COULD RATS POSE A HEALTH RISK FOR PEOPLE LIVING IN VANCOUVER’S DOWNTOWN EASTSIDE?
UNDERSTANDING THE ECOLOGY OF RATS AND RAT-ASSOCIATED ZOONOSES IN AN INNER-CITY NEIGHBOURHOOD

by

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ABSTRACT

Urban rats (*Rattus* spp.) are an important source of zoonotic pathogens, yet there is a paucity of integrated, interdisciplinary, ecosystem-based research on rat-associated zoonoses (RAZ). The goal of this project was to begin to characterize the public health risks associated with rats by studying the ecology of rat populations and the zoonotic pathogens that they carry in an inner-city neighbourhood of Vancouver, Canada. By characterizing rat populations within our study area, we were able to identify a number of factors which could influence the ecology of RAZ. We were also able to design a tool to predict rat abundance based on characteristics of the urban microenvironment, which may be useful for predicting RAZ prevalence in the future. Although we found that *L. interrogans* (a common RAZ) was present in our study area, other zoonoses thought to be endemic in rat populations worldwide (Seoul hantavirus, *Rickettsia typhi*, and *Bartonella* spp.) were conspicuously absent. However, rats were found to carry other potentially zoonotic organisms (*Clostridium difficile* and methicillin-resistant *Staphylococcus aureus*) for which they are not the ‘traditional’ reservoir. Finally, we found that by integrating data regarding rat ecology and RAZ, we were able to gain a more comprehensive picture of how these pathogens circulate within rat populations. Overall, this research illustrates the importance of a comprehensive and holistic approach for obtaining a better understanding of RAZ, and highlights the need for ongoing research and surveillance.
Published Materials:

Several of the chapters in this thesis were written as stand-alone manuscripts for publication in a peer-reviewed academic journal. My contributions to each manuscript are detailed below.

Chapter 1: Literature Review


CGH, CMJ, and DMP designed the study. CGH created the search matrix, identified literature for inclusion in the review (in conjunction with KLP), extracted and analyzed the data, and wrote the manuscript. All co-authors contributed to manuscript revisions.


CGH, TK, and DMP designed the study. CGH designed the survey with the assistance of TK. CGH deployed the survey, analyzed the data, and wrote the
manuscript. AYTF and KLP helped to deploy the survey and collate the survey data. All co-authors contributed to manuscript revisions.

Chapter 3: The Characteristics of Wild Rat (*Rattus* spp.) Populations from an Inner-City Neighbourhood with a Focus on Factors Critical to the Understanding of Rat-Associated Zoonoses.

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CGH, CMJ, and DMP helped to design the study. CGH performed the fieldwork, collected and analyzed the data, and wrote the manuscript. KLP and AYTF assisted with the fieldwork and data collection. CMJ assisted with the data analysis. All co-authors contributed to manuscript revisions.


CGH, TK, CMJ and DMP designed the study. CGH collected and analyzed the data, and wrote the manuscript. AYTF and KLP assisted with the fieldwork. JB and JR performed the PCR testing. SM assisted with the GIS mapping and spatial analysis. All co-authors contributed to manuscript revisions.
Chapter 6: Ecology of *Leptospira interrogans* in Norway Rats (*Rattus norvegicus*) in an Inner-City Neighbourhood of Vancouver, Canada.


CGH, PT, CMJ, TK, and DMP designed the study. CGH collected and analyzed the data, and wrote the manuscript. AYTF and KLP assisted with the fieldwork. JB and JR performed the PCR testing. SM assisted with the GIS mapping and spatial analysis. All co-authors contributed to manuscript revisions.

Chapter 8: Bacteria Isolated From Conspecific Bite Wounds in Norway and Black Rats: Implications for Rat Bite-Associated Infections in People


CGH, PT, CMJ, and DMP designed the study. CGH conducted the literature review (in conjunction with KLP), analyzed the data (in conjunction with PT), and wrote the manuscript. EZ performed the bacterial cultures. All co-authors contributed to manuscript revisions.

Chapter 9: Carriage of Methicillin-Resistant *Staphylococcus aureus* by Wild Urban Norway and Black Rats (*Rattus norvegicus* and *Rattus rattus*).

CGH, JSW, PT, TK, CMJ, and DMP designed the study. CGH collected and analyzed the data, and wrote the manuscript. JSW performed the MRSA culture and typing. VM performed the MRSA WGS. RMM, VM, and PT assisted with the WGS analysis. LH, MGR, and GNA-R provided human MRSA isolates for comparison. All co-authors contributed to manuscript revisions.

Chapter 10: Carriage of *Clostridium difficile* by Wild Urban Norway and Black Rats (*Rattus norvegicus* and *Rattus rattus*)


CGH, JSW, PT, CMJ, and DMP designed the study. CGH collected and analyzed the data, and wrote the manuscript. JSW performed the *C. difficile* culture and typing. SM assisted with the GIS mapping and spatial analysis. All co-authors contributed to manuscript revisions.

Ethics Approval:

The following is a list of UBC Research Ethics Board Certificates Pertaining to the work included in this dissertation.
All activities pertaining to the trapping and handling of rats was approved by the University of British Columbia’s Animal Care Committee (A11-0087).

Collection, analysis, and publication of data pertaining to the pest control professional survey were approved by the University of British Columbia’s Behavioral Research Ethics Board (H11-00989).
# TABLE OF CONTENTS

Abstract ......................................................................................................................................... ii  
Preface .......................................................................................................................................... iii  
Table of Contents ....................................................................................................................... viii  
List of Tables ............................................................................................................................... xi  
List of Figures ............................................................................................................................. xii  
List of Abbreviations ................................................................................................................ xiv  
Acknowledgements ................................................................................................................ xvi  
Dedication ................................................................................................................................ xvii  
Chapter 1: Introduction ..............................................................................................................1  
1.1 Background ........................................................................................................................... 1  
1.2 Rationale ............................................................................................................................ 2  
1.3 Study Setting ...................................................................................................................... 3  
1.4 Conceptual Framework ........................................................................................................ 4  
1.5 Knowledge Gaps ................................................................................................................ 7  
1.6 Objectives and Aims .......................................................................................................... 8  
1.7 Study Design .................................................................................................................... 11  
1.8 Summary ........................................................................................................................... 12  
Chapter 2: Literature Review ..................................................................................................14  
2.1 Synopsis ............................................................................................................................... 14  
2.2 Introduction ....................................................................................................................... 14  
2.3 Methods ............................................................................................................................. 15  
2.4 Results ............................................................................................................................... 16  
  2.4.1 Overview of Rat-Associated Zoonoses ....................................................................... 16  
  2.4.2 Common Epidemiologic Themes for the Above Zoonoses ....................................... 25  
2.5 Discussion .......................................................................................................................... 33  
Chapter 3: Using Experiential Knowledge to Understand Urban Rat Ecology: A Survey of Canadian Pest Control Professionals ..................................................................35  
3.1 Synopsis ............................................................................................................................... 35  
3.2 Introduction ....................................................................................................................... 36  
3.3 Methods ............................................................................................................................. 37  
3.4 Results ............................................................................................................................... 38  
3.5 Discussion .......................................................................................................................... 44  
Chapter 4: The Characteristics of Wild Urban Rat Populations with a Focus on Factors Critical to the Understanding of Rat-Associated Zoonoses ...........................................49  
4.1 Synopsis ............................................................................................................................... 49  
4.2 Introduction ....................................................................................................................... 49
4.3 Methods ........................................................................................................... 52
4.4 Results .............................................................................................................. 59
4.5 Discussion ....................................................................................................... 66

Chapter 5: A Mixed Methods Approach to Exploring the Relationship Between Norway Rat (Rattus norvegicus) Abundance and Features of the Urban Environment in an Inner-City Neighbourhood ................................................................. 73
5.1 Synopsis ........................................................................................................... 73
5.2 Introduction .................................................................................................... 74
5.3 Methods ........................................................................................................... 76
5.4 Results .............................................................................................................. 83
5.5 Discussion ....................................................................................................... 87

Chapter 6: Ecology of Leptospira interrogans in Wild Urban Norway Rats .......... 92
6.1 Synopsis ........................................................................................................... 92
6.2 Introduction .................................................................................................... 93
6.3 Methods ........................................................................................................... 94
6.4 Results .............................................................................................................. 97
6.5 Discussion ..................................................................................................... 103

Chapter 7: No evidence of infection with Seoul hantavirus, Bartonella spp., and Rickettsia typhi in Wild Urban Norway and Black Rats ................................................. 109
7.1 Synopsis ......................................................................................................... 109
7.2 Introduction .................................................................................................. 109
7.3 Methods ......................................................................................................... 110
7.4 Results ............................................................................................................ 113
7.5 Discussion ..................................................................................................... 113

Chapter 8: Bacteria Isolated From Conspecific Bite Wounds in Norway and Black Rats: Implications for Rat Bite-Associated Infections in People ................................................. 117
8.1 Synopsis ......................................................................................................... 117
8.2 Introduction .................................................................................................. 118
8.3 Materials and Methods .............................................................................. 119
8.4 Results ............................................................................................................ 121
8.5 Discussion ..................................................................................................... 124

Chapter 9: Carriage of Methicillin-Resistant Staphylococcus aureus by Wild Urban Norway Rats .............................................................................................................. 129
9.1 Synopsis ......................................................................................................... 129
9.2 Introduction .................................................................................................. 130
9.3 Methods ......................................................................................................... 131
9.4 Results ............................................................................................................ 135
9.5 Discussion ..................................................................................................... 144

Chapter 10: Carriage of Clostridium difficile by Wild Urban Norway and Black Rats 147
10.1 Synopsis.......................................................................................................................... 147
10.2 Introduction..................................................................................................................... 148
10.3 Methods............................................................................................................................ 149
10.4 Results.............................................................................................................................. 152
10.5 Discussion: ....................................................................................................................... 157

Chapter 11: Conclusion........................................................................................................... 163
11.1 Summary of Findings....................................................................................................... 163
11.2 Synthesis and Implications............................................................................................ 166
11.3 Strengths and Limitations.............................................................................................. 170
11.4 Avenues for Further Research....................................................................................... 173
11.5 Recommendations......................................................................................................... 178
11.6 Conclusions.................................................................................................................... 181

References..................................................................................................................................184

Appendices................................................................................................................................216
  Appendix A : Pest control professional rat survey................................................................. 216
  Appendix B : A systematic environmental observation tool to predict relative rat abundance in urban centres............................................................................. 223
  Appendix C : Guidance document for the systematic environmental observation tool................................................................................................................... 231
Table 2-1: A summary of the main zoonotic pathogens associated with Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) in urban centers. .................................................. 24
Table 2-2: Conclusions from a review of the literature regarding the ecology of zoonotic pathogens associated with Norway rats and black rats (*Rattus norvegicus* and *Rattus rattus*) in urban centers. ............................................................................................................. 34
Table 5-1: Relationship between features of the urban environment and relative abundance of Norway rats (*Rattus norvegicus*) as measured by trap success. ......................... 83
Table 6-1: Baseline characteristics and associations with *L. interrogans* PCR status among Norway rats (*Rattus norvegicus*). ................................................................................................................................. 98
Table 6-2: Unadjusted and adjusted odds ratios for being *L. interrogans* PCR-positive among Norway rats (*Rattus norvegicus*). ............................................................................................................................. 101
Table 8-1: Bacteria isolated from 40 bite-wound-related skin and soft tissue infections in Norway and black rats (*Rattus rattus* and *Rattus norvegicus*). ......................................................... 123
Table 9-1: Characteristics of 22 MRSA isolated obtained from wild Norway rats (*Rattus norvegicus*). ..................................................................................................................................... 137
Table 9-2: Relationship between MRSA-status, season, and morphometric characteristics among a population of wild Norway rats (*Rattus norvegicus*). ................................................................. 138
Table 10-1: Relative frequency of *Clostridium difficile* ribotypes (with toxin profile and toxinotype) isolated from urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). ................................................................................................................................. 154
Table 10-2: Baseline characteristics and associations with *Clostridium difficile* status among urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). ................................................................. 156
Table 10-3: Relationship between rat characteristics and *Clostridium difficile* status among Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). ................................................................. 157
Table 11-1: Conclusions from the research described in this dissertation and avenues for further study. ............................................................................................................................. 182
LIST OF FIGURES

Figure 1-1: Conceptual framework for the ecology and emergence of rat-associated zoonoses. .................................................................................................................................................. 5

Figure 4-1: A. Spatial distribution of trapped Norway rats (Rattus norvegicus) and trap success in each city block. B. Spatial clusters of relatively high and low rat abundance on Getis-Ord G* analysis. For the GiZScore, a high z-score indicates spatial clustering of high values and a low z-score indicates spatial clustering of low values. A z-score near zero indicates no significant clustering................................................................. 61

Figure 4-2: Seasonal variation in the proportion of immature, parous, and pregnant/lactating Norway rats (Rattus norvegicus). .............................................................................. 63

Figure 6-1: Number of L. interrogans-positive vs. -negative Norway rats (Rattus norvegicus) in each study location. Prevalence of L. interrogans is noted above each bar. 99

Figure 6-2: Distribution of L. interrogans-positive Norway rats (Rattus norvegicus) and clusters of high and low L. interrogans prevalence in an inner-city neighborhood of Vancouver, Canada. Observed vs. expected number of L. interrogans-positive rats with relative risk and p values noted for each cluster. Inset = Map of Vancouver with location of study site........................................................................................................................................ 102

Figure 8-1: Bacterial species isolated from 40 bite wound-related skin and soft tissue infections in Norway and black rats (Rattus norvegicus and Rattus rattus). ................... 122

Figure 9-1: Number of MRSA-positive vs. -negative Norway rats (Rattus norvegicus) in each study location. Prevalence of MRSA is noted above each bar. .............................. 136

Figure 9-2: Maximum likelihood phylogenetic tree of all MRSA samples isolated from Norway rats (Rattus norvegicus). Branch color represents cluster: Green=Cluster 1; Pink=Cluster 2; Yellow=Cluster 3; Orange=Cluster 4. Labels give isolate ID and date. MLST, spa type, and presence of PVL shown in right columns. Abbreviations: ST=Sequence Type; tNew=New spa type with 3 repeat insertion from t008. Scale bar represents the average number of substitutions per site.................................................. 140

Figure 9-3: Number of variant nucleotide positions between every MRSA sample isolated from Norway rats (Rattus norvegicus) calculated from reference based assembly. ..................................................................................................................................................... 141

Figure 9-4: Maximum likelihood phylogenetic tree of all ST8 Norway rat (Rattus norvegicus) and human MRSA samples. Labels give isolate ID, sample location and date. Text color represents sample isolation location: Red=Rat; Dark blue=Human DTES; Pale blue=Human other. MLST, spa type and presence of PVL shown in right columns. Abbreviations: DTES=Downtown East Side; CA=Community-acquired; HA=Hospital-acquired; ST=Sequence Type; tNew=New spa type with 3 repeat insertion from t008. Scale bar represents the average number of substitutions per site........................................... 142
Figure 9-5: Geographic distribution of MRSA-positive Norway rats (*Rattus norvegicus*) from an inner-city neighborhood of Vancouver, Canada, and clusters of high and low MRSA prevalence by whole genome sequence (WGS) group. Observed vs. expected number of MRSA-positive rats with relative risk and p values noted for each cluster. Inset = Map of Vancouver with location of study site .................................................. 143

Figure 10-1: Number of *Clostridium difficile* -positive vs. -negative rats in each study location. Prevalence of *C. difficile* is noted above each bar ................................................................. 153

Figure 10-2: A) Geographic distribution of *Clostridium difficile*-positive Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). B) Distribution of *C. difficile* ribotypes with international designations or previously identified in our laboratory and clusters of high prevalence. C) Distribution of ‘novel’ *C. difficile* ribotypes and clusters of high prevalence. Inset = Location of study site ........................................................................... 155
LIST OF ABBREVIATIONS

AHC – Animal Health Centre
AIC – Akaike’s information criterion
BIC - Bayesian Information Criterion
DTES – Downtown Eastside
GLMM – Generalized linear mixed model
IDU – Injection drug user
MLR – Multiple logistic regression
MRSA – Methicillin-resistant *Staphylococcus aureus*
MRSP – Methicillin-resistant *Staphylococcus pseudintermedius*
NML – National Microbiology Laboratory
OVC – Ontario Veterinary College
PCP – pest control professional
PCR – Polymerase chain reaction
qPCR – Real time polymerase chain reaction
RAZ – Rat-associated zoonoses
SROs – Single room occupancy hotels
SSO – Systematic social observation
WGS – Whole genome sequencing
ZINB – Zero-inflated negative binomial model
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DEDICATION

For Randy and Cambria.

You are my greatest source of joy and inspiration.
1.1 Background

According to the World Health Organization, 75% of emerging infectious diseases recognized over the past 10 years are zoonotic (transmitted from animals to humans) (1). This has led to a realization that zoonotic diseases, particularly those associated with wild animals, pose a significant threat to human health (2, 3). Urban centres are of special concern with regard to zoonotic disease emergence because cities provide optimal habitat for certain wild species (4), leading to increased contact with humans and, potentially, zoonotic disease transmission. Of the wild animals found in cities, rats (Rattus spp.) are, perhaps, the most notorious because of the frequency and severity with which they infest urban centres and because of their propensity towards close association with people (5, 6).

It is well recognized that, historically, rats have been the source of a number of important zoonotic diseases, such as the bubonic plague (7). However, for many, it will come as a surprise to learn that rats continue to be the source of a variety of pathogens responsible for significant human morbidity and mortality in cities around the world. For example, between 2009 and 2011, there were at least 12 internationally reported outbreaks of rat-associated leptospirosis in nine countries (with over 2,000 cases in an outbreak of leptospirosis in Manila, Philippines) and nine outbreaks of rat-associated plague in six countries (with a case fatality rate of 30% for an outbreak of plague in Madagascar) (8).

