance associated with the random effect of the city block was calculated using: $\text{ICC} = \frac{\text{variance}^{\text{block}}}{\text{variance}^{\text{block}} + \pi^2/3}$, as previously described by (61). All statistics were carried out in the open source software RStudio (Version 1.0.136, 2009-2016) using the lme4 package for regression (62).

4.3 Results

4.3.1 Sample Description

22 out of 402 (5.5%, 95% CI 3.5%-8.3%) rats included in this analysis were positive for MRSA. The distributions of sex, sexual maturity, weight, length, and the number of bite wounds in this sample are described in Table 4.1 and Figure 4.1.

4.3.2 Statistical Analysis

Five rats were excluded because they were missing values of potential confounders and one rat was excluded because it was caught in the period before the intervention and recaptured in the period after the intervention (note that rerunning the final model including this individual rat did not substantially change the results; the effect of the intervention in intervention blocks remained statistically insignificant). Holding sex and dichotomous length constant, a rat caught in the intervention block after the intervention did not have a significantly different odds of carrying MRSA than a rat caught before the intervention (Table 4.2). Further, no period in any
block type was associated with a significant change in the odds of MRSA carriage as compared to the odds of carriage before the intervention. In this final model, the random effect of the block had a variance of 2.685, therefore =44.9% of the total model variance was due to the random effect of the block.

4.4 Discussion

This study suggests that kill-trapping, a rodent control technique, does not significantly impact the odds that individual rats carry MRSA. Specifically, this study found that six weeks after a kill-trapping intervention, rats caught in city-blocks subjected to kill-trapping did not have a significantly changed odds of MRSA carriage relative to rats caught in the period before kill-trapping. Furthermore, there was no change in the odds of carriage after the intervention in either control blocks or in non-kill blocks flanking intervention blocks. These results indicate that our kill-trapping intervention did not have an impact on the odds that individual rats tested positive for MRSA.

MRSA may not have been affected by the kill-trapping intervention because rats may pick up the bacteria from their environments and there may be a low level of rat-to-rat transmission. It may be the case that the acquisition of environmentally mediated pathogens like MRSA are not easily susceptible to population disturbances, while pathogens that have higher levels of horizontal rat-rat transmission are.

The number of bite wounds, length, and weight were all statistically significant predictors of MRSA status, independent of the period or the block type of capture, suggesting a link between these morphological characteristics and MRSA carriage. As length and weight are rough indicators of a rat’s chronological age (81), perhaps this association reflects a greater probability of exposure in older rats due to a longer exposure to the environment from which MRSA is likely acquired. Furthermore, the association with the number of bite wounds may indicate that rats with more open wounds are more likely to get cutaneous/soft tissue MRSA infections [and subsequently carry MRSA in their saliva through grooming in and around these wounds]. Alternatively, the number of bite wounds may also be a rough indicator of age, in that older rats have simply had more time to acquire wounds.
One limitation of this study was that it did not characterize MRSA isolates to the strain level and therefore could not detect the impact of kill-trapping on the odds of carriage of specific strains. This is important because rats may become colonized by different strains of MRSA from different sources (16) and thus the impact of kill-trapping may not be the same on all strains. Additionally, the protocol used here to isolate and confirm MRSA has not been validated for rats specifically, though in a comparison of common methods used for MRSA surveillance in people, this method had the highest positive predictive power (82). Furthermore, there are very few studies on MRSA in rats (16,43,45) and no two have used the same protocols. As such, we chose a test that had a specified analytical sensitivity and specificity (80) in human clinical samples and was relatively easy to use. Importantly, because Himsworth et al. (16) found that rats in the DTES did not carry novel strains of MRSA (they carried strains known to be carried by people), the analytical specifications, given above, of this test are likely similar for these samples from rats.

In conclusion, this study found that kill-trapping did not have a detectable impact on the isolation of MRSA from the oropharynx or rectum of urban rats. This may be the case because MRSA acquisition is mediated by environmental contamination rather than rat-to-rat transmission. As a result, disturbances like kill-trapping, that may act upon a population by disrupting social behaviours, may not have a large impact on the carriage of MRSA.
**Description of Sample of Norway Rats (n=402)**

<table>
<thead>
<tr>
<th>MRSA Status</th>
<th>Positive (n=22)</th>
<th>Negative (n=380)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (68%)</td>
<td>199 (52%)</td>
</tr>
<tr>
<td>Female</td>
<td>7 (32%)</td>
<td>181 (48%)</td>
</tr>
<tr>
<td></td>
<td>[Female]</td>
<td>[Female]</td>
</tr>
<tr>
<td>Sexual Maturity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>16 (73%)</td>
<td>220 (58%)</td>
</tr>
<tr>
<td>Juvenile</td>
<td>6 (27%)</td>
<td>160 (42%)</td>
</tr>
<tr>
<td></td>
<td>[Juvenile]</td>
<td>[Juvenile]</td>
</tr>
<tr>
<td>Number of Bite Wounds</td>
<td>0 [Median]</td>
<td>0 [Median]</td>
</tr>
<tr>
<td></td>
<td>0-9 [Range]</td>
<td>0-13 [Range]</td>
</tr>
</tbody>
</table>