These numbers are likely a vast underestimate of the human health burden associated with rats because: 1) with the exception of plague, rat-associated zoonoses (RAZ) are generally not reportable and are, therefore, not recorded in national health databases (9); and 2) many rat-associated zoonotic diseases have non-specific clinical
presentations in people and are, therefore, underdiagnosed or misdiagnosed (10-12). For example, although leptospirosis can manifest in renal failure or fatal pulmonary haemorrhage, the majority of infected people experience an undifferentiated febrile illness (10).

Finally, since rat-associated health risks have been shown to intensify in association with the growth of cities and increased urban poverty, and given unprecedented rates of global urbanization (over half the global population currently resides in urban centers) (13), rat-related issues are likely to increase in the future.

1.2 Rationale

Despite anecdotal evidence that rats are thriving in cities around the world, there is a paucity of contemporary research on urban rats and rat-associated zoonotic risks. Nowhere is this knowledge gap more prominent than in Canada, where there has been only one published study on urban rats and/or RAZ. This study, conducted in 1984, attempted to document the presence of parasites and bacterial zoonoses in rats residing within a landfill in Richmond, British Columbia (14). The researchers trapped 43 rats and tested them for ectoparasites (fleas, lice, and mites) and gastrointestinal parasites (tapeworms, roundworms, and protozoa) via visual inspection, and for Salmonella spp. via fecal culture. They also tested five rats for exposure to Leptospira interrogans via serology. Of these 43 rats, 77% and 91% had ectoparasites and gastrointestinal parasites, respectively; 4.7% were positive for Salmonella spp.; and two of the five rats tested for L. interrogans were seropositive. The significance of this study, however, is lessened by the small sample size, limited and now dated methodologies for disease analysis, and an absence of concurrent data on the rat population sampled.

The paucity of Canadian data on RAZ is problematic because: 1) without scientific data on the ecology of urban rats and the pathogens they carry, it is impossible
to even begin to assess the health threats that rats pose to Canadians, or to develop efficient and effective strategies to monitor and mitigate those threats; 2) the non-specific illness caused by rat-borne zoonotic pathogens can lead to underdiagnosis or misdiagnosis of infected humans, particularly where health professionals are not aware of the presence of these pathogens in their jurisdictions; and 3) given that the prevalence and/or distribution of RAZ appear to be increasing in association with global urbanization and urban poverty, Canadians may be vulnerable to future incursions or epidemics associated with these pathogens if we remain ignorant of ecology of rats and RAZ in our country.

1.3 Study Setting

Field studies of rats and RAZ in Canada are clearly needed in order to address the aforementioned knowledge gaps. However, despite being advantageous in terms of ensuring data generalizability, conducting several such studies simultaneously in multiple Canadian cities was outside the scope this project, particularly given the complexity of the problem, the field and laboratory work required, and the absence of baseline data.

For this reason, we chose to focus on the City of Vancouver—specifically on Vancouver’s Downtown Eastside (DTES). The DTES is generally a low-income neighbourhood with high rates of drug addiction, HIV infection, unemployment, and homelessness (15). However, the DTES is also one of the most diverse areas of the city and includes residential areas of varying density and socio-economic status, a large open air illicit drug market, a variety of retail and restaurant venues, parks, squares, and community gardens, a significant number of construction and revitalization programs, industrial areas, a waterfront and working port, and a large Asian ethnic area (15). The fact that this diversity is contained within a relatively small geographic
area makes the DTES ideal for studying urban issues, including rat infestations and rat-human interactions.

Most importantly, given that the DTES is Vancouver’s oldest and most impoverished neighbourhood (15), it is likely to produce an environment that supports larger rat populations compared to other areas of the city (i.e., due to building disrepair, accumulated waste, etc.) (16, 17). Additionally, poverty, as well as concurrent health issues and behaviours, such as injection drug use in public spaces and HIV/AIDS (15), could make DTES residents more vulnerable to rat-associated health risks compared to the general population (16). Indeed, initial discussions with the City of Vancouver and DTES residents revealed that rat infestations and rat-human interactions are perceived to be a significant issue contributing to perceived health and quality of life disparities afflicting the DTES.

For these reasons, the DTES is ideal for Canada’s first comprehensive study of rats, their diseases, and the health risk that they may pose to people both from a scientific standpoint, because this ‘high risk’ neighbourhood could effectively be a ‘sentinel’ for rat associated health risks in Canada, and from a social justice standpoint, because DTES residents are likely at greatest risk for rat-associated zoonoses, and perhaps most likely to benefit from any interventions generated as the result of this study.

1.4 Conceptual Framework

The conceptual framework developed for this project (Figure 1-1) is based on the ‘One Health’ or ecosystem-based approach to zoonotic disease research (18-22). This approach stems from the philosophy that zoonotic disease emergence is the result of a complex network of interactions among the infectious agent (and occasionally vectors), the animal host, humans, and the environment (18-22).
Figure 1-1: Conceptual framework for the ecology and emergence of rat-associated zoonoses.

The pyramid represents the fact that detected human disease is only the ‘tip of the iceberg’ (18). This is because those human cases that are detected represent only a fraction of individuals who are actually diseased, which, in turn, represents only a fraction of people who are exposed/infected (but did not subsequently develop clinical illness) (18). In turn, pathogen spillover from animals to humans is the endpoint of an extensive set of events within the animal host and ecosystem (18, 22). Therefore, in contrast to diseases that circulate in humans only, traditional anthropocentric approaches, particularly those that focus on detected disease in people, are ineffective.
for the study of zoonoses (19, 20). Additionally, focusing only on human infection is, by its very nature, reactionary, and does little to predict, prevent, or address zoonotic disease emergence (19).

In order to best understand, monitor, and mitigate zoonotic disease risks, we must utilize a more holistic approach. This approach should begin by seeking to understand the ecology of the animal reservoir host and to identify the zoonotic pathogens circulating in host populations (as well as any involved vectors, if applicable) (18, 23, 24). Next, we must characterize the ecology of those pathogens in the context of the host ecology, as disease ecology in the reservoir host is a direct determinant of the risk of pathogen transmission to people (2, 18, 22). Human exposure/infection is also determined by the probability and nature of human interactions with the reservoir host, which is independently influenced by reservoir host ecology (as well as human behaviors) (18, 20). Only once these aspects (i.e., the base of the pyramid) have been addressed, will we be in a position to effectively attempt to identify and interpret the presence of, and risk factors associated with, zoonotic disease in people.

It is important to note that the pyramid is situated within a circle that represents the local environment. This symbolizes the fact that zoonotic disease ecology is contextual, and influenced by local environmental characteristics (both physical and socio-economic) (18, 22). In the case of RAZ, local factors might include features of the built urban environment (e.g., land use, building disrepair), infrastructure management (e.g., garbage and sewer systems), and neighbourhood socioeconomic status. The local ecosystem is in turn, influenced by the broader national or global environment (18, 22). Broader environmental factors could include climate change and urbanization. These environments are important to consider not only because they influence current ecosystems, but also because they are subject to changes, often anthropogenic in origin,
which can influence the ecology of zoonotic pathogens (18, 20, 22). Indeed, urbanization is recognized to be an important driver of zoonotic disease emergence (18, 20).

Given this conceptual framework, priority areas for the study of RAZ should include: 1) describing the ecology of urban rat populations (including how the urban environment might influence rat ecology); 2) identifying the zoonotic pathogens circulating within those rat populations; and 3) characterizing the ecology of circulating zoonotic pathogens in rats. This was the focus of the present study, i.e., to analyze, in detail, the bottom two sectors of the pyramid, as well as the inner circle enclosing the pyramid itself, thus creating a solid baseline for further future research on urban RAZ.

1.5 Knowledge Gaps

Given the conceptual framework and priority areas detailed above, and after a review of the published literature regarding rats and RAZ (Chapter 2), the following emerged as the most pressing knowledge gaps requiring immediate address:

1) The ecology of rodents is known to be a direct determinant of pathogen dynamics in rodent populations, yet there are little contemporary data on the characteristics of urban rat populations, and many studies of RAZ do not account for the ecology of the rat population under study. This hinders the development of a comprehensive understanding of pathogen ecology in rat populations, prevents comparability and synthesis of results generated by different studies, and can lead to erroneous conclusions with regard to RAZ dynamics.

2) It is known that the ecology of urban rats and RAZ are strongly influenced by features of the urban environment (i.e., the availability of food and harborage); however, the specific environmental factors that exert the most significant impacts on rat populations have yet to be identified and characterized. Additionally, there
are no well-described tools or methodologies for conducting an in-depth evaluation of the relationship between urban rats and their environment.

3) Urban rats are known to be the natural reservoir for a variety of zoonotic bacteria (e.g., *Leptospira interrogans*, *Rickettsia typhi*, certain *Bartonella* spp., *Streptobacillus monilliformis*) and viruses (e.g., Seoul hantavirus, SEOV). However, there is little data on the ecology of these pathogens in urban rat populations. This is problematic given that the ecology of RAZ in rats is a direct determinant of the risk of pathogen transfer to humans, and given that an understanding of pathogen ecology in the reservoir host is essential to developing efficient and effective strategies to monitor and mitigate disease emergence.

4) Much of the study on urban RAZ has been narrowly focused on the aforementioned pathogens, for which rats have long been recognized to be an important source. There has been little research to determine if rats could be the source of other ‘unexpected’ zoonotic or potentially zoonotic organisms; thus the true extent of zoonotic risks associated with urban rats remains poorly characterized.

1.6 Objectives and Aims

In order to address these knowledge gaps, the Vancouver Rat Project was developed. The overarching goal of this project was to begin to characterize rat-associated health risks in the DTES. Specifically, the research focused on the following objective and aims:

Objective 1: To gather together what is already known about rat and RAZ ecology.

Aim 1: To identify and synthesize the published literature regarding RAZ ecology with a focus on rats and RAZ in urban centres. This aim is addressed in Chapter 2 (Literature Review).
Aim 2: To develop a preliminary understanding of the characteristics and significance of rat infestations in Vancouver by gathering the experiential knowledge of pest control professionals. This aim is addressed in Chapter 3 (Using Experiential Knowledge to Understand Urban Rat Ecology: A Survey of Canadian Pest Control Professionals), which presents the results of a survey given to Canadian pest control professionals to gather their professional opinions on a variety of topics related to rats and rat infestations. These topics included frequency and severity of rat infestations in different settings/locations, perceived factors that promote rat infestations, perceived health risks posed by rats, and rat control methods.

Objective 2: To characterize the ecology of rat populations within the DTES.

Aim 1: To describe the characteristics of rat populations within the study area with a focus on factors that could influence the ecology of zoonotic pathogens or our understanding of that ecology. This aim is addressed Chapter 4 (The Characteristics of Wild Urban Rat Populations with a Focus on Factors Critical to the Understanding of Rat-Associated Zoonoses), which describes the trapping study that formed the basis for this project. Included here is a description of the characteristics of the rat populations under study, including rat population density, distribution, and demographic characteristics, reproductive seasonality, factors influencing body mass and inter-specific aggression, and how trapping methodology might influence the characteristics of the trapped population (vs. the source population).

Aim 2: To begin to understand how the environment might influence RAZ ecology by identifying features of the urban microenvironment that influence rat abundance. This aim is addressed in Chapter 5 (A Mixed Methods Approach to
Exploring the Relationship Between Norway Rat (*Rattus norvegicus*) Abundance and Features of the Urban Environment), which describes the development and implementation of a tool that characterizes and quantifies features of the microenvironment within a city block (e.g., land use, building disrepair, exposed garbage, presence of vegetation, etc.). Included in this chapter, as well, is a description of how the data collected by the tool were used to understand the relationship between environmental factors and rat abundance within the study area.

**Objective 3: To characterize the ecology of zoonotic or potentially zoonotic pathogens in DTES rat populations.**

**Aim 1: To identify the presence and characterize the ecology of ‘known’ RAZ (i.e., zoonotic pathogens commonly associated with rats) within DTES rats, including *Leptospira interrogans*, *Rickettsia typhi*, *Bartonella* spp., *Seoul hantavirus*, and bacteria associated with rat bites.** In Chapter 6 (Ecology of *Leptospira interrogans* in Wild Urban Norway Rats), the ecology of *L. interrogans* in DTES rat populations is described. In Chapter 7 (No Evidence of Infection with *Seoul hantavirus*, *Bartonella* spp., and *Rickettsia typhi* in Wild Urban Norway and Black Rats), the apparent absence of *Rickettsia typhi*, *Bartonella* spp., and SEOV in DTES rat populations is discussed. In Chapter 8 (Bacteria Isolated from Conspecific Bite Wounds in Norway and Black Rats: Implications For Rat Bite-Associated Infections in People), the bacteria that could be transmitted to people through rat bites was investigated by studying intra-specific bite-related wound infections in rats, and management of rat bite wounds in people is reviewed.

**Aim 2: To determine if urban rats carry the potentially zoonotic organisms methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*,**
with which they are not traditionally associated. This aim is addressed in Chapter 9 (Carriage of Methicillin-Resistant *Staphylococcus aureus* by Wild Urban Norway Rats), in which the presence and ecology of MRSA in DTES rats is described, while Chapter 10 (Carriage of *Clostridium difficile* by Wild Urban Norway and Black Rats) describes the presence and ecology of *C. difficile* in DTES rats.

1.7 Study Design

Preliminary Work

A systematic review of the published literature was conducted to gain a preliminary understanding of rats and RAZ. Subsequently, in order to gain a better understanding of rats and rat infestations in Canada, a survey was used to collect the professional opinions of Canadian pest control professionals (PCPs), a group that likely has the most practical experience with rats and rat infestations in urban areas. Collaborations with members of the British Columbia Structural Pest Management Association also informed the design of the trapping study, as local PCPs were able to provide advice regarding the most efficient and effective trapping methodologies.

Fieldwork and Rat Ecology Study

The fieldwork, which formed the base for the project as a whole, involved extensive trapping of rats throughout the DTES over the course of one year. A variety of data were collected in the field, including the location and date where each rat was trapped, as well as the morphometric and demographic features of trapped rats (e.g., species, mass, sex, sexual maturity, etc.). Data regarding the environmental characteristics of each city block included in the study were also collected.
Analysis of data gathered through the trapping study resulted in a better understanding of the ecology of rat populations in the DTES, and informed subsequent studies of RAZ ecology. Additionally, the trapping study, which used a trap-removal methodology (i.e., trapped rats were euthanized and collected vs. set free), facilitated the collection of samples for pathogen testing.

**Laboratory Work and Zoonotic Pathogen Studies**

Each trapped rat underwent a full post-mortem examination. This involved collecting a variety of data, including volume of body fat, pregnancy status, etc. It also involved a standardized tissue collection procedure. Blood and tissue samples were subsequently tested for the presence of zoonotic pathogens (or antibody against those pathogens), including pathogens traditionally associated with rats (based on the literature review) and those with zoonotic potential but not previously identified in rat populations. Spatial and statistical analyses were used to develop an understanding of RAZ ecology in DTES rats.

1.8 Summary

This dissertation consists of ten chapters. Chapters 2 and 3 detail the preliminary work described above. Chapter 2 is a review of the published literature regarding RAZ ecology with a focus on describing the zoonotic pathogens that have been associated with rats in the past, and on identifying common themes among the ecologies of those zoonoses. Chapter 3 describes the results of a survey to gather the professional opinions of PCPs regarding rats and rat infestations. Chapters 4 and 5 describe the results of the rat ecology study. Specifically, Chapter 4 described the field-work and the characteristics of DTES rat populations, while Chapter 5 describes the environmental factors that appear to influence rat abundance within the DTES. Finally, Chapters 6-10
describe the laboratory work and the results of the rat disease analysis. Chapters 6-8 describe the presence (or absence), as well as the ecology of zoonotic pathogens traditionally associated with rats, while Chapters 9 and 10 describe carriage of potentially zoonotic pathogens that have not previously been identified in urban rat populations.
Chapter 2: Literature Review

2.1 Synopsis

Urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) are the source of a number of pathogens responsible for significant human morbidity and mortality in cities around the world. These pathogens include zoonotic bacteria (*Leptospira* spp., *Yersina pestis, Rickettsia typhi, Bartonella* spp., *Streptobacillus* spp.), viruses (Seoul hantavirus, hepatitis E virus), and parasites (*Angiostrongylus cantonensis*). A more complete understanding of the ecology of these pathogens in people and rats is critical for determining the public health risks associated with urban rats, and for developing strategies to monitor and mitigate those risks. Although the ecology of rat-associated zoonoses is complex, due to the multiple ways in which rats, people, pathogens, vectors, and the environment may interact, common themes can still be identified. This review summarizes the ecology of zoonoses associated with urban rats with a view to identifying similarities, critical differences, and avenues for further study.

2.2 Introduction

Urban rats (*Rattus* spp.) are known to be the source of a variety of zoonotic pathogens responsible for significant human morbidity and mortality in cities around the world. Although there are a number of published papers regarding RAZ, the results of these studies have yet to be comprehensively collated and synthesized; therefore the current state of knowledge regarding RAZ in urban centres remains obscure.

The objective of this review was to summarize, evaluate, compare, and contrast the peer-reviewed and published literature regarding the ecology of RAZ in Norway rats (*Rattus norvegicus*), black rats (*Rattus rattus*), and people living in urban centers,
with a view to synthesizing what is already known, and identifying knowledge gaps to address in the future.

2.3 Methods

Databases utilized included: Medline, Embase, Web of Science, BIOSIS Previews, and Zoological Record Plus. Text word searches (including ‘wildcards’ to capture term variations, e.g., zoono*) were conducted using keywords pertaining to rats (rat, rats, Rattus rattus, Rattus norvegicus, Norway rat*, brown rat*, black rat*, roof rat*), rat-associated zoonoses (zoono*, zoonotic disease, leptospi*, Weil’s disease, hanta*, hantavirus, Seoul hantavirus, Seoul virus, hemorrhagic fever with renal syndrome, plague, Yersin*, Rickettsia, typhus, murine typhus, Streptobacillus monilliformis, rat bite fever, Haverhill fever, Bartonell*, Salmonell*, Campylobacter*, E. coli, hepatitis E virus), and the urban environment (urban, city, cities, metropol*). Groups of keywords were combined using Boolean operators. Medline and Embase were also searched using Medical Subject Headings (rats, zoonoses, urban health, urban populations, and cities). Papers in languages other than English were excluded. The literature search was conducted between December 2011 and February 2012 and identified a total of 704 papers for initial consideration.

To ensure that the review was focused on the most up-to-date research, all papers published prior to 1990 were excluded (n = 209). Remaining papers (n=495) were organized according to the amount of information they contributed regarding the ecology of zoonotic pathogens associated with Norway and black rats. Papers with significant ecologic content (e.g., reviews, observational studies, modeling studies, large case series, etc.) were retained (n = 213) and all other papers (e.g., case studies, studies with low sample size, studies focused exclusively on pathogenesis, pathogen genetics, treatment methodology, etc.) were excluded (n = 282). For this step, papers were
screened by two reviewers (CG Himsworth and KL Parsons), and decisions (inclusion vs. exclusion) were arrived at by consensus. Finally, papers not focused on Norway and black rats in urban centers (e.g., studies with a primarily rural focus, studies focused on other rodent species, etc.) were excluded (n = 71). Additional sources (n = 19) were added through citation searching and to fill specific information gaps. A total of 161 papers were reviewed in detail.

Data from these papers was extracted and synthesized based on the methodology for narrative synthesis (25). The goal of narrative synthesis is to identify themes common across research regarding a particular subject, which can then be used to identify commonalities and critical differences among included papers.

2.4 Results

2.4.1 Overview of Rat-Associated Zoonoses

Bacterial zoonoses

*Leptospira interrogans*

*Leptospira interrogans*, a gram-negative spirochete bacterium, is considered to be the most widespread zoonotic pathogen in the world (26). Different strains (serovars) are adapted to different mammalian hosts (10, 27, 28), and although almost any animal can be a *Leptospira* spp. carrier, rats are the most common source of human infection, particularly in urban environments (26-29, 29). The serovars most commonly associated with rats are Icterohemorrhagiae and Copenhageni (28).

In the animal reservoir, *Leptospira* spp. colonizes the kidneys and is shed in the urine (27, 28). People may become infected through direct contact with rat urine or indirectly through contact with contaminated soil or water (10, 26, 27, 30). Leptospirosis (i.e., infection with *Leptospira* spp.) most commonly causes an undifferentiated febrile illness (10), but may progress to Weil’s disease, a syndrome characterized by jaundice,
hemorrhage, and renal failure (10, 28, 30) with a mortality rate of 5-15% (29-31).

*Leptospira* spp. is also associated with a pulmonary hemorrhage syndrome (10, 28) that has a mortality rate of up to 50% (28). Some studies have suggested that rat-associated serovars may be more pathogenic to humans compared with serovars from other animal hosts (32), while others have found this not be the case (33).

Although *Leptospira* spp. is ubiquitous in rat populations (10, 27, 28, 30), human disease is most common in the tropics (10), particularly in Southeast Asia, Oceania, the Indian subcontinent, the Caribbean, and Latin America (26).

**Yersina pestis**

*Yersinia pestis*, a gram-negative bacillus (34, 35), is the etiologic agent of the plague. Norway and black rats are the most common source of the pathogen in cities (36), and the bacterium is spread between rats, and from rats to people, via fleas (35). *Xenopsylla cheopis* (the oriental rat flea) is the classic vector in urban centers (30, 35-37), although several flea species, particularly those in the genus *Xenopsylla*, can be competent vectors (35, 38). While feeding on an infected rat, fleas ingest the bacteria, which subsequently proliferate and form a blockage in the digestive tract (35, 37). This blockage prevents blood meals from entering the stomach resulting in starvation, which causes fleas to bite repeatedly and regurgitate bacteria into subsequent hosts (35, 37).

When an infected flea bites a human, the bacteria spread to local lymph nodes and replicate, causing them to swell and create the characteristic bubo (34, 35), from which bubonic plague gets its name. This is followed by systemic spread of the bacteria, with replication in the internal organs (35). Clinically, bubonic plague is characterized by headache, chills, fever, and malaise, which may progress to sepsis and death (34). Mortality rates may reach 20% in developing countries where access to health care is minimal (39). While the bubonic form of plague is most common, the pneumonic form
(associated with person-to person aerosol transmission) may occur during the height of an outbreak (39, 40).

Although rats are an important reservoir for human infection (35, 36, 41, 42), plague is lethal to rats themselves; therefore they are not thought to maintain *Y. pestis* in nature (35). Some researchers have suggested that more resistant rodent species (e.g., mice and voles) are required to form enzootic foci, and that it is the transmission of plague from these enzootic hosts to susceptible rat populations that results in explosive outbreaks and human infection (35). Persistence of plague in a particular location depends upon the presence of suitable maintenance hosts, as well as a climate conducive to flea activity and bacterial replication (35), which may be the reason why the distribution of plague is limited compared to other RAZ. The most significant foci of enzootic plague are located in Africa, Southeast Asia, and South America (35). These countries also have the highest incidence of human infection (35).

**Rickettsia typhi**

*Rickettsia typhi* is a gram-negative obligate intracellular bacterium of white blood cells (43). It is believed to be present in rat populations worldwide (43, 44) and is transmitted among rats, and from rats to people, by fleas, particularly *X. cheopis* (30, 43). After the flea feeds on an infected rat, *R. typhi* multiplies in the flea’s intestinal tract and is shed in the feces (43). Infection occurs when flea feces are inoculated into the skin through the flea bite or via scratching (43). Unlike *Y. pestis*, *R. typhi* does not adversely affect the flea and may even be transmitted vertically from the infected flea to its offspring (44).

*R. typhi* causes murine typhus in people, which is generally a self-limiting disease (43, 44) characterized by fever, headache, lethargy, myalgia, arthralgia, nausea,
vomiting, and a characteristic skin rash (43, 45). Mortality is approximately 1% and 4%, with and without appropriate antimicrobial therapy, respectively (43, 44).

**Bartonella spp.**

*Bartonella* spp. is a gram-negative red blood cell-associated coccobacillus (46, 47). This rapidly expanding genus currently has 30 characterized species, with more being discovered with each study (46, 48). A number of mammalian species can serve as reservoirs, and different species of the bacterium appear to be adapted to different hosts. Norway and black rats can be infected with several related species including *B. elizabethae, B. trobocorum, B. rochalimae, B. phocensis,* and *B. rattimassiliensis* (49), and simultaneous infection with multiple species is not uncommon (50, 51).

Rat populations worldwide are thought to be infected with *Bartonella* spp. (46, 47), however the degree to which rats are a source of infection for people is not known (30). Thus far, there have been a limited number of published cases of human disease attributed to rat-associated *Bartonella* spp. (47), including cases of endocarditis and neuroretinitis associated with *B. elizabethae,* and febrile illness attributed to *B. rochalimae* (46, 48). Not much is known about the source of these human infections (47).

It is suspected that *Bartonella* spp. is transmitted among rats and from rats to people by arthropod vectors, particularly fleas (52). Rat-associated *Bartonella* spp. has also been identified in lice, mites, and ticks (52-54); however, the role of these vectors in *Bartonella* spp. ecology remains to be determined. Some studies have also suggested that *Bartonella* spp. may be transmitted vertically from infected rodents to their offspring (48).
**Streptobacillus monilliformis**

*Streptobacillus monilliformis*, a pleomorphic gram-negative bacillus (55, 56), is part of the normal flora of the rat oropharynx, and is thought to be present in rat populations worldwide (30, 55, 56).

*S. monilliformis* can be transmitted to people through the bite of an infected rat, which causes the aptly named rat bite fever, and through ingestion of food contaminated by rats, which causes Haverhill fever (30, 55). Infection with *S. monilliformis* in people manifests as fever, headache, chills, vomiting, skin rash, and polyarthritis, although pharyngitis and vomiting may be more pronounced with Haverhill fever (30, 55, 56). If left untreated, *S. monilliformis* infection can progress to septicemia and the mortality rate is 7-13% (30, 55, 56).

**Other bacteria**

Rats are capable of carrying and shedding *Escherichia coli* (57-59), *Salmonella* spp. (57, 60), and *Campylobacter* spp. (57), all of which are important causes of gastrointestinal disease in people. Additionally, studies have shown that rats are frequently colonized by antibiotic-resistant strains of these bacteria (57-60), although the public health significance of these findings remains unknown. Rats are also competent hosts for several *Borrelia* spp., and may contribute the ecology of urban Lyme disease, particularly in Eurasia (61-63).

**Viral zoonoses**

**Seoul hantavirus**

Seoul hantavirus (SEOV) is an RNA virus of the Genus *Hantavirus*, Family *Bunyaviridae* (12, 30). Rats are the primary reservoir for SEOV (30), which is shed in the urine, saliva, and feces (64). The virus is spread among rats through environmental
contamination, social contact, and intraspecific aggression (e.g., biting) (65). In male
Norway laboratory rats, infection with SEOV actually increased aggressive behaviour,
thus facilitating virus transmission (Klein et al. 2004). Transmission of the virus from
rats to people is thought to result from aerosolization and inhalation of rat excreta,
although exposure via contaminated food or fomites is possible (12).

Seoul hantavirus is one of several rodent-associated hantaviruses that cause
hemorrhagic fever with renal syndrome (HFRS) (66). Approximately 20% of all HFRS
cases are thought to be due to SEOV infection, while 70% are due to Hantaan virus, for
which the striped field mouse (Apodemus agrarius) is the reservoir, and the remaining
10% are due to other hantaviruses (12). In some areas, however, (e.g., Huludao, China)
SEOV is the most common cause of HFRS (67).

HFRS is characterized by fever, myalgia, and headache, progressing to
multisystemic hemorrhage and renal failure (12, 66, 68). The mortality rate for HFRS is
5-10% (12), and survivors may develop chronic renal impairment upon recovery from
the acute phase (66). Infection with SEOV may also lead to hepatic dysfunction not
observed with other hantavirus species (65, 66, 68)

Although SEOV is thought to have a worldwide distribution in rat populations
(12, 30, 68), HFRS is largely limited to Asian countries, including China, Russia, and
Korea (12, 68). That being said, human exposure to SEOV (as evidenced by
seropositivity) has been documented in countries outside of Asia (69) and, in the USA,
seropositivity has been associated with proteinuria and hypertensive renal disease (66,
70, 71).

Other viruses

Hepatitis E virus (HEV) is a recently recognized viral zoonosis (72) and a
common cause of acute hepatitis in people (73). Although pigs are thought to be the
primary reservoir (72), studies have shown that rats may also be HEV carriers (72, 74, 75). New research, however, has shown that HEV in rats is only partially related to that found in humans and pigs (76). Additionally, rat HEV does not appear to be transmissible to non-human primates, and human HEV does not appear to be transmissible to rats (76), suggesting that rat HEV is not zoonotic.

Rats are increasingly being investigated as a source of emerging viruses. For example, one researcher suggested that the severe acute respiratory syndrome (SARS) virus may have been spread by black rats during a 2003 outbreak in a Hong Kong apartment complex (77).

Parasitic zoonoses

*Angiostrongylus cantonensis*

Norway and black rats are the main reservoirs for this parasitic nematode, the adults of which reside in the pulmonary arteries (78, 79). Eggs are passed in the feces and develop to an infectious stage in a mollusk intermediate host (i.e., land snails or slugs) (79). Humans can become infected by consuming the intermediate host, a paratenic host (i.e., a host that is not needed for parasite development but that can aid in maintenance or transmission, such as amphibian or crustacean that consumed the intermediate host), or vegetation contaminated by mollusk mucus (79). Once ingested by a person, the larvae migrate to the central nervous system or eyes where they cause eosinophilic meningitis or ocular angiostrongyliasis (78, 79). The parasite is primarily found in Southeast Asia, the South Pacific, Australia, and the Caribbean, although endemic foci can also be found in Africa, the USA, and South America, and cases may occur sporadically in other areas (e.g., Europe) (78-80).
Other parasites

Rats are the reservoir for the zoonotic tapeworms *Hymenolepis* spp. and *Rodentolepis* spp., which reside in their intestinal tract (81-83). Larval stages can be transmitted among rats, from rats to humans, and, occasionally, among humans, via the feces or through consumption of an arthropod intermediate host (84, 85). In people, the parasite may develop in the intestine and cause enteritis (84-86). Rats are also a reservoir for *Capillaria* spp., a nematode that infects the rat liver (81, 82, 87). Eggs are released into the environment upon the death of the rat, or through the feces of a predator that consumes an infected rat (88). Infection in humans is usually asymptomatic but can result in hepatitis (88).

Although not a direct source of infection for people, rats are an intermediate host for *Toxoplasma* spp. and can serve as a source of infection for cats and other animals, which, in turn, are a source of infection for people (89-91). Rats are also being investigated as a potential reservoir for zoonotic *Cryptosporidium* spp., a gastrointestinal parasite of people (92).
Table 2-1: A summary of the main zoonotic pathogens associated with Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) in urban centers.

<table>
<thead>
<tr>
<th>Zoonotic organism</th>
<th>Associated disease in rats</th>
<th>Associated disease in people</th>
<th>Method of transmission between rats</th>
<th>Method of transmission from rats to people</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leptospira interrogans</em></td>
<td>None</td>
<td>Febrile illness, Weil’s disease (jaundice and renal failure), pulmonary hemorrhage</td>
<td>Direct or indirect contact with urine</td>
<td>Direct or indirect contact with rat urine</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>Yersinia pestis</em></td>
<td>Septicemia (often fatal)</td>
<td>Bubonic plague (febrile illness, lymphadenitis, sepsis)</td>
<td>Fleas</td>
<td>Fleas</td>
<td>Primarily Asia, Africa, and South America</td>
</tr>
<tr>
<td><em>Rickettsia typhi</em></td>
<td>None</td>
<td>Febrile illness, rash</td>
<td>Fleas</td>
<td>Fleas</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>Bartonella spp.</em></td>
<td>None</td>
<td>Febrile illness, endocarditis, neuroretinitis</td>
<td>Fleas</td>
<td>Fleas</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>Streptobacillus monilliformis</em></td>
<td>None</td>
<td>Haverhill fever and rat bite fever (febrile illness, rash, polyarthritis, pharyngitis, sepsis)</td>
<td>Close contact</td>
<td>Direct contact with rats or ingestion of food contaminated by rats</td>
<td>Worldwide</td>
</tr>
<tr>
<td><em>Seoul hantavirus</em></td>
<td>None</td>
<td>Febrile illness, hemorrhagic fever with renal syndrome (hemorrhage, shock, renal failure)</td>
<td>Contact with urine, feces, or saliva</td>
<td>Direct or indirect contact with rat urine, feces, or saliva</td>
<td>Worldwide (human disease primarily in Asia)</td>
</tr>
<tr>
<td><em>Angiostrongylus cantonensis</em></td>
<td>Granulomatous pneumonia</td>
<td>Febrile illness, eosinophilic menigitis, ocular angiostrongyliasis</td>
<td>Indirect. Consumption of mollusk intermediate host.</td>
<td>Indirect. Consumption of intermediate host, contaminated vegetation, or paratenic host</td>
<td>Primarily SE Asia, South Pacific, Australia, and the Caribbean, occasionally found elsewhere</td>
</tr>
</tbody>
</table>
2.4.2 Common Epidemiologic Themes for the Above Zoonoses

The changing face of RAZ

The frequency and distribution of RAZ has changed over time in association with changes in human populations. In particular, increasing urbanization and urban poverty have resulted in the emergence, or re-emergence, of RAZ in urban centers. For example, although leptospirosis has long been considered a primarily rural or occupational disease, the incidence of urban leptospirosis is increasing (26, 29, 93). Similarly, the incidence of SEOV HFRS in Chinese cities is increasing, and this increase has been attributed to urban population growth, in combination with concomitant increases in urban rat populations and rat-human contact (67). Additionally, several African cities, particularly in Madagascar, have experienced a re-emergence of urban plague since the 1980s (39, 94).

Interestingly, some zoonoses that have historically been associated with urban centers are now moving farther afield. In the USA, for example, murine typhus, although still present in many urban centers (44, 95-97), is shifting to a suburban focus involving cat fleas and opossums (43, 44). In New Zealand, *R. typhi* infection in people is most strongly associated with exposure to rats at rural holiday homes (45).

The role of climate, season, and weather in RAZ

Climate, season, and weather play strong roles in the ecology of many RAZ by influencing human exposure to zoonotic organisms and through their effect on the biology and ecology of rats, vectors, and pathogens. In the case of *Leptospira* spp., heavy precipitation and flooding facilitate dispersal of leptospires in the environment, increasing human contact with the bacterium (26). Leptospirosis is, thus, most common in tropical areas with high annual rainfall (31), and the incidence of disease is highest in
the wet season (29, 98). As well, studies of urban leptospirosis have shown that cases are associated with proximity to water bodies and flood plains (26, 27, 99-103), and that people suffering from the disease report increased contact with flood water compared to those not affected (29).