Table 4.1 Description of the sample of 402 Norway rats from which MRSA swabs were taken.
Final Model of the effect of the intervention (n=402)

<table>
<thead>
<tr>
<th>Confounders</th>
<th>Levels</th>
<th>Estimate</th>
<th>SEestimate</th>
<th>ORadj</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.9524</td>
<td>0.5180</td>
<td>2.591923</td>
<td>0.0660</td>
<td>0.98-7.66</td>
</tr>
<tr>
<td>Dichotomous Length</td>
<td>Length&lt;31.35cm</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>Length≥31.35cm</td>
<td>1.2370</td>
<td>0.5331</td>
<td>3.445262</td>
<td>0.0203</td>
<td>1.28-10.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The Intervention</th>
<th>Levels</th>
<th>Estimate</th>
<th>SEestimate</th>
<th>ORadj</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention Variable</td>
<td>Before Intervention, all Block Types (n=191)</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>After Intervention, Control Blocks (n=120)</td>
<td>0.4435</td>
<td>0.7479</td>
<td>1.558151</td>
<td>0.5531</td>
<td>0.39-8.56</td>
</tr>
<tr>
<td></td>
<td>After Intervention, Non-Kill Flanking Blocks (n=44)</td>
<td>-1.1666</td>
<td>1.1156</td>
<td>0.311424</td>
<td>0.2957</td>
<td>0.016-1.97</td>
</tr>
<tr>
<td></td>
<td>After Intervention, Intervention Blocks (n=47)</td>
<td>-0.5903</td>
<td>1.2125</td>
<td>0.554161</td>
<td>0.6263</td>
<td>0.026-4.90</td>
</tr>
</tbody>
</table>

a – Reference group

Table 4.2 Summary of the final model of the effect of the kill-trapping intervention on the odds that individual rats carry MRSA.

Levels of the intervention variable refer to the trapping period (before or after the intervention) and the block-type (control, non-kill flanking, or intervention) each rat was caught in.
Figure 4.1 Distributions of the weight and length of the 402 rats included in the statistical analysis.

Red lines mark the medians of each distribution.
5.1 How can we interpret the results of these studies?

We found that killing rats at the level of a city block was followed by a significantly increased odds of *L. interrogans* carriage among the remaining rats within that block. This effect was not observed in control blocks nor was it observed in non-kill flanking blocks. In contrast, there was no significant change in the odds of capturing rats carrying MRSA, in any block type, following kill-trapping.

There are several potential explanations for these results. With regard to the odds of *L. interrogans* carriage, it is possible that the results were, at least in part, due to trapping bias. For example, it has been suggested that dominant rats, (which may be more likely to carry *L. interrogans*) are less likely than other rats to enter traps (30). If this was the case, our intervention may have preferentially removed non-colonized rats [since dominant rats may be more likely to carry *L. interrogans*], leaving a higher proportion of colonized rats to be sampled in the trapping period after the intervention as compared to before the intervention. Interestingly, some have suggested the opposite, that dominant rats may actually be more likely than subordinates to enter traps (30,31). If this is the case, then our initial trapping may have induced trap shyness preferentially in the proportion of the rat population that is less likely to carry *L. interrogans*. For instance, rats that don’t enter the traps may be able to learn trap avoidance by being in the vicinity of the rat while it is trapped (40); it is therefore possible that the non-dominant rats that may have been less likely to enter traps in the period before kill-trapping were even less likely to enter traps after kill-trapping due to learned shyness. In this scenario, we would catch an even higher proportion of dominant rats [who may be more likely to carry *L. interrogans*] to subordinate rats in the period after the intervention as compared to before it. This being said, dominant and submissive rats have differing morphometric characteristics (e.g., body mass, bite wounds, etc.). By controlling for these factors during multivariate modelling, we were likely able to account for at least some of these potential biases. As such, this suggests that the
increased odds of *L. interrogans* carriage may have been a result of increased intraspecific transmission subsequent to the kill-trapping intervention.

With regard to MRSA, the lack of a significant finding may have two explanations. First, this study may not have been sufficiently statistically powered to detect a change in the odds of MRSA carriage. For instance, we only captured 22 MRSA positive rats spread out over both the periods before and after the intervention and over 36 city-blocks. It is possible that if there was a change in the odds that rats carried MRSA, this study may not have sampled enough rats to detect it. Secondly, it may be the case that environmentally acquired pathogens like MRSA are not as readily susceptible to pest control techniques as are socially transmitted pathogens like *L. interrogans*.