The incidence of SEOV HFRS in China is highest in April and lowest in September (67), which is thought to reflect seasonal differences in rat abundance and rat-human contact (67). Annual temporal variation in plague transmission, on the other hand, is most likely a result of seasonal changes in flea population dynamics (42, 94, 104)). Similarly, murine typhus cases occur predominantly during warm weather, which promotes high flea populations (43).

*Yersina pestis*, itself, requires sufficient ambient heat in order to activate genes that cause it to block the flea gut and trigger the chain of events required for transmission (94). Should the temperature be too hot (i.e., >28°C), however, fleas can clear the blockage and will not transmit the bacterium (35). This may be one of the reasons why plague epidemics tend to subside at high temperatures (35).

What remains to be determined is the potential impact of climate change, particularly since the incidence and distribution of other rodent-associated zoonotic diseases, such as Lyme disease, is changing rapidly in association with changing climate (105, 106). Climate change could influence the ecology of RAZ in a variety of ways, for example by precipitating changes in pathogen distribution, vector abundance, transmission pathways, and pathogen prevalence/load in rats and vectors (106).

**Ecology of RAZ in rat populations**

An understanding of the ‘behaviour’ of zoonotic pathogens in rat population is crucial for identifying which rats or rat populations pose the greatest health risk for people. For example, for an individual rat, the probability of infection with *Leptospira*
spp., SEOV, and hepatitis E virus, increases with age (10, 107-109), likely because of increased opportunity for exposure. For SEOV, in particular, infection and viral shedding is most common in old, large male rats (110), because intraspecific aggression appears to play an important role in virus transmission (69). At a population level, pathogen prevalence may decrease at the height of juvenile recruitment, which is the time when the greatest number of young, uninfected rats leave the nest and enter the population (111). Overall, the above suggests that well established, mature, and stable rat populations may pose the greatest health risk to people.

That being said, the impact of externally imposed changes in rat population dynamics (e.g., large scale poisoning or trapping campaigns) on the ecology of RAZ is unclear. For other infectious diseases in wild populations, control methods based on culling animals may actually cause an increase in disease prevalence by disrupting normal population ecology and thereby enhancing disease transmission (112). Anthropogenic changes in rat populations may have similar unintended effects. For example, previous plague epidemics have revealed that large rat-die offs consistently precede human casualties (36, 37, 40, 104). This is a result of the fact that X. cheopis (the primary plague vector) prefers to feed on rats but will feed on humans (thus transmitting the disease) when the rat population collapses (37). Thus culling of rats during a plague outbreak may actually increase the incidence of disease in people by removing the rat flea’s preferred host (37, 113).

**Geographic distribution of RAZ**

The prevalence of many zoonotic pathogens in rat populations is highly variable among cities. Studies of *Leptospira* spp. in Norway rats (using culture) have shown that the prevalence of infection was 80% in Salvador, Brazil (114), 17% in Tokyo, Japan (115), 21% in Medillin, Columbia (116), and 96% in Buenos Aires, Argentina (117). Similarly,
the prevalence of *Bartonella* spp. infection in black rats (using culture and/or PCR) was 43% in Nepal (118), 24% in Israel (119), and 32.3% in Bangladesh (120). There is also marked variation in prevalence of infection among cities in the same country (111), and even among different locations within a city (107, 115). For example, a study of *Leptospira* spp. in Copenhagen, Denmark, showed that, despite robust rat populations in all locations sampled, one location had no positive animals, while the prevalence in other locations ranged from 48-89% (107). In Buenos Aires, Argentina, the prevalence of SEOV seropositive rats ranged from 0 – 26.1% at different sites within the city (110). Variation in pathogen prevalence even over a short geographic distance suggests that site-specific prevalence of zoonoses in rat populations may be more relevant from a public health standpoint than aggregated city- or country-wide measures.

The reasons for this heterogeneity in pathogen prevalence are unclear but could be attributable to limited rat homeranges, with disease transmission occurring primarily among family groups (121). Alternatively, features of the physical microenvironment in which rats reside (e.g., land use, availability of soil and vegetation, presence of water bodies etc.), may affect pathogen prevalence through their effect on rat, vector, or pathogen ecology (122). Finally, it is possible that some of these differences may be artifactual (i.e., due to variation in study methodologies).

This later point is particularly problematic when comparing studies documenting zoonotic pathogen exposure in people. For example, the prevalence of SEOV exposure in humans (as evidenced by seropositivity) in the USA varied from 34-74% in Baltimore, to 31-41% in New Orleans, to 5-9% in Houston, to 0-3% in San Francisco (69). However, marked variation in the composition of study populations, in combination with a lack of information on or adjustment for demographic, behavioral, and socio-economic characteristics, hinders study comparability and identification of the precise reasons behind this variation.
Risk factors for infection and disease in people

Although many of the reasons behind geographic variations in the prevalence of RAZ remain unclear, it is possible to identify risk factors for exposure to these pathogens in people. Among the most important of these risks factors is country of origin. Both epidemic and endemic rat-associated zoonotic diseases are more common in developing vs. industrialized nations (26). This may be partially due to the climate in developing countries, which tends to be warmer and wetter, and thus more conducive to the transmission of RAZ (31). Perhaps the stronger determinant, however, is a higher rate of urban poverty in these areas (28, 29, 123). For example, during an outbreak of leptospirosis in Salvador, Brazil, people residing in urban slums were four times more likely to get leptospirosis compared to non-slum dwellers (29). Additionally, people living in poor, densely populated areas of Madagascar are more likely to get bubonic plague compared to the general population (104). These findings are hardly surprising given that impoverished urban populations experience inadequate housing, infrastructure, and sanitation, which promote rat infestations, enable close contact between rats and people, and facilitate pathogen transmission (42). Additionally, impoverished populations often have limited access to medical care, hindering zoonotic disease diagnosis and treatment (30).

Paradoxically, in Peru, populations with the highest rate of exposure to Leptospira spp. had comparatively low rates of disease, suggesting that prior exposure to the bacterium leads to protective immunity (123). Barcellos et al. (2001) suggest this as the reason why people residing in the highest risk areas for Leptospira spp. infection in Rio de Janeiro, Brazil, were actually less likely to develop clinical leptospirosis during an outbreak.

Even within developed countries, urban poor may be at an increased risk for exposure to and infection with RAZ because of declining infrastructure (i.e., urban
decay), poor standards of living, inadequate hygiene, IV drug use, homelessness, and immunosuppression (e.g., HIV/AIDS; (96)). For example, Childs et al. (1992) found that 16% of people from an inner city population in Baltimore, USA, had been exposed to *Leptospira* spp. and that seropositivity was associated with low income and African American ancestry. Additionally, there have been several cases of clinical leptospirosis in homeless people from Baltimore, all of which were associated with exposure to rats in alleyways (124). However, two studies of SEOV exposure in Baltimore IV drug users showed that the prevalence of antibody against the virus was low (<1%) and not significantly different from that found in people visiting the emergency room or a sexually transmitted disease clinic (who were assumed to be at lower risk of exposure) (125, 126).

Although impoverished populations may be at increased risk, people have the potential to acquire RAZ regardless of their socioeconomic status. For example, a study in Tokyo, Japan, identified at least 13 cases of autochthonous Weil’s disease (i.e., acquired in Japan vs. another country) in non-impoverished individuals between 2002 and 2008 (115).

Finally, it is important to note that it is the home (vs. work or social) environment that appears to play the most important role in determining an individual’s risk of exposure to RAZ (32, 127). For example, studies have shown that seeing rats around the household, particularly groups of rats, is an independent risk factor for *Leptospira* spp. exposure and infection (29, 101, 102, 128).

One question yet to be addressed is whether immunosupression could significantly affect the risk of RAZ. For example, it has been established that HIV infection can predispose individuals to infections with a variety of zoonotic agents associated with other animal species, such as *Mycobacterium bovis*, *Cryptosporidium* spp., and *Giardia* spp. (129). Given the variety of conditions that can lead to
immunosupression, from HIV/AIDS to medical intervention for cancer and rheumatologic disease, the degree to which these conditions influence the risk of RAZ in urban populations warrants further study.

Burden of disease in people

Although RAZ represent a diverse group of organisms, their clinical manifestations are strikingly similar and frequently non-specific (10, 26, 27). This lack of clinical specificity, in combination with lack of awareness among health care practitioners can lead to high rates of misdiagnosis and underdiagnosis (27, 31), particularly in developed countries where these diseases are less common (10, 97, 124). A lack of access to reliable diagnostic tests in many laboratories may further hinder the detection of RAZ (39), (55, 56). Finally, in many cases, the patients themselves are unaware of rat or vector exposure, which means that a detailed history may not be helpful in making the diagnosis. For example, many people with murine typhus do not recall being bitten by fleas (43, 45, 97) and a significant proportion of those with so-called rat bite fever do not recall having contact with a rat (55, 56).

Underdiagnosis and misdiagnosis is problematic as it may lead to delayed or inappropriate treatment, causing increased mortality from otherwise survivable diseases (29, 39) and chronic debilitating illness in otherwise healthy working-age individuals (29, 31, 99). Underdiagnosis and misdiagnosis can also lead to underreporting of RAZ, making it difficult to estimate or deal with the health burden associated with these diseases. Under-reporting can also be a result of different infectious disease reporting systems. For example, leptospirosis was eliminated from the list of nationally notifiable diseases in the USA in 1995 (124, 130), whereas Germany implemented a mandatory reporting system for infectious diseases, including leptospirosis, in 2001(127).
Effect of RAZ in rats

Despite the range of clinical manifestations associated with RAZ in people, with
the exception of *Y. pestis* (35), all of the aforementioned pathogens cause asymptomatic
infections in rats (27, 28, 82, 131). Indeed, not only do infected rats appear clinically
normal, so close is the adaptation between many of these zoonotic pathogens and their
hosts that infection may not even result in tissue pathology or elicit a functional
immune response. For example, studies have found that there are no significant
differences in the microscopic appearance of tissues from rats with and without renal
*Leptospira* spp. infection (131) and many rats infected with *Leptospira* spp. or *Bartonella*
spp. have no detectible antibody response to the pathogen (114, 116, 132, 133). Similarly,
rats infected with SEOV can remain persistently infected even in the face of neutralizing
antibody (12). Indeed Easterbrook et al. (2007b) showed that SEOV can actually modify
the rat immune system to maintain infection and viral shedding. In the case of *S.
moniliformis*, the bacterium is considered to be part of the normal flora of the rat
oropharynx (55, 56).

One implication of inconsistent immune responses in rats is that, for those
wishing to study RAZ in their rodent hosts, the choice of diagnostic test is critical to the
validity and meaning of research findings. For example, given that many rats infected
with *Leptospira* spp. do not mount an antibody response (114, 116, 132, 133), the use of
serology could underestimate the prevalence of infection. Additionally, studies have
shown that many rats have *Leptospira* spp. antibody against serovars that are non-
homologous to those with which they are infected and/or serovars known to be
maintained in another, non-rat species (107, 114, 116, 132, 132), suggesting that
serostatus may be completely unrelated to infection status. In contrast, for SEOV, there
appears to be good concordance between serology and pathogen presence (by PCR or culture) (134).

### 2.5 Discussion

Overall, it is clear that urban Norway and black rats can pose a significant health risk to people through the transmission of zoonotic diseases, and that this risk is likely to increase with increased urbanization and urban poverty (e.g., because of increased habitat available for urban rats and increased contact between rats and disadvantaged people). It is also clear the ecology of RAZ is difficult to understand as it is based on the complex interactions of a number of factors, including rats, people, pathogens, vectors, and the urban environment. Although the literature to date has provided a solid foundation for our knowledge of RAZ, there are a number of gaps that require further consideration (Table 2-2). Only by developing a complete and comprehensive understanding of urban-rat-associated zoonoses will we be able to accurately estimate the health risks posed by urban rats, and develop informed and effective strategies to monitor and mitigate those risks.
Table 2-2: Conclusions from a review of the literature regarding the ecology of zoonotic pathogens associated with Norway rats and black rats (*Rattus norvegicus* and *Rattus rattus*) in urban centers.

<table>
<thead>
<tr>
<th>Knowledge gained from previous study of rat-associated zoonoses</th>
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</thead>
<tbody>
<tr>
<td>• The ecology of RAZ is changing in association with global urbanization and urban poverty.</td>
</tr>
<tr>
<td>• Climate, season, and weather influence the ecology of RAZ through their effects on distribution of and interactions among rats, vectors, and zoonotic pathogens, which ultimately influence human exposure to RAZ.</td>
</tr>
<tr>
<td>• The ecology of zoonotic pathogens in rat populations influences the risk of pathogen transmission between rats and people.</td>
</tr>
<tr>
<td>• The distribution and prevalence of RAZ appears to be heterogeneous, even over a short geographic distance.</td>
</tr>
<tr>
<td>• Developing nations and impoverished populations within developing and developed nations are at highest risk for rat-associated diseases.</td>
</tr>
<tr>
<td>• With the exception of plague, infection with zoonotic organisms does not cause clinical illness in rats, and immune responses to zoonotic organisms in rats may be variable or absent.</td>
</tr>
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<table>
<thead>
<tr>
<th>Remaining knowledge gaps</th>
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</thead>
<tbody>
<tr>
<td>• What is the true prevalence and distribution of RAZ in rats and people? Why does the prevalence of exposure to and infection with RAZ in rats and people vary among geographic locations?</td>
</tr>
<tr>
<td>• Is it possible to identify specific features of the urban microenvironment that influence RAZ prevalence?</td>
</tr>
<tr>
<td>• What is the true constellation of factors influencing RAZ ecology in rats and people? Can we develop a better understanding of how these factors interact to influence RAZ ecology?</td>
</tr>
<tr>
<td>• What is the impact of climate change or other environmental changes on the ecology of RAZ in urban centers?</td>
</tr>
<tr>
<td>• How do rat control methods impact the ecology of RAZ? Could epidemiologic information regarding RAZ be used to create targeted rat control measures geared to prevent zoonotic pathogen transmission, specifically?</td>
</tr>
<tr>
<td>• How does immunosuppression affect the risk of RAZ in people?</td>
</tr>
<tr>
<td>• What is the true health burden caused by RAZ in people?</td>
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Chapter 3: Using Experiential Knowledge to Understand Urban Rat Ecology: A Survey of Canadian Pest Control Professionals

3.1 Synopsis

Rats (*Rattus rattus* and *Rattus norvegicus*) are among the most prolific and widespread urban pest species in the world. However, there is relatively little contemporary data regarding the ecology of rats in urban centers. Practical constraints associated with field studies suggest the need for more efficient methods of data collection, one of which could involve pest control professionals, who have considerable experiential knowledge of urban rats. The objective of this study was to gather data regarding urban rat ecology through a survey of Canadian pest control professionals and to determine whether such a survey could be useful for the collection of ecological data regarding pest species. Survey results suggested that rat infestations fluctuate seasonally and that there are a variety of environmental factors that may attract rats, particularly exposed garbage, abandoned buildings, and compost. Respondents suggested that rat infestations are most frequent and/or severe in residential areas, commercial areas dealing with food, as well as in neighbourhoods of low socioeconomic status. The majority of respondents believed that rats pose at least a moderate health risk to the general public and to themselves, and they also believed that although poison baiting is the cheapest and easiest method of rat control, environmental modification is the most effective way to control rat infestations in the long term. Despite issues with low response rates, these results suggest that pest control professionals may be a valuable source of data regarding urban pest species.
3.2 Introduction

Despite the ubiquity of rats in urban centers, there is a relative paucity of contemporary data regarding urban rat ecology. A lack of understanding regarding urban rat infestations is problematic as it hinders the development of informed and effective rat control strategies as well as scientific assessments of rat-associated public risks (e.g., the risk of disease transmission from rats to people). The scarcity of research on this topic may be partially attributable to the considerable time and resources required to conduct field studies, suggesting the need for more efficient methods to collect ecological data on urban rats. We suggest that one of these methods should involve efforts to collect the knowledge and perspectives of pest control professionals (PCPs).

Pest control professionals are at the frontlines of rat control and have extensive experiential knowledge regarding urban rat infestations. This professional experience typically involves a variety of different situations over a significant period of time, a scenario that is particularly valuable because it is hard to reproduce experimentally. Additionally, PCPs have insight into relative efficacy of different rat control methods and into public perceptions regarding rats, giving them the potential to be source of information on a variety of rat-related topics. Pest control professional knowledge, however, is frequently overlooked in scientific circles, and, to our knowledge, there have been no previous studies attempting to collate and disseminate it. The aim of this study was to assess the feasibility of using PCPs as a source of ecologic data on urban rats, and to survey PCPs to collect their professional opinions regarding urban rat infestations and associated issues.
3.3 Methods

Two versions of the survey were used. For the first version, members of the Structural Pest Management Association (SPMA) of British Columbia, Canada, were invited to participate in a voluntary online survey between June 1 and September 30, 2011. The SPMA is the province’s official professional association for members of pest control industry who deal with pests infesting structures (vs. agricultural pests). Membership in the SPMA is voluntary and includes individuals involved in all aspects of the industry (i.e., technicians, company owners, retailers, etc.). The online survey consisted of 30 questions that focused on topics including: an individual’s roles in the pest control industry, their company’s rat control business, frequency and severity of rat infestations in different settings/locations, perceived factors that promote rat infestations, perceived health risks posed by rats, and rat control methods.

Low participation and completion rates for the first version of the survey led to the development of a second, shorter, paper-based version (see Appendix A), which was distributed at the Canadian Pest Management Association’s (CPMA) Annual meeting, March 8-10, 2012. The CPMA is the national professional organization for those involved in the structural pest management industry. All pest control professionals (PCPs) who attended the meeting were invited to participate in the survey (included in the registration package), which consisted of 17 questions covering roughly the same topics as the first version.

For both versions of the survey, survey deployment was preceded by an advertising campaign to increase awareness of the survey. For the first version, this included oral presentations at SPMA meetings, postings on the SPMA website homepage, and e-mails to SPMA members. For the second version, this included an article in a national pest control industry magazine and an oral presentation at the CPMA. These activities were undertaken in collaboration with SPMA and CPMA.
administrators and members, who were active participants in the generation and deployment of the survey.

3.4 Results

The two surveys shared nine identical questions. The online survey contained 21 distinct questions not included in the paper-based survey, while the paper-based survey contained eight distinct questions not included in the online survey. Eight questions from the online survey were excluded because of insufficient response rate or because they caused significant confusion. These primarily included questions pertaining to frequency/number of rat infestations over different periods of time and in specific geographic locations, which proved difficult to answer.

Differences in survey content, as well as varying degrees of survey completion, resulted in variation in the number of responses for each question. Given the variable response rates across survey items (i.e., changing the denominator), for ease of readability, responses are, for the most part, presented as proportions. Additionally, although quantitative summaries of responses are provided where appropriate, for some questions qualitative summaries are provided in order to increase clarity.

Respondent characteristics

Twenty-five individuals responded to the online survey. Of these, 10 individuals completed the majority of the questions. The remaining participants answered less than five questions and were excluded. Eight individuals responded to and completed the paper-based survey. All eight responses were included.

Responses from a total of 18 individuals were included in the analysis. Of these 18 individuals, there were 10 company owners, three managers, three technicians, one company president, and one technical support auditor. Respondents represented a
total of 17 companies, all but one of which were located within BC. One respondent indicated that s/he worked for the Manitoba branch of a national pest control company that also operates in BC. Of the 16 pest control companies identified that operate in BC, only three were located outside of the Greater Vancouver Regional District (GVRD). These three companies were located in Vancouver Island, Northern BC, and the Fraser Valley. The cities encompassed within the GVRD are Burnaby, Coquitlam, Delta, Langley, Maple Ridge, New Westminster, North Vancouver, Pitt Meadows, Port Coquitlam, Port Moody, Richmond, Surrey, Vancouver, West Vancouver and White Rock.

**Rat control business**

Fifty percent of the respondents indicated that less than 25% of their company’s business was associated with rats (vs. other pest species), while 44% of the respondents indicated 25-50% of their company’s business was associated with rats. Only one respondent indicated that as much as 50-75% of his/her company’s business was associated with rats.

Eighty three percent of respondents indicated that the number of rat infestations they respond to changes with the seasons. Fall was reported to be the busiest season with regards to rat control followed by winter, then spring, then summer. Thirty three percent of the respondents commented on this seasonal variation. They suggested that colder weather causes rats to move indoors, increasing contact between rats and people and leading to increased calls to PCPs.

**Factors influencing rat infestations**

To assess the perceived relative importance of factors influencing rat infestations, each factor was ranked on a scale from one to five, with one being “not important” and
five being “very important”. Overall perceived factor importance was determined by the percentage of the maximum score that that factor received. The factors perceived to be most important (≥ 80% of maximum score) were presence of exposed garbage (95%), abandoned buildings (93%), compost (87%), and building disrepair (80%). Factors perceived to be of moderate importance (60-80%) were presence of perishable food (71%), gardens (71%), low socioeconomic status of a neighbourhood (68%), parks (67%), presence of freshwater (65%), and high population density (64%). Factors perceived to be of relatively low importance (< 60%) were low human activity (i.e., areas not used by people) (53%), presence of saltwater (47%), and cracked pavement (43%).

**Rat infestations in different settings**

Respondents indicated that they responded to rat infestations more frequently in residential areas followed by commercial areas associated with food (e.g., restaurants and groceries). Commercial areas not associated with food and industrial areas were serviced less frequently.

Rat infestations were reported to be most severe (i.e., greater number of rats per infestation) in commercial areas associated with food, while infestations were reported to be less severe in commercial areas not associated with food and residential areas, followed by industrial areas. Respondents indicated that, in their experience, there were often less than 10 rats per rat infestation in all locations other than commercial areas associated with food, where infestations of 20-50 rats were more common.

**Perceived rat-associated health risks**

With regard to perceived health risks posed to the general public, 20% of respondents believed that rats pose a severe health risk, 47% believed that rats pose a moderate health risk, and 20% believed that rats pose a minimal health risk. Only one
respondent believed that rats do not pose any health risk to the general public. Respondents indicated that they believed that health risks may be exacerbated by indoor infestations and well established infestations (vs. new or small infestations), as well as the age and health status of individuals exposed to rats.

With regard to perceived health risks posed to PCPs, one of eight respondents believed that rats pose a severe health risk, four believed that rats pose moderate health risk, one believed that rats pose a minimal health risk, and two believed that rats pose no health risk. Again, respondents commented that they believed that health risks increase when dealing with indoor infestations.

**Public perceptions of rats**

Seven of eight PCPs who responded to a question regarding public perception of rats indicated that their clients were moderately to highly concerned about rat infestations on their property, while one respondent indicated that clients were only mildly concerned.

Fifty three percent of respondents indicated that their clients were primarily concerned about rat infestations because of perceived health risks, while 40% of respondents indicated that their clients were concerned about rats for aesthetic reasons (i.e., because clients found rats distasteful). One respondent indicated that s(he) did not know the reasons for client concern.

Six of seven respondents indicated that the general public is relatively ill-informed about rats, while one responded indicated that the general public is moderately knowledgeable about rats.
Rat control

Poison baiting was selected as the easiest and cheapest way to eliminate rat infestations by five of eight and seven of eight respondents, respectively. However, four of eight respondents indicated that poison baiting is the least effective way to eliminate rat infestations in the long term. Another four of eight respondents indicated that trapping and removal is least effective in the long term. PCPs commented that these two control methods are not effective in the long term because they deal with the results of a rat infestation (i.e., the rats themselves) rather than the cause (i.e., the factors promoting and sustaining the infestation).

Seventy five percent of respondents indicated that environmental modification is the most effective way to eliminate rat infestations in the long term. Respondents commented that environmental modifications (e.g., removal of rubbish and outdoor structures that could provide harborage for rats, “rodent-proofing” of buildings, etc.) are most effective because they remove the factors that promote and sustain rat infestations, thus cutting them off at the ‘source.’

Four of the respondents noted that the best way to eliminate rat infestations is to utilize a combination of different methods (e.g., poisoning and trapping) with environmental modification as the core of the control strategy. They also indicated that the most appropriate methods of rat control depend on specifics of each infestation. For example, one respondent indicated that the use of poison bait was inappropriate for indoor residential situations, perhaps because of the possibility that people or pets could come into contact with the bait. PCPs also recommended that a post-management program be instituted after elimination of an infestation and that this program should involve a series of inspections to confirm successful extermination and prevent re-infestation.
Rat infestations in and around Vancouver

The following questions pertain to Vancouver, BC’s largest city. With regard to the average severity of rat infestations to which they responded, PCPs who serviced these areas reported that infestations were generally mild to moderate (<25 rats per infestation), both within the city of Vancouver and throughout the GVRD. In Vancouver, there was an apparent trend towards decreased severity of rat infestations in neighbourhoods of higher socioeconomic status. Six PCPs responded to questions specifically regarding Vancouver’s Downtown Eastside (DTES), the city’s poorest neighbourhood. Three of six respondents agreed with the statement “rat infestations are more common in the DTES compared to other parts of Vancouver,” while the remaining three respondents were neutral. Four of six respondents agreed with the statements “rat infestations are more severe in the DTES compared to other areas of Vancouver” and “rat infestations are harder to control in the DTES compared to other areas of Vancouver,” while the remaining two respondents were neutral. Three of six respondents disagreed with the statement “current levels of rat control in the DTES are sufficient,” while three of six respondents were neutral. Two of six respondents agreed with the statement “rats and people come into contact more often in the DTES compares to other areas of Vancouver,” while the four of six respondents were neutral.

Respondents commented that severity of rat infestations is most associated with local environmental conditions (e.g., infrastructure disrepair, lack of sanitation, etc.) rather than geographical location per se. Indeed, one respondent noted that frequency and severity of rat infestations may vary within a neighbourhood depending on property-level environmental factors.
3.5 Discussion

This study, which sought to assess the feasibility of surveying pest control professionals in order to obtain their perspectives on rat infestations and associated issues, yielded a low response rate. Despite this, however, several findings of interest were identified. For example, the results obtained suggest that, from the point of view of pest control professionals: 1) rat infestations fluctuate seasonally, possibly as a result of rat migration between the indoor and outdoor environments; 2) there are a variety of environmental factors that may attract rat infestations, the most important of which appear to be presence of exposed garbage, abandoned buildings, and compost; 3) local ecological factors are generally more important than neighbourhood-level factors with regard to influencing rat infestations; 4) however, residential areas and commercial areas dealing with food experience more frequent and/or severe rat infestations compared to commercial areas not dealing with food and industrial areas; 5) neighbourhoods of lower socioeconomic status appear to experience more rat infestations compared to wealthier areas; 6) rat infestations appear to be more severe, more common, and harder to control within Vancouver’s Downtown Eastside compared to other areas of the city; 7) rats are perceived to pose at least a moderate health risk to the general public and to pest control professionals; 8) poison baiting is one of the least effective management tools for eliminating rat infestations in the long term, although it is the cheapest and easiest; 9) environmental modification is the most effective rat control method in the long term.

Several of these findings are in agreement with previous studies regarding the ecology of urban rats. For example, Frantz (1976) found that the activities of urban rats changes across the seasons, and that rats have a tendency to move indoors at the onset of inclement weather (135), which may explain the increase in rat complaints in the fall and winter reported by PCPs in our study. What is particularly interesting about these
finding is that it suggests that previous studies (121, 136, 137) that were conducted in outdoor environments only, and that suggested that rat activity declines in the winter, may in fact have been measuring outdoor to indoor migration rather than a decrease in rat populations and activity per se.

With regard to environmental factors influencing rat infestations, participants in this study indicated that site-specific environmental characteristics appear to be more important than neighbourhood-level factors. This is in agreement with previous studies that have found that the occurrence of rat infestations reflects local environmental factors, such as availability of food and harborage and presence of barriers to rat movement (121, 137-139). Indeed, Traweger (2005) found that, by compiling local environmental factors that promote rat infestations into a Geographic Information Systems map, one could successfully identify suitable urban rat habitat and model the distribution of rats in a city.

With regard to specific local environmental factors, exposed garbage and abandoned buildings have previously been found to be strong promoters of rat infestations as they supply the food source and harborage necessary to maintain urban rat populations (140-142). However, other factors previously found to be important in promoting rat infestations, such as running water, natural soil (in the form of green spaces), and high population density (121, 122, 143), were perceived to be of moderate to low importance in our study. One of the most interesting findings in this study was the perceived importance of compost in promoting rat infestations, which is in contrast to previous studies that have found that rats are generally not attracted to compost piles (122). The potential for compost to attract rats has been a concern voiced by our collaborators with the City of Vancouver and may become a growing concern as the rate of composting in urban centers increases (144).
This study also found that land use (e.g., commercial, residential, etc.) appeared to influence urban rat infestations. Land use is a factor that is seldom addressed in studies of urban rat ecology. Interestingly, one of the few studies to address the effect of land use (145) also found that rat infestations were most common in residential areas. Given that land use has the potential to be one of the most easily identifiable factors associated with rat infestations, the influence of land use on urban rat populations warrants further study.

Despite the emphasis on local environmental factors, our study did indicate that rat infestations tend to be more prevalent and problematic in neighbourhoods of lower socioeconomic status. This is congruent with previous studies, which have identified low neighbourhood socioeconomic status as a consistent predictor of rat infestations (143, 146). It is believed that crowded, unsanitary, and dilapidated housing conditions prevalent in these neighbourhoods support rat infestations by providing abundant food and harborage (121, 143). Additionally, residents of these neighbourhoods might have fewer resources to deal with these infestations compared to wealthier counterparts. This may explain why PCPs in our study were of the opinion that rat infestations are more common, more severe, and harder to control in Vancouver’s Downtown Eastside (a community of low socioeconomic status) when compared to other areas of the city.

Controlling, eradicating, and preventing rat infestations in urban centers can be a significant challenge. Although rat populations can be greatly reduced by using poison bait or trapping and removal (147, 148), populations often return to pre-control levels after baiting and trapping have ceased (135, 140, 147). This supports the PCP’s assertions that baiting and trapping are not effective in the long term. The low cost and ease of poison, as suggested by PCPs, may explain why it is still in popular use. However, its relative ineffectiveness, in combination with potential detrimental effects, including poisoning of non-target species, (149, 150), suggests that other methods
should be used instead of or in combination with poison. The PCPs in this study suggested that environmental modification ought to be the core of any rodent control strategy, an assertion that is supported by previous studies which have found that, in order to successfully reduce rat populations in the long term, the carrying capacity of the environment (i.e., the availability of harborage and food) must be reduced (135, 138).

With regard to capturing a snapshot of public knowledge and opinions regarding rats, according to PCPs in this study, their clients are not very knowledgeable about rats but are concerned about the health risks (i.e., risk of infectious disease transmission) associated with them. PCPs are also concerned that they themselves may be at risk for rat-associated diseases, which is an occupational health hazard that has not been fully addressed. These concerns are not unfounded given that rats are the source of a number of zoonotic pathogens (pathogens transmissible from animals to people) responsible for human illness in cities around the world (30). Thus these concerns suggest the need for increased education and awareness among the public regarding rats, and among both PCPs and the public regarding rat-related health risks and methods to monitor and mitigate those risks. Given the close contact between PCPs and the public, educated PCPs may be an optimal group to provide public education.

The most significant limitation of this study was the low response rate. For the first round of the survey, this was most likely a result of the complexity of questions and the time required to complete the survey (approximately 30 minutes), as well as the online format, which required PCPs to complete the survey on their own time. For the second round, the survey was shortened, simplified, and deployed during the CPMA annual conference; however, the low response rate persisted. Given that both rounds were preceded by considerable advertising and industry awareness campaigns, and given the support and positive feedback we received from the PCP community, we can
only attribute the low response rate to a lack of motivation, possible resulting from insufficient incentive. For our study, those who completed the survey were entered into a prize draw; however, it may have been better to compensate each individual for the time taken to complete the survey. We were not able to collect any information on non-respondents, therefore it was not possible to determine how the low response rate may have biased our results. Future research should seek to determine optimal methods for engaging the PCP community in research of this kind. Additionally, although we hoped to collect data from a number of Canadian cities through the CPMA survey, all but one respondent were from BC. This may limit the generalizability of our results to other areas in Canada. Finally, it is important to temper these data with the knowledge that PCP opinions may be subject to bias. Specifically, PCP experience will be dependent on the infestations they are asked to respond to, and the hiring of a PCP is dependent on the circumstances of the infestation and the characteristics of the individual reporting it. For example, the apparent relationship between compost and rat infestations may be a result of public discontent associated with new municipal composting programs.

Despite the low response rate, however, there was a significant degree of agreement among PCPs, suggesting that these results may be representative of PCP professional opinions. These opinions are particularly valuable as it represents contemporary and practical experience with urban rat infestations and may thus be more relevant for those wishing to develop ecologically-based urban rat control programs compared to more scholarly sources of ecological data. To our knowledge this is the first study to survey PCPs regarding urban rat issues and one of the first to use PCP professional opinions to collect data regarding urban pest ecology. Our results suggest that, if practical issues regarding non-response can be overcome, PCPs may be a good source of information regarding pest species.
Chapter 4: The Characteristics of Wild Urban Rat Populations with a Focus on Factors Critical to the Understanding of Rat-Associated Zoonoses

4.1 Synopsis

Rodent ecology is a primary determinant of the dynamics of zoonotic pathogens in rodent populations and the risk of pathogen transmission to people, yet many studies of rat-associated zoonoses do not account for the ecological characteristics of urban rat populations. This hinders the development of an in-depth understanding of the ecology of rat-associated zoonoses, limits comparability among studies, and can lead to erroneous conclusions. We conducted a year-long trapping-removal study to describe the ecological characteristics of urban rat populations in the DTES. The study focused on factors that might influence the ecology of zoonotic pathogens in these populations and/or our understanding of that ecology. We found that rat population density varied remarkably over short geographical distances, which could explain observed spatial distributions of rat-associated zoonoses and have implications for sampling and data analysis during research and surveillance. Season appeared to influence rat population composition even within the urban environment, which could cause temporal variation in pathogen prevalence. Body mass and bite wounds, which are often used in epidemiologic analyses as simple proxies for age and aggression, were shown to be more complex than previously thought. Finally, we found that factors associated with trapping can determine the size and composition of sampled rat population, and thus influence inferences made about the source population. These findings may help guide future studies of rats and rat-associated zoonoses.

4.2 Introduction

Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) are commensal rodents with a virtually worldwide distribution (151). They are highly adapted to
coexisting with human populations, and are particularly ubiquitous in the urban environment (6, 140, 151). Rat infestations are problematic in urban settings because rats are the source of a number of zoonotic pathogens.

Rodent ecology is a primary determinant of zoonotic pathogen dynamics in rodent populations and of the risk of pathogen transmission from rats and people (16, 152). Yet many studies of RAZ do not account for the characteristics of rat populations during sampling or data analysis (49, 117, 132, 133, 153). This is problematic because it hinders the development of a comprehensive understanding of pathogen ecology in rat populations, prevents comparability and synthesis of results generated by different studies, and can lead to erroneous conclusions with regard to RAZ dynamics.