### 5.2 What are the implications of these findings?

The different impact that kill-trapping had on *L. interrogans* as compared to MRSA, may be due to the differing modes of rat-to-rat transmission of these zoonotic pathogens. This is important because it suggests that kill-trapping may impact the levels of various RAZ in different ways. For example, it may be the case that while putative environmentally acquired pathogens like MRSA are not easily susceptible to change as a result of culling, other pathogens that are horizontally transmitted through specific social interactions, like *L. interrogans*, may be more readily affected. Therefore, it is possible that knowledge of RAZ epidemiology within a rat population can help predict the likelihood that culling will impact a specific pathogen. For example, hantavirus, which is transmitted via aggressive interactions (24), may be highly susceptible to change after rat culling while RAZ like *E. coli* and *Salmonella*, which are likely environmentally acquired (83), may not be. From a practical perspective, however, this information has limited application. For instance, people working in the field to reduce rat populations and their associated health risks often do not have knowledge of the pathogens the animals are carrying prior to performing control. For this reason, it would be prudent for people performing rat culling to assume that at best their methods will be largely ineffective at decreasing the disease risk and at worst, they could actually increase those risks.
This finding has important implications for those engaging in pest control (e.g., the public, PCPs, public health professionals, municipalities, etc.), particularly those who engage in pest control for the purpose of reducing human health risks. More often than not, rat control is reactive, meaning, that control is not enacted until after complaints are received (29). Further, these reactive methods usually rely on killing techniques, such as rodenticides and kill-trapping (29,30). Importantly, these after-the-fact approaches, by definition, occur after the rat population already exists and they often leave a proportion of the population alive (29,84). If these control attempts are performed in a rat population that is endemically infected with a RAZ like *L. interrogans*, based on the results of this study, control efforts may paradoxically increase health risks by increasing infection levels among surviving rats. Importantly, this effect may not be limited to the specific location where rat control is taking place. For example, some rats may move around a city block and enter multiple buildings (38). Killing rats in one building could theoretically disrupt the social structure of its colony in another location, thereby eliciting change in the transmission of RAZ in areas spatially separated from the control efforts.

5.3 Limitations

These studies have several important limitations. First because of the small sample size, we were not able to assess the impacts of kill-trapping on black rats. Secondly, this study was not designed to estimate rat abundance at any level of trapping. This is important because we cannot reconcile the heightened risk from the increased odds of *L. interrogans* carriage after kill-trapping with the potential decrease that might result from the kill-trapping reducing the overall population size. For example, it may be the case that the increased odds of *L. interrogans* carriage was offset by the reduced probability of contact between people and rats. However, this seems unlikely as we removed less than 20 rats in each city block and block level/area specific abundance has been noted in other studies to be above 100 individuals (25,85). Along these same lines, I anecdotally, observed many dozens of rats foraging/feeding together in single parking lots, single green spaces, and single yards, both before, during, and after intervention periods, indicating that the block level prevalence of rats may have been very high throughout this study.
Finally, this study was not able to account for the actual risk to the human population. In general, it is very difficult to define and quantify the health risk that rats pose to people, particularly in the DTES. For instance, Himsworth et al. (15), suggested that while people living in the DTES may be at an increased risk of contracting Leptospirosis, there is a lack of apparent human cases in that neighbourhood. This may be due to a combination of underdiagnoses (since injection drug use, homelessness, and pre-existing conditions like HIV make general febrile illness’s like most Leptospirosis cases a common problem), and misdiagnoses (both because of the general common-place occurrence of fever with unknown causes and a general lack of awareness by healthcare practitioners in urban areas)(3). However, we can conclude that while the change in risk to the human population is not directly quantifiable here, it is difficult to purport, in light of the results in this dissertation and other studies assessing the impact of culling animals on disease (26-28), that culling animal reservoirs of pathogens is the best or even a safe way to approach controlling the health risks posed by rats.

5.4 Recommendations

Given that reactive control is ineffective and that it may increase the levels of some RAZ in the remaining rats, those engaging in rat control have two options to consider: 1) ensure that the entire rat population is eliminated, or 2) use an altogether different approach to control. Choosing option number one comes with several caveats. First, research shows that reactive control is often unable to completely remove rat infestations (29,86). This is a result of the fact that, within the area where the control effort is occurring, a certain portion of the rat population will not enter traps or will not consume poison bait (30). Further, these control efforts are often limited to specific buildings and/or locations (vs. an entire city block) and may not address the entire infestation (29,30). Finally, even if total eradication is achieved, unpredictable changes in pathogen prevalence/transmission may still occur. For example, Amman et al. (28) found that total elimination of bats from a cave in Uganda was not effective at reducing the risks of Marburg virus [for which the bats were the reservoir]. Specifically, they found that after elimination, the new population of bats that resurged in the cave had a significantly higher prevalence of Marburg virus than did the culled population (the cause of this increase was
unclear). Further, the authors suggested that this heightened prevalence in the bat population precipitated the largest outbreak of Marburg virus in people that had ever occurred in Uganda.