The characteristics of rat populations can influence the ecology of RAZ and/or our understanding of RAZ ecology in a variety of ways. For example, the prevalence of many zoonotic pathogens among rats can vary remarkably even over a short geographic distance (107, 115). The reasons for this are unclear but could be related to geographic variations in rat population density, independent of, or in combination with, environmental factors. Although past research has indicated that urban rats form tight colonies with small home ranges (154), there are very little data regarding how rats are distributed across the modern urban landscape.

The ecology of RAZ could also be influenced by temporal factors, particularly season. Seasonal changes in rodent population dynamics have been shown to influence the ecology of rodent-borne zoonotic pathogens in more sylvatic settings (155, 156). However, there is considerable conflict in the literature regarding the impact of season on rats and RAZ in urban ecosystems (138, 157-159), which, being largely under human control, would seem less prone to natural seasonal variations.

In addition to extrinsic factors, the characteristics of rats themselves can influence the likelihood of infection with a RAZ. Body mass is one of the most common variables used to analyze and interpret data on RAZ, with mass being used as a proxy for
chronological age (107, 116, 123, 158). However, there are a variety of factors other than age that could influence body mass, such as population of origin (160, 161), health status (162), and access to resources (138, 163, 164). The factors contributing to body mass in urban rats have not been well described.

Rat behaviour and social interactions are also likely to have significant impacts on disease transmission but are very difficult to capture and quantify during field research. One aspect of behaviour that can be identified is intra-specific aggression resulting in visible cutaneous bite wounds. Past research has identified a positive correlation between Seoul hantavirus infection and the presence of cutaneous bite wounds in urban rats (64). However, it is difficult to say whether this indicates that the virus is transmitted through biting, or whether bite wounds are a reflection of some underlying characteristic of the individual, behavioural or physiologic, which is driving infection. As with body mass, the factors affecting wounding in urban rats have not been fully investigated.

Finally, most studies of RAZ use data generated through trapping to make inferences about population and disease ecology (157, 158, 165). These inferences are based on the assumption that all animals in a population are equally likely to enter any given trap at any given time (i.e., that trapping itself does not create any systematic bias in the characteristics of the study population when compared to the source population). However, there are a variety of factors that could influence if and when an animal enters a trap, including trapping methodology, trap environment, and characteristics of the individual rat (163). Given marked variation among studies with regard to how trapping is carried out (116, 158, 166, 167), it is important to better understand how trapping might influence the number and characteristics of rats trapped.

The overall objective of this study was to describe the characteristics of rat populations in the DTES, with a focus on factors that could influence the ecology of zoonotic pathogens in these populations and/or our understanding of pathogen
ecology. Specifically, we aimed to: 1) Describe rat population density and distribution across the study area; 2) Describe seasonal variations in rat population size and structure; 3) Identify the factors that contribute to body mass in urban rats; 4) Identify the factors that influence the presence and number of cutaneous bite wound in rats; and 5) Describe how factors associated with trapping can influence the success of rat collection and the characteristics of the rat population collected.

4.3 Methods

Study area

The study area included 43 contiguous city blocks covering an area of approximately 82 ha. These blocks constitute the core of what is traditionally considered the DTES. Also included was 1 private property (3 ha) within an international shipping port at the northern border of the study area.

Trapping methodology

Initially, it was hoped that population size could be estimated by tracking decline in catch-per-unit effort (168, 169). However, after attempting to use this method in the first five blocks under study, we found that it was only effective in city blocks with very large rat populations. More importantly, the prolonged period of trapping activity drew unwanted attention from the public, leading to significant equipment vandalism. For this reason, we elected to switch to a trap success index to measure relative rat abundance among blocks using shorter trapping periods and fewer traps. This method is based on calculating the total number of rats trapped compared to the total number of traps set (168-170). Although trap success methods do not allow calculation of absolute population size, we were more interested in comparisons among blocks, therefore an index of relative rat abundance was considered sufficient.
Each city block and the port site was assigned to a randomly selected 3-week study period over the course of 1 year (September 2011 – August 2012), in order to capture seasonal variations in rat ecology (170). Within each block, approximately 20 Tomahawk Rigid Traps for rats (Tomahawk Live Trap, Hazlehurst, WI) were set out along each side of the back alley that bisected the block. After several episodes of vandalism that involved crushing traps, stainless steel trap covers were designed. These trap covers were locked to the trap and to a chain that secured the trap and cover to an immovable object in order to prevent theft. Traps were evenly spaced where possible, but had to be placed on public property in a location where they did not obstruct traffic and could be secured. Additionally, given that rats prefer to travel along solid surfaces (164), traps were placed alongside a wall whenever possible. Where this was not possible, traps were placed against the nearest solid object (e.g., a fence or dumpster).

For the private property at the international shipping port, 56 traps were placed in 8 locations, which included both indoor and outdoor areas where site employees had seen rats. Also within this property, rats trapped by a private pest control professional in areas not accessible to researchers were also collected.

Traps were pre-baited (filled with bait but fixed open) for 1 week in order to ensure rats were acclimatized to the traps and bait prior to the onset of trapping. Pre-baiting was followed by active trapping for 2 consecutive weeks. Traps were set at 4:00 pm and checked at 9:00 am the following day. Traps were not set during the day in order to reduce interactions between rats/traps and people during the period of time when the alleys are most used by area residents. Trapping was conducted on weekdays and traps were baited but fixed open on weekends. Baits used included combinations of peanut butter, bacon fat, oats, and flour, which were rolled into balls and frozen for easier handling. Fresh apple slices were provided as a water source.

Trapped rats were transferred from the trap to a rodent inhalation narcosis chamber (Sciencelab.com Inc., Houston, Texas) where they underwent isoflurane
anesthesia, followed by blood collection via intracardiac puncture and intracardiac pentobarbital euthanasia. All rodent procedures were conducted in the back of a study van in order to protect the researchers and rats, and to prevent public disturbance. Rats collected by the private pest control professional at the port were trapped using snap-type kill traps.

Data collection

Georeferenced aerial photographs were used to identify and map the location of each trap within the study area using ArcGIS 10.0 (ESRI, Redlands, USA). For each trap, the number of rats caught in that trap and the proximity of the trap to the nearest building (i.e., directly against a solid wall vs. away from a solid wall) was also recorded.

For each night of trapping in each block, we recorded the number and location of traps set, rats trapped, and traps sprung for other causes (e.g., traps sprung or damaged by people, trapping of non-target species, etc.). The temperature average (°C) and precipitation (mm) for the preceding day were obtained from Environment Canada’s National Climate Data and Information Archive (www.climate.weatheroffice.gc.ca).

After euthanasia, the following measurements were collected from each rat in the field: mass (g), body length (nose to anus, cm), sex, and sexual maturity [animals were considered sexually mature if they had scrotal (vs. inguinal) testes or a perforate (vs. imperforate) vagina (170)]. Rats were identified to species based on external morphology (171) and each rat was palpated over the lumbar spine and pelvic bones and assigned a body condition score based on the scale developed by Hickman and Swan (162). The presence and number of bite wounds in the skin was recorded, as was the date and location (block and trap) where each rat was trapped.

Rat carcasses frozen at –30°C and shipped to the Animal Health Centre (AHC), British Columbia Ministry of Agriculture, Abbotsford, where they were thawed and underwent a standardized necropsy and tissue collection protocol. During the
necropsy, sex and sexual maturity were confirmed. When there was a conflict in the assessment of sex or maturity between the field and laboratory researchers, sex and maturity were recorded as ‘undetermined.’ For females, the uterus was examined for pregnancy or placental scars and the mammary chain for evidence of lactation (well developed mammary gland tissue and/or bare, prominent mammae) (170). A female was considered parous if she was pregnant, had placental scars, and/or was lactating. Embryos were counted when present. Volume of subcutaneous and visceral fat were assessed and used to rate rats on a three point ‘fat scale’ generated for the purposes of this study: poor condition (score of 0) = minimal to no visible internal fat; moderate condition (score of 1) = moderate internal fat; good condition (score of 2) = abundant internal fat. Fat score for an individual animal was arrived at via consensus among at least 2 team members until all members were comfortable using the scale and a subjective degree of consistency had been achieved, at which point, scores were assigned by the individual team member performing the necropsy.

**Data analysis**

All statistical analyses were conducted using R (R Development Core Team, Vienna, Austria) and an alpha level of 0.05 was used to determine statistical significance. Packages utilized in R included ‘pscl’ for zero-inflated negative binomial models, ‘lme4’ for generalized linear mixed models, and ‘survival’ for Cox proportional-hazards models. For all models, individuals with missing values for any of the variables under consideration were excluded. For multivariate models, the goal was to construct the most parsimonious model that predicted the outcome of interest. For each multivariate model, the final model was arrived at by manual stepwise regression using Akaike’s Information Criteria (AIC) to compare candidate models (the final model was that with the lowest AIC) (172). Collinearity among explanatory variables was identified using Spearman’s rank correlation (ρ) (172). Highly correlated variables
(ρ > 0.8) were modeled separately (172). For each final model, appropriate diagnostics were performed based on the model form to ensure that underlying statistical assumptions were met (172, 173).

Population density and distribution

A trap success index was used to measure relative rat abundance in each block (168-170). For each block, this index was calculated by dividing the total number of rats caught by the total trap effort (# traps set x #nights) adjusted according to the method described by Nelson and Clark (174). This method involves subtracting half a trapping unit from the total trap effort for each trap spring for any cause (e.g., trapping of a rat, trapping of a non-target species, tripping of a trap). This adjustment was considered particularly important because of the marked variation in rat abundance among blocks, and because of the frequency with which traps were sprung by non-target species and by members of the public, both of which can significantly bias trap success indices if they are not taken into account (174). Because each block under study was roughly the same area (1.2 ha), and because the home range of rats in urban centers is thought to be limited to a city block (175, 176), our trap success indices could be considered to approximate relative population density (168), further increasing comparability. Limited movement of rats among blocks also reduces the possibility of rats migrating into or out of blocks as a result of trapping activities (175), particularly given the relatively short trapping period. Relative abundance was not calculated at the port site because it was not possible to trap rats in this location in the uniform and systematic manor employed in the city blocks.

Variation in the number and location of rats trapped across the study area was visualized spatially in ArcGIS, and Getis-Ord Gi* was used to identify clusters of high and low values for number of rats trapped using the trap as the unit of analysis, the
number of rats trapped in that trap as the outcome, a fixed distance band of 148 m (the average length of an alley in the study area), and a Manhattan distance method.

**Seasonality**

In order to determine whether relative rat abundance varied according to season, a zero-inflated negative binomial model was used to evaluate the relationship between trap success in a block (with the block being the unit of analysis) and the season in which trapping took place (September – November = fall, December – February = winter, March – May = spring, June – August = summer).

In order to detect seasonality in population structure, bivariate logistic or linear generalized linear mixed models (GLMMs) were used to examine the relationship between sexual maturity (immature vs. mature rats), parity, pregnancy/lactation, and number of embryos (outcome variables) and season (explanatory variable), while controlling for clustering by block of origin (i.e., the city block in which the rat was trapped). Note that, for all analyses, parity and pregnancy/lactation were examined among sexually mature females only. Number of embryos was examined among pregnant females only.

**Body mass**

In order to identify the components that could contribute to mass in rats, we used linear regression to examine the relationship between mass (outcome variable) and length, sex, sexual maturity, body condition score, fat score, block of origin, and season of capture (explanatory variables) in a bivariate and multivariate manner. Block of origin was added as a fixed effect and the outcome variable, mass, was natural logarithm-transformed to satisfy model assumptions.
Two methods of assessing nutritional condition were used in this study: palpation (162) and visual assessment of internal fat stores. These measures were compared using Spearman’s rank correlation.

**Cutaneous bite wounds**

Logistic GLMMs (controlling for block of origin) were used to examine the relationship between wound presence (explanatory variable) and mass, length, sex, sexual maturity, fat score, body condition score, pregnancy, lactation, and season of capture (explanatory variables) in a bivariate and multivariate manner.

Among rats with one or more bite wounds, bivariate and multivariate poisson GLMMs were used to examine the relationship between number of bite wounds and the aforementioned explanatory variables.

**Factors influencing trappability**

Given that rats prefer to travel along walls and avoid open spaces (164), we examined the relationship between the number of rats trapped in any one trap (0 vs. > 0) and whether that trap was placed against or away from a solid wall using a logistic GLMM (controlling for block of origin).

In order to determine how ambient temperature and precipitation might influence trap success on a given block-night of trapping (one block-night of trapping is trapping in one block for one night), the relationship between trap success on a block-night (explanatory variable - dichotomized into 0 vs. > 0) and average temperature and precipitation (explanatory variables) was examined using a logistic GLMM to control for the random effect of both block of origin and trap day (i.e., when the rat was trapped during the 2 week trapping period).

In order to determine how rat demographic characteristics might influence trapability, bivariate and multivariate logistic GLMMs (controlling for block) were used...
to examine the relationship between mass, length, sex, sexual maturity, fat score, body condition score, presence and number of bite wounds, pregnancy, and lactation (explanatory variables) and trap day (outcome variable). Trap day was dichotomized to day 1 vs. days 2-10 of trapping.

4.4 Results

Characteristics of trapped rats

A total of 725 rats were collected over the course of the project. Of those 685 (94.5%) were Norway rats and 40 (5.5%) were black rats. Thirty-two of 40 black rats (80.0%) were trapped at the port site, while the remainder (n = 8), were trapped in the city blocks. In contrast, 674 of 685 (98.4%) of Norway rats were trapped in the city blocks and only 11 Norway rats (1.6%) were trapped at the port.

Among the Norway rats, 381 (56.3%) were male and 397 (63.9%) were sexually mature (sex and maturity could not be determined for 8 and 64 rats, respectively). Median mass and nose-to-rump length was 142.2 g (range = 20.0 – 466.2 g) and 17.5 cm (range = 8.5 – 26.0 cm), respectively. Median body condition on palpation was 2.5/5 and median fat score was 1/2. Bite wounds were present in 169 (24.7%) rats. Among rats with bite wounds, the median number of wounds was 2 (range 1-15). Among sexually mature females (n = 155), 97 (62.6%) were parous, 32 (20.6%) were visibly pregnant and, 78 (50.3%) were lactating. Among pregnant females the median number of embryos was 9 (range = 1 -15).

Among the black rats 19 (47.5%) were male and 21 (61.8%) were mature (sexual maturity could not be determined for 6 rats). Median mass and body length were 76.4g (range = 24.4 – 259.8g) and 13.8 cm (range = 8.5 – 21.0 cm), respectively. Median body condition on palpation was 2/5 and median fat score was 0/2. Bite wounds were present in 8 (20%) of rats. Among rats with bite wounds, the median number of wounds was 1
(range = 1-3). Among sexually mature females (n = 11), 2 (18.2%) were parous, 1 (9.1%) was pregnant, and 1 (9.1%) was lactating. The pregnant female had 6 embryos.

For subsequent analyses, rats trapped at the port (n = 43, 32 black rats and 11 Norway rats) were excluded because the port trapping scheme was more opportunistic than systematic, and therefore not comparable to that undertaken in the city blocks. Additionally, given that only 8 black rats were trapped outside the port, and given that the ecology of black and Norway rats is known to differ, only Norway rats trapped outside the port (n = 674) were included for further consideration. Henceforth, Norway rats trapped in the city blocks will be referred to as ‘rats.’

**Population density and distribution**

Relative rat abundance varied remarkably among blocks, with trap success ranging from 0 to 0.94 (median 0.04) and geographic clusters of high and low rat abundance were observed (Fig. 4-1).
Figure 4-1: A. Spatial distribution of trapped Norway rats (*Rattus norvegicus*) and trap success in each city block. B. Spatial clusters of relatively high and low rat abundance on Getis-Ord Gi* analysis. For the GiZScore, a high z-score indicates spatial clustering of high values and a low z-score indicates spatial clustering of low values. A z-score near zero indicates no significant clustering.
Seasonality

Of the 43 blocks included in the study, 9 were trapped in the fall, 10 in the winter, 14 in the spring and 12 in the summer. There was no significant association between season and rat abundance.

Pregnant and lactating rats, as well as sexually immature rats, were found throughout the year, suggesting that reproduction and juvenile recruitment occur year round. However, there was temporal variation in the proportion of the population composed of pregnant/lactating and sexually immature rats. The proportion of immature rats in the population was lowest in the fall, increasing through the winter, spring, and summer (Fig. 4-2). The proportion of pregnant/lactating rats was highest in the fall, decreasing through the winter and spring before increasing again towards the summer. Parity closely mirrored pregnancy, with the proportion of parous females also being highest in the fall and decreasing through the winter and spring before increasing in the summer.

After controlling for the effect of block, compared to the fall, the odds a rat being sexually immature were 4 times greater in the spring (OR = 3.68, 95% CI = 1.17 – 11.56) and 5 times greater in the summer (OR = 5.03, 95% CI = 1.17 – 21.44). There was no significant difference between the fall and winter. The odds of a sexually mature female being pregnant or lactating in the spring was 1/4 that of the fall (OR = 0.28, 95% CI = 0.10 – 0.82). There was no significant difference in the proportion of pregnant and lactating sexually mature females in the fall compared to summer or winter. The odds of an adult female being parous in the spring was 1/5 that of the fall (OR = 0.20, 95% CI = 0.07 – 0.59). There was no significant difference in the proportion of parous females in the fall compared to summer or winter. There was no association between season and number of embryos in pregnant females.
Body mass

On bivariate analyses, increased log mass was associated with male sex ($\beta = 0.16$, 95% CI = 0.05 – 0.28), sexual maturity ($\beta = 1.33$, 95% CI = 1.27 – 1.40), increased fat score ($\beta = 0.69$, 95% CI = 0.64 – 0.74), increased body condition score ($\beta = 0.23$, 95% CI = 0.15 – 0.30), increased length ($\beta = 0.18$, 95% CI = 0.17 – 0.18), season and block of origin (data not shown). There was no significant association with body condition score.

Maturity and length were highly correlated ($\rho = 0.81$) and were modeled separately. The final multivariate model included length ($\beta = 0.17$, 95% CI = 0.16 – 0.17), fat score ($\beta = 0.05$, 95% CI = 0.02 – 0.07), and block of origin [for example, rats in block 31 weighed less than those in block 6 (β = - 0.19, 95% CI = -0.09 – -0.29) while those in block 19 weighed more than those in block 6 (β = 0.06, 95% CI = 0.01 – 0.12)].

Body condition score on palpation has a significant but weak correlation with fat score as assessed through post-mortem examination ($\rho = 0.22$, P < 0.001).
Cutaneous bite wounds

Among the 674 rats, 166 (24.6%) had bite wounds. On bivariate analysis wound presence was associated with male sex (OR = 1.70, 95% CI = 1.15 – 2.49), sexual maturity (OR = 11.58, 95% CI = 6.10 – 22.00), increased mass (OR = 1.14, 95% CI = 1.11 – 1.16 per 10g), increased length (OR = 1.46, 95% CI = 1.36 – 1.58), and increased fat score (OR = 3.11, 95% CI = 2.37 – 4.10). Among mature females, wound presence was associated with pregnancy (OR = 3.20, 95% CI = 1.40 – 7.30) and lactation (OR = 2.96, 95% CI = 1.39 – 6.29). There was no significant association with season or body condition score.

Among the 166 rats with bite wounds, 82 (49.4%) had 1 wound, 31 (18.7%) had 2 wounds, 18 (10.8%) had 3 wounds, 15 (9.0%) had 4 wounds, 8 (4.8%) had 5 wounds, 7 (4.2%) has 6 wounds, 4 (2.4%) had 7 wounds, and 1 had 15 wounds. The rat with 15 wounds was considered an outlier and excluded from the analysis. On bivariate analysis, increased wound number was associated with season (RR = 1.20, 95% CI = 0.91 – 1.55 for spring vs. fall, RR = 0.94, 95% CI = 0.62 - 1.43 for summer vs. fall, RR = 1.54, 95% CI = 1.18 – 2.02 for winter vs. fall), increased mass (RR = 1.02, 95% CI = 1.01 – 1.03 per 10g), increased length (RR = 1.06, 95% CI = 1.02 – 1. 10), and increased fat score (RR = 1.18, 95% CI = 1.01 – 1.37) among rats with at least one bite wound. There was no significant association with sex, sexual maturity, body condition score, pregnancy, or lactation.

The final multivariate model for wound presence included sex (OR = 1.75, 95% CI = 1.09 – 2.81 for males vs. females), length (OR = 1.50, 95% CI = 1.38 – 1.63), and season (OR = 7.40, 95% CI = 2.62 – 20.95 for spring vs. fall, OR = 7.30, 95% CI = 3.52 – 15.15 for summer vs. fall; OR = 2.31, 95% CI = 0.92 – 5.79 for winter vs. fall). The final multivariate model for wound number included sex (RR = 1.24, 95% CI = 0.98 – 1.58 for males vs. females), mass (RR = 1.02, 95% CI = 1.02 – 1.03 per 10g), and season (RR = 1.55, 95% CI = 1.35 – 1.79 for spring vs. fall; RR = 1.07, 95% CI = 0.70 – 1.66 for summer vs. fall, RR = 1.54, 95% CI = 1.17 – 2.02, 0.39 – 3.93 for winter vs. fall). Note that the
following variables were highly correlated and therefore modeled separately: maturity and length ($\rho = 0.81$), mass and length ($\rho = 0.97$), mass and maturity ($\rho = 0.80$), wound presence and wound number ($\rho = 0.99$).

**Factors influencing trappability**

Of the 878 traps placed over the course of the project, no rats were trapped in 612 of the trap locations (69.7%). For the remaining 266 trap locations where rats were caught, the median number of rats trapped was 2 (range = 1 – 9). Five hundred ninety seven (68.0%) of the traps were placed immediately adjacent to a solid wall, while 281 (32%) were not. Trap placement was not significantly associated with the number of rats caught.

There were a total of 354 block-nights of trapping. No rats were caught on 186 (52.5%) of those block-nights. There was no association between trap success on any given block-night (0 vs. > 0) and temperature or precipitation.

There were up to 12 days of trapping in a trapping period and we recorded the day of trapping within a trapping period for 649 rat captures. Of those, 144 (22.2%) rats were trapped on the first day of trapping, 95 (14.6%) on day 2, 77 (11.9%) on day 3, 74 (11.4%) on day 4, 66 (10.2%) on day 5, 83 (12.8%) on day 6, 60 (9.2%) on day 7, 44 (6.8%) on day 8, 4 (0.6%) on day 9, and 2 (0.3%) on day 10.

On bivariate analysis, the odds of being trapped on the first day of trapping (vs. subsequent days) was associated with increased mass (OR = 1.03, 95% CI = 1.01 – 1.05 per 10 g), increased length (OR = 1.11, 95% CI = 1.05 – 1.17), maturity (OR = 2.29, 95% CI = 1.43 – 3.66), and the presence of skin wounds (OR = 1.60, 95% CI = 1.03 – 2.50). There was no significant association with sex, body condition score, fat score, wound number, pregnancy, or lactation.
The final model contained only length, although the bivariate models containing maturity and mass were roughly equivalent. Note that maturity, length, and mass were highly correlated (\( \rho \geq 0.08 \)) and therefore modeled separately.

4.5 Discussion

The results of this study show that urban rat populations have a variety of ecologic features that should be taken into account when studying RAZ.

For example, our data show that Norway rat population density varies remarkably over a short geographic distance. Not only did density vary among adjacent city blocks but rats appeared to be distributed non-uniformly along the length of the alley. This heterogeneous distribution is presumably a result of variation in the microenvironment of a city block, particularly as it pertains to resource availability (122, 177). Given that urban rats are territorial and have a small home range (164, 175), small spatial variations in the abundance of suitable food and harborage could create marked variation in carrying capacity across the urban landscape (138).

The heterogeneous distribution of rats across the urban landscape may, in part, be responsible for observed spatial variations in the presence and prevalence of RAZ (178). This, in turn, suggests that clustering of rats by city block, or whatever unit best approximates the colony, is an important consideration when designing sampling and analytic protocols for research. For example, it suggests that intensive sampling of rats across a study area is likely necessary in order to achieve adequate representation of populations of interest. This approach is in contrast to many past studies of RAZ, which appear to use less rigorous and/or more opportunistic sampling methods (123, 133, 153, 159, 179). It also suggests that it is important to control for geographic clustering of rat populations during any data analysis, which does not appear to occur in most studies of rats and their pathogens.
In contrast to observed spatial variation, relative rat abundance did not vary significantly with season. Additionally, pregnant/lactating, parous, and immature animals were present throughout the year, which suggests a lack of strict reproductive seasonality. However, relative population composition did appear to vary significantly among seasons. Specifically, the proportion of mature females that were parous or pregnant/lactating was higher in the summer and fall and lower in the winter and spring, while the proportion of sexually immature rats in the population was lowest in the fall and higher in the winter, spring, and summer. Seasonal variations in reproduction and population demography could influence the ecology of RAZ in a number of different ways. For example, behavioural changes related to mating could influence pathogen transmission, while seasonal changes in the relative proportion of immature vs. mature animals could alter pathogen prevalence (156, 180), and thus the risk of pathogen transmission to people. For example, infection with some RAZ, including Leptospira spp. (107, 181) and SEOV (64, 157), is more common in older rats, suggesting that juvenile recruitment might dilute the prevalence of these pathogens in certain seasons.

Many rodents demonstrate seasonal variations in reproduction, population density, and demography, including several species of bandicoots (Bandicota spp.), mice (Mus pp), and rats (Rattus spp.) (170). However, there has been considerable conflict among studies regarding if and how urban rat populations are influenced by season (138, 182), and it remains unclear to what degree these discrepancies are a result of methodological inconsistencies vs. true geographic or temporal variation. Given that our findings are based on a single year of study, we cannot definitively conclude that the rat populations under study demonstrate reproductive seasonality (183). However, it appears prudent to account for season in studies of urban rats and RAZ.

Body mass in rats is often used as a proxy for age in rats, but research has shown this relationship is only approximate and often inaccurate, especially for the youngest
and oldest rats in a population (154). Additionally, previous studies have found significant variation in the growth rate of rats among different habitats, which has been attributed to some combination of genetic heterogeneity and variation in resource availability among different locations (160, 161, 184). The results of our study, particularly the relationship between mass and length, as well as sexual maturity, support the conclusion that, although age is important determinant of mass, a number of factors independent of age (such as body fat and block of origin) can also influence mass.

Similarly, the presence and number of bite wounds were associated with a variety of demographic factors, such as sex, mass/length, and season. Specifically, bite wounds appear to be most common in longer/heavier rats and male rats, which is consistent with past behavioral studies that suggest that intra-specific aggression is most common among mature males (164). The association between bite wounds and season, while controlling for mass/length, suggests that there might be seasonal variations in the incidence of intra-specific aggression. Past research has suggested that aggression among male rats is often related to competition for oestrus females (154); however, the temporal relationship between wounding and pregnancy was less clear in our data. It is interesting to note that in other rodent species, intra-specific aggression also appears to be more common in older males and in certain seasons, although this may vary according to species and location (185, 186). As with rats (64, 187), intra-specific aggression among males appears to play a role in pathogen transmission among other rodent species (185, 186).

It is of note that the relationship between mass, wounding, and other demographic and environmental factors varied depending on whether they were included in bivariate or multivariate models. This suggests that mass and wounding are complex variables, and that this complexity should be taken into account when using these variables in epidemiologic and ecologic studies. Much of the past research on
RAZ has been based solely on bivariate modeling techniques (116, 158). However, given that ecologic factors have the potential to confound one another, as well as the probability of infection with a zoonotic pathogen, it seems prudent to use more complex statistical methods in the future (e.g., multivariate models) to avoid the identification of erroneous associations.

In this study, we used two different methods to assess nutritional condition. These included a published body condition scoring system utilizing external palpation of the rat’s body (162) and a fat store system (generated for this study) using visual assessment of internal fat stores at necropsy. Scores generated using these two methods were only weakly correlated, and while fat score was associated with a number of different variables mentioned above, far fewer variables were associated with body condition score. Overall, we believe fat score to be the superior variable as it is the most direct reflection of nutritional condition and we found it easier to assess in a consistent manner. We are not certain why the body condition scoring system was not successful in our study, but it could be related to the fact that this system was developed for laboratory animals (vs. wild rats, who are generally leaner) or because of our relative inexperience in using this method.

This study employed a trap-removal methodology, which has been used commonly in the past for the study of rats and RAZ (143, 169, 182, 188, 189). However, one of the issues common to all trapping-based studies is that it can be difficult to distinguish population characteristics from factors that affect trapability. In our study, we were concerned that trap placement and weather (temperature or precipitation) might affect our ability to trap rats. However, we found that none of these variables were significantly associated with trap success. It was interesting that the majority of rats were caught in the first few days of trapping. Indeed, the number of rats trapped dropped remarkably after the first day with 22.2% of all rats trapped on trap day 1 vs. 14.6% on day 2. This number dropped to < 1% by trap day 10. This suggests shorter
trapping periods (i.e., of 3 – 4 days in duration) might be sufficient to collect an adequate sample of urban rats.

That being said, it is important to consider that the characteristics of rats entering the traps may change over the course of the trapping period. For example, we found that sexually mature rats, heavier rats, and longer rats were more likely to be trapped on the first trap day vs. subsequent days. Given that many zoonotic pathogens are more common in older animals (16), short trapping periods might be appropriate for detecting RAZ in rat populations. However, more prolonged trapping might be necessary to obtain a representative sample of the population for epidemiologic studies, and to detect certain pathogens that are more common in young rats (e.g., Clostridium difficile (190)).

The aforementioned changes in the characteristics of the study population over the trapping period is interesting given that a week of pre-baiting was conducted in advance of active trapping. Pre-baiting is often used in studies of rats and other rodents in order to allow animals to overcome neophobic responses to traps and bait and thus ensure that all members of trappable population are equally likely to be captured once active trapping is initiated (163, 191-193). The fact that older animals appear to have been trapped first might suggest that more mature, and thus more dominant rats were excluding immature and thus more submissive rats from the food resources within the traps (therefore younger rats began entering traps only when the older animals had been removed) (154). Many studies of urban rats do not appear to employ pre-baiting (116, 132, 165), and it would be interesting to know how this might bias the characteristics of the trapped population.

Although the specific location of a trap within an alley did not appear to greatly affect the trap success for Norway rats, it is difficult to determine if an overall focus on ground-based outdoor trapping influenced our ability to capture black rats. Among the trapped population, Norway rats comprised 95% of the captures with the remainder
being black rats. Most of the black rats were captured at the port area, where trapping was predominantly conducted inside structures. In contrast, 98% of the rats trapped in the city blocks were Norway rats, where traps were set on the ground outside of buildings. Norway rats are thought to reside primarily in underground burrows (177, 194), while black rats are more likely to live in human-made structures (194, 195). Where these two species co-exist they are thought to remain segregated, with the larger and more aggressive Norway rats excluding the smaller and more timid black rats from ground-based resources (164, 196). For these reasons our trapping methodology in the city blocks may have resulted in underrepresentation of black rats within the study area. That being said, in many cities, Norway rats are thought to have displaced black rats completely (194) so it is possible that black rats are actually rare within the majority of the study area. It should also be noted that rats in this study were identified to species based on external morphologic characteristics; therefore ‘cryptic’ Rattus spp. may have been overlooked. These cryptic species are morphologically indistinguishable from Norway and/or black rats, and therefore can only be recognized using genetic techniques (197).

Overall, the results of this study shed some light on the characteristics of urban rat populations that could influence the ecology and study of RAZ. However, there are still a variety of aspects requiring further elucidation. For example, although we were able to show that rat population density varies by city block, further research will be needed to identify the precise reasons behind this variation. There is some indication that the heterogeneous distribution of rats across the urban landscape is a result of variations in the environment with regard to resource availability (138, 177). However, rat behaviour and/or genotype, could also play a role in abundance and distribution (195). Indeed, information on the genetic structure of rat populations, which has received little attention in the past, has the potential to deepen our understanding of both rat and RAZ ecology. Additionally, although we were able to identify seasonal
variation in population structure, we could not account for seasonal variations in rat
behaviour, vector ecology, or rat-human interactions, all of which can influence RAZ
ecology (67, 94, 180). Perhaps most importantly, the use of a trap-removal methodology
and the fact that the study took place over one year meant that we were not able to
capture a variety of longitudinal changes in rat populations, for example, inter-annual
variation and changes due to anthropogenic modification in the urban environment
(e.g., urban decay and re-development). Scientists are well aware that long term
environmental alterations, such as climate change, are likely to have significant impacts
on the ecology of wild animals and their diseases (198). However, little attention has
been paid to the changes that occur in urban ecosystems, which would seem much
more dramatic and rapid, particularly from the perspective of urban rats. For this
reason we suggest that cities around the world should invest in surveillance and
research that aims to understand and monitor local rat populations now and into the
future.
Chapter 5: A Mixed Methods Approach to Exploring the Relationship Between Norway Rat (*Rattus norvegicus*) Abundance and Features of the Urban Environment in an Inner-City Neighbourhood

5.1 Synopsis

Urban rats (*Rattus* spp.) are among the most ubiquitous pest species in the world. Previous research has shown that rat abundance is largely determined by features of the environment; however, the specific urban environmental factors that influence rat population density within cities have yet to be clearly identified. Additionally, there are no well described tools or methodologies for conducting an in-depth evaluation of the relationship between urban rat abundance and the environment. In this study, we developed a systematic environmental observation tool using methods borrowed from the field of systematic social observation. This tool, which employed a combination of quantitative and qualitative methodologies, was then used to identify environmental factors associated with the relative abundance of Norway rats (*Rattus norvegicus*) in an inner-city neighbourhood of Vancouver, Canada. Using a multivariate zero-inflated negative binomial model, we found that a variety of factors, including specific land use, building condition, and amount of refuse, were related to rat presence and abundance. Qualitative data largely supported and further clarified observed statistical relationships, but also identified conflicting and unique situations not easily captured through quantitative methods. Overall, the tool helped us to better understand the relationship between features of the urban environment and relative rat abundance within our study area and may useful for studying environmental determinants of zoonotic disease prevalence/distribution among urban rat populations in the future.
5.2 Introduction

Norway rats (Rattus norvegicus) are among the most widespread rodent species, inhabiting every continent except for Antarctica (154). The ubiquity of this species is largely attributable to their remarkable ability to quickly and successfully adapt to new environments and resources (154). Norway rats are best suited to close cohabitation with people, and are considered a true commensal species as few populations are found in a truly sylvatic state (154).

Their propensity to exploit human settlements has caused Norway rats to become a particularly ubiquitous and problematic pest in cities around the world. Within urban settings, Norway rats can damage property, contaminate food, and act as a source of infectious disease for people (16, 154). For these reasons there is considerable interest in identifying and understanding the factors that promote or deter rat infestations in urban centers.

Previous studies have indicated that the environment is probably the single most important determinant of rat density and distribution across the urban landscape (138, 154). More specifically, it is clear that rats require suitable harborage and food sources in order to establish an infestation (138, 154). However, the adaptability of Norway rats means that it is often difficult to identify what specific features of the urban environment combine to create optimal rat habitat.