Option number two represents a paradigm shift in the way that urban rodent control is most often carried out. As such, this option should be constructed based upon specific scientific evidence. For example, evidence suggests that control should be proactive, rather than reactive (29,84,87). Unlike reactive control, proactive methods attempt to prevent rat infestations from occurring, rather than dealing with them after they have become established (29,84,87). This, on its own would mitigate the risk of RAZ because without rats there are no RAZ. However, we can acknowledge that it is not always possible to prevent rat infestations from occurring in all urban areas, because, taking Vancouver as an example, they already exist.

Where rat infestations must be eliminated, rat control should take place on a biologically relevant scale (i.e., an entire city block). For example, when rat complaints are received from a building, PCPs should survey the surrounding area (the entire city block) for rat signs. If rat signs are observed beyond the building of interest, control should expand to include all potentially infested areas. Additionally, control should not rely solely on methods for culling rats, like rodenticides and kill-trapping. Current scientific models of urban control center on the idea of limiting the carrying capacity (ecological-based pest management or EBPM) of the environment such that harborage and foodstuffs for rats are reduced drastically (29,84). The general idea of this control is to reduce birth rates, increase natural mortality, and to force rats to look elsewhere for resources. For example, these methods require removing/sealing garbage and compost, sealing outdoor toilets, plugging holes to buildings, eliminating access to restaurants and other food establishments, and filling in burrows.

Proactive methods like EBPM are often limited by political will, public-to-government coordination, lack of a centralized enforcement agency, and lack of leadership and responsibility (84). Successful programs have been extremely well coordinated, have involved the public, biologists, pest-control professionals and the municipal government, and they have had centralized leadership, clear responsibilities, and have had strict accountability (84). For example, to ensure that there are no foodstuffs/harbourage available in a city block requires first that the public residing in that block are on board with the efforts so that they comply with rules such as sealing garbage and compost. Furthermore, the participation of the municipal
government is required to ensure that people comply with the new rules and to enforce them when they do not. Skilled PCPs and biologists are required to continually assess and enact the control efforts.

5.5 Conclusions and Future Directions

The central question of this thesis, how does rat culling impact RAZ in rat populations, can be represented by a simplistic pathway; kill-trapping rats → rat carriage of RAZ → human risk. It is important to iterate that this work focused only on the first part of this pathway (i.e., how does killing impact the carriage of RAZ within rat populations), and it was not designed to assess how killing changed the risk that rats pose to people. As a result, our study only indicates that after kill-trapping, the risk of human exposure to *L. interrogans* is increased *if and when* a person encounters a rat and I cannot comment on any change in the overall risk to people (for the various reasons stated in the limitations section above). This leaves us with the very important question as to whether kill-trapping, through changing the odds of *L. interrogans* carriage in the non-killed portion of the rat population, increases the risk to people.

Perhaps future studies should investigate the second step in the simplistic pathway above; does rat-culling change the human risk of contracting Leptospirosis? We can imagine planning such a study. First, we would have to choose an area, such as urban slums in Brazil, where the human risk of Leptospirosis is both real and quantifiable. Second, we would need to set up a kill-trapping protocol and monitor the levels of the disease in the local human population to observe any changes. However, given the results of this dissertation, we must ask, would such a study be ethical? We now know, by investigating the first link in this pathway, that killing has the potential to increase the levels of carriage of *L. interrogans* in the remaining rats. At the very least, this means that culling is not directly decreasing the risk that each rat poses to people.

Combining the results of this study with other research, we now know that; 1) kill-trapping is an ineffective method for reducing or removing rats, 2) kill-trapping may not decrease the risk of RAZ carriage in rats and it may even increase that risk, and 3) alternative methods that are effective at removing and keeping rat infestations away do exist. Therefore, we cannot ethically investigate how kill-trapping may change the RAZ risk that rats pose to people, both because scientific evidence suggests that such trapping has no benefits (other than being the cheapest and easiest option) and because it has the potential to increase that very risk. Further, there are known alternatives to these methods that are
effective at reducing and removing rats. Instead, this dissertation can be viewed as a final nail closing the coffin over outdated ineffective rat-culling techniques, and pushing us towards more holistic methods (section 5.4) that evidence suggests are far more efficacious.
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Infectious Diseases; 2016 Nov 26;16:1–8.