Researchers have employed a variety of methods in an effort to identify environmental features that promote or deter rat infestations. For example, a study in Buenos Aires, Argentina, compared the relative abundance of Norway rats in several locations demonstrating varying degrees of human modification (195). They found that Norway rats were more common in shantytowns and parklands compared to industrial-residential neighbourhoods and natural preserves (195). Another study in the same city found that Norway rats were more abundant in shantytowns compared to parklands (147). In Salzburg, Austria, Norway rats were trapped in a variety of urban
locations with distinct environmental features (121). This study found that Norway rats were most common in habitats with water, vegetation, natural soil, and organic waste, while rats were rarely found in heavily built-up areas and areas frequently utilized by people (121).

The main drawback of the aforementioned approaches is that they are capable of capturing only ‘high-level’ environmental impacts on rat populations (i.e., differences in rat abundance between different ecosystems). Rat population density and distribution, however, may vary considerably even over very short geographic distances within a single urban ecosystem (143, 199), suggesting that minute differences in the micro-environment of a city block could determine its capacity to support rat populations. These micro-environmental data, however, are relatively difficult to collect in a systematic fashion, and is seldom captured by cursory observation or pre-established databases.

A similar problem has been encountered in the social and health sciences, where researchers have attempted to identify links between an individual’s health, welfare, behaviour, etc., and the features of the neighbourhood in which they reside. In order to overcome this problem, researchers have developed the science of systematic social observation (SSO) (200, 201). The tools employed by SSO require the researcher to closely observe and quantify multiple different aspects of a city block, resulting in the creation of a comprehensive dataset that captures the complexity of the block environment (200, 201). These tools have revolutionized our understanding of impact of the urban environment on the people who reside within it.

Given that the urban environment also influences rat populations, it seems possible that a similar tool could be developed and used to predict rat abundance. There are several studies that have used a similar approach to study rats. However the tools and methodologies employed by these studies had several limitations including the following: capturing a limited number of environmental features (202), being
specific to rural (203) or developing areas (135, 204), and being difficult to reproduce (135, 204).

The goals of this study were: 1) to develop a systematic environmental observational tool that captures and quantifies features of a developed urban microenvironment that could influence rat populations; 2) to develop an approach to using that tool that includes analysis of both quantitative and qualitative data; and 3) to use that tool/approach to identify environmental factors associated with relative rat abundance within an inner-city neighborhood of Vancouver, Canada.

5.3 Methods

Development of an environmental observation tool

The environmental observation tool (see Appendix 2) was modeled after the SSO tool developed by Parsons et al. (2010). We utilized scales, items, and constructs from SSO tools (201, 205-207) that measured factors with the potential to influence rat populations, as well as from prior environmental observation studies of rat abundance, specifically those developed by (135, 202, 204). We also developed new items based on a review of the literature regarding rat ecology (154) and our field observations. The final tool included 58 items covering 6 major domains: (1) land use characteristics, (2) property condition, (3) green space characteristics, (4) alley surface characteristics, (5) presence of waste, and (6) alley use (i.e., for loitering and transportation). Additionally, there was a free form section at the end of the tool where observers could make notes about the block under evaluation. Particular attention was paid to identifying and describing features that were perceived to be associated with rat presence/abundance, as well as unusual or unique features of the block under study.

In order to facilitate the use of the tool in this study, and in the future, each domain and item was accompanied by detailed instructions regarding its purpose.
and/or use. Additionally, a supplementary document was produced with photographic examples of different items and ratings (see Appendix 3).

**Tool implementation**

The 43 city blocks were included in the study. The port was not included because trapping was not conducted in the same uniform and systematic method as the city blocks, preventing comparability of rat abundance at the port vs. in the blocks.

Two observers were trained in the use of the tool, and these raters completed their observations together between 8:00 am and 4:00 pm, with final ratings for each item being arrived at via consensus. To complete their observations, observers evaluated all aspects of the block including block faces (i.e., the area of the block facing a street), alleyways, and aerial photographs.

**Dependent Variable**

Given the relatively small number of black rats trapped in the city blocks, and the fact that the ecology of black rats can differ from that of Norway rats (154), black rats were excluded from the analysis.

The unit of analysis was the city block (n = 43). The dependent variable was trap success rate in each block, with trap success rate being equal to the number of rats trapped in a block over the entire trapping period divided by the total trap effort over that period. The total trap effort (# traps set x # nights) was adjusted according to the method described by Nelson and Clark (174).

Given that each block under study had roughly the same geometric surface area (1.2 ha) and was trapped in the same manner, and given that rat populations are thought to be relatively isolated to the level of a city block with minimal movement of animals among blocks (154), it is appropriate to use the city block as the unit of analysis.
and the number of rats trapped offset by trap effort as a proxy for relative population density/rat abundance.

To confirm the absence of geographic autocorrelation of rat abundance among the blocks, a georeferenced map of block outlines within the study area was imported into ArcGIS 10.0 (ESRI, Redlands, USA). Global Moran’s I was used to test the null hypothesis that trap success (# rat trapped/trap effort) within a block was randomly distributed (i.e., no spatial autocorrelation) using inverse distance for conceptualization of spatial relationships and a Euclidean distance method.

**Predictor variables**

A total of 59 potential predictor variables were included for initial consideration. Each of the 58 items on the environmental observational tool was used to create 1 predictor variable (note that variables were numbered according to the item numbering in the observation tool). The season in which the block was trapped (fall = September – November, winter = December – February, spring = March – May, summer = June – August) was also included as a predictor.

Seven variables (variable nos. 1, 2, 5, 13, 25, 31, and 41) were eliminated initially because they captured the same and/or less information as subsequent variables. For example, variable 13 is a nominal-categorical variable that characterizes the predominant housing type in a block, with 8 categories that each correspond to a class of housing. However, variable nos. 14-19 are ordinal variables that capture both the presence of each class of housing in a block, as well as the proportion of each block occupied by each class of housing.

Next, variables representing different aspects of the same concept were consolidated into a single new composite variable representing the weighted average of the underlying component variables. For example, variable nos. 26 – 30 all represent
building condition (the underlying concept of interest), and pertain to the proportion of each block occupied by buildings in ‘extremely poor’, ‘poor’, ‘fair’, ‘good’, and ‘excellent’ condition. These variables were combined into a single composite variable, as were variable nos. 32 - 36 and 42 – 44.

   Predictor variables where > 95% of observations had the same score (variable nos. 8, 12, 18, and 54) were deemed non-discriminatory and removed. Variable no. 46 (number of rat holes) was also removed because it was deemed to be a sign of rat abundance rather than a cause.

   A total of 37 predictor variables were considered for statistical modeling.

Model building

The goal of the model-building protocol was to identify the most parsimonious set of predictor variables that best explained the outcome, and all predictor variables were given equal consideration going into the model building procedure (i.e., we had no a priori hypotheses regarding the relative importance of specific predictors). Similarly, we had no a priori hypothesis regarding interactions among predictors, and, given the large number of potential predictors (n = 37) relative to the number of observations (n = 43), no interaction terms were included for consideration.

   A zero-inflated negative binomial (ZINB) model was used to model the relationship between the predictors and outcome in order to account for excess zeros (no rats were trapped in 11 of 43 blocks) and overdispersion in the outcome variable. This ZINB model combined a binary model with a logit link to model the odds of not trapping any rats (zero-inflation model), and a negative binomial model with a log link to model the number of rats trapped offset by the natural logarithm of the trap effort (count model). The unit of analysis was the city block (n = 43).
The majority of the predictor variables captured the proportion of the block occupied by an environmental factor of interest, and were therefore modeled in a linear manner. For several ordinal variables (variable nos. 48, 49, 57, and 58) where the spacing between categories was more subjective (e.g., ‘none’ vs. ‘some’ vs. ‘a little’ vs. ‘a lot’), bivariate models treating the variable in a linear and categorical manner were generated and compared using the Bayesian Information Criterion (BIC) (172). For all 4 predictors, the linear form of the variable was superior to the categorical form in both the binary and count portions of the model, therefore these variables were treated as numeric variables. Season was treated as a categorical variable.

In order to narrow down the pool of potential predictors for inclusion in a multivariate model, a program was created in R (R Development Core Team, Vienna, Austria) that performed automated combined forward and backward selection on each part of the model independently using Akaike's Information Criterion (AIC) to select the best potential group of predictors for further consideration (172). Briefly, in order to select the binary model, the negative binomial model was set at 1 (offset by the natural logarithm of the trap effort). The null binary model (a binary model with no predictors) was used as the starting point, and predictors were added sequentially based on their associated AIC (i.e., the predictor that produced the lowest AIC for the binary model was added first). After each addition, the model was checked to determine if removal of one or more predictors included in the model affected the AIC. If removal of the predictor(s) decreased the AIC for the binary model, then the predictor(s) was eliminated. If removal increased the AIC, then the predictor(s) was retained. This process was repeated until addition or elimination of any subsequent predictors could not further decrease the binary model AIC. The selection procedure described above was then repeated for the count model holding the binary model at 1. All 37 predictor variables, including season, were allowed to compete equally for both components of the model, with the stepwise selection procedure ensuring that only those variables that
improved model fit (according to the AIC) were retained. This process should control for confounding among predictors because it will select the variables with the strongest relationship to the outcome in the presence of all other predictor variables.

Variables identified through automated selection for the binary and count components of the ZINB model were then combined in a single model and manual forward and backward selection was performed using the BIC to maximize model fit and parsimony (as the BIC penalizes model complexity more heavily than the AIC) (172). The final model was that with the lowest BIC.

To investigate collinearity among explanatory variables in the final model, Spearman’s rank correlation ($\rho$) was used to examine bivariate relationships between each of the included predictors (172). Any highly correlated variables ($\rho > 0.8$) were modeled separately, with only the variable making the most significant contribution to the model (based on the BIC) being retained (172). Finally, the variables included in the final model were used to build a negative binomial (NB) model and the Vuong test was used to compare the NB and ZINB models with the null hypothesis that the two models were equivalent and the alternative hypothesis that the ZINB model was superior.

**Qualitative analysis**

Thematic and narrative qualitative analysis (208) was used in order to: 1) provide insight into the results of the quantitative analysis, 2) capture information not captured by the quantitative aspects of the tool, 3) provide increased resolution for environmental data by allowing the observer to note special features within the block (vs. the quantitative aspects of the tool, which pertained to the block as a whole), and 5) aid in triangulation for the purposes of identifying the environmental characteristics most significantly associated with rat abundance.
Data included in the qualitative analysis included written notes recorded in the environmental observational tool, as well as field notes recorded during a two-day long debrief at the end of the project. This debriefing involved re-visiting each block included in the study area and discussing observer opinions of the block environment and its relationship to rat abundance.

Handwritten notes were transcribed into Microsoft Excel (Microsoft, USA) and labeled with block number and block trap success. Emergent thematic analyses of early observations were discussed among the two observers and served to inform the focus of subsequent observations, as well as ongoing analyses. The coding framework employed a priori codes derived from the quantitative observational tool, as well as emergent codes based upon ongoing observational work. All qualitative data were reviewed, and text segments related to each individual code were categorized/classified (to identify commonalities among blocks and special features within a block). Subsequent coding passes were used to refine and expand code categories and to identify instances of negative evidence.
5.4 Results

Quantitative findings

There was no significant autocorrelation of rat abundance among blocks (p = 0.14) based on Global Moran’s I, supporting our assertion that the city blocks could be treated as independent units. The final ZINB model included one variable in the zero-inflation portion of the model and six variables in the negative binomial portion of the model (Table 5-1). None of the variables included in the final model were highly correlated (ρ < 0.45 for all comparisons) and the Vuong test indicated that the ZINB model was superior to the NB model (P = 0.03).

Table 5-1: Relationship between features of the urban environment and relative abundance of Norway rats (Rattus norvegicus) as measured by trap success.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable description</th>
<th>β</th>
<th>SE</th>
<th>P-Value</th>
<th>exp (β)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Count Model (negative binomial with log link)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Proportion of block occupied by abandoned parcels</td>
<td>0.70</td>
<td>0.32</td>
<td>0.027</td>
<td>2.02</td>
<td>1.08 – 3.77</td>
</tr>
<tr>
<td>14</td>
<td>Proportion of block occupied by single family houses</td>
<td>-0.67</td>
<td>0.25</td>
<td>0.007</td>
<td>0.51</td>
<td>0.31 – 0.83</td>
</tr>
<tr>
<td>19</td>
<td>Proportion of block occupied by housing over commercial</td>
<td>0.65</td>
<td>0.21</td>
<td>0.002</td>
<td>1.91</td>
<td>1.27 – 2.87</td>
</tr>
<tr>
<td>c26t30</td>
<td>General building condition</td>
<td>-2.01</td>
<td>0.58</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04 – 0.42</td>
</tr>
<tr>
<td>48</td>
<td>Amount of garbage/trash/junk/litter</td>
<td>0.83</td>
<td>0.28</td>
<td>0.003</td>
<td>2.29</td>
<td>1.33 – 3.93</td>
</tr>
<tr>
<td>58</td>
<td>Amount of transport</td>
<td>-0.99</td>
<td>0.24</td>
<td>&lt; 0.001</td>
<td>0.37</td>
<td>0.23 – 0.59</td>
</tr>
<tr>
<td></td>
<td><strong>Zero-Inflation Model (binomial with logit link)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Proportion of block occupied by institutional parcels</td>
<td>-3.42</td>
<td>1.44</td>
<td>0.012</td>
<td>0.03</td>
<td>0.002 – 0.55</td>
</tr>
</tbody>
</table>
The final model indicated that as the proportion of a block occupied by institutional parcels (i.e., those dedicated to not-for-profit services, such as churches, outreach centres, etc.) increased, the odds of not trapping any rats decreased (Table 5-1). As the proportion of a block occupied by single-family homes increased, the trap success rate decreased. In contrast, as the proportion of the block occupied by housing over commercial and by abandoned parcels (i.e., those with structures that were previously used but are now vacant and not undergoing construction or demolition) increased, the trap success rate increased. As general building condition increased, trap success rate decreased, whereas trap success rate increased as the as the amount of garbage/trash/junk/litter in the alley increased. Finally, as the use of an alley as a transportation corridor increased, trap success rate decreased.

Qualitative findings

Relationship between rat abundance and land use

Observers often reported trapping rats around abandoned buildings, demolished buildings, and vacant lots. In one block, a large parcel filled with debris from a demolished building was observed to be heavily infested with rats.

Blocks with low trap success tended to be described as being primarily residential (particularly single family homes) or primarily industrial (including industrial sites producing food), while blocks with high trap success tended to be described as being more mixed-use. Observers noted that there was no clear association between the presence of facilities dealing with food (e.g., restaurants, groceries, etc.) and rat presence/abundance.

Relationship between rat abundance and socioeconomic status of a block

Observers reported that rats were much more abundant in the impoverished DTES neighbourhood than the adjacent more affluent Gastown neighbourhood.
Observers also reported that blocks composed primarily of single-family homes appeared to be much more affluent than other residential areas, and that many of the low-rise apartments and housing over commercial was low income housing and single-room occupancy hotels (SROs). Low-income apartments, SROs, and outreach centres for impoverished community members were more commonly reported in blocks with high trap success. Also, within a block, observers often reported trapping rats near SROs.

**Relationship between rat abundance and property/building condition**

Buildings in disrepair were much more commonly reported in blocks with high trap success vs. blocks with low trap success. Also, within a block, observers often reported trapping rats around buildings in disrepair. Conversely, buildings and properties were much more commonly reported to be in good to excellent condition in blocks with low trap success. However, observers reported trapping rats around buildings in good repair and vice versa. Observers noted that all aspects of a block had to be assessed to adequately evaluate repair. In some blocks, the street face appeared to be in good repair while the alley face was in very poor repair.

**Relationship between rat abundance and green space**

Well maintained green space and food gardens were more often reported in blocks with low trap success. Unkempt green space was reported in blocks with high and low trap success. However, within a block, observers often reported trapping rats near unkempt green space.

**Relationship between rat abundance and waste**

Observers reported debris, strewn garbage, and overflowing garbage bins much more commonly in blocks with high trap success. Within a block, observers also reported trapping rats in close proximity to areas with accumulated refuse and around
dumpsters. Blocks with low trap success were most commonly described as clean with very little garbage. However, observers also noted that some blocks with high trap success were relatively clean. Observers commented that rats were not caught around organic waste bins or compost piles.

Relationship between rat abundance and condition of the alley surface

Blocks with high trap success tended to be described as having pavement in disrepair, cracked pavement, unpaved areas, and rat holes, while blocks with low trap success tended to be described as being well paved and having pavement in good repair. Within an alley, observers reported trapping rats around areas where the pavement was in disrepair and/or deeply cracked, around unpaved areas, and around rat holes, regardless of the condition of the rest of the alley. However, some blocks with high trap success were reported to have good alley surface condition and vice versa.

Sources of harborage for rats

Observers suggested that most rats likely live in buildings or in underground burrows (rat holes were commonly seen in blocks with high trap success). However, observers also reported trapping rats near areas with stored wood pallets, plastic barrels, and other containers. In four blocks, observers reported frequently catching rats near rat corridors (i.e., access points, such as gaps between buildings, which connected the alley face to deeper aspects of the block).

Effect of human activity on rat presence

Heavy use of alleyway by people (particularly people loitering) was reported in blocks with high and low trap success, but was more commonly reported in blocks with high trap success.
Observers noted that the alleyways more heavily used for loitering tended to be dirtier (i.e., have more accumulated refuse).

5.5 Discussion

Using quantitative and qualitative analysis, we were able to identify a variety of environmental factors associated with rat abundance. We also found that once observers were comfortable with the use of the tool, its implementation was relatively efficient and straightforward.

As has been found in previous studies using SSO, the combination of quantitative and qualitative analysis was particularly beneficial. Not only did the qualitative data support and elucidate the quantitative results, these data also provided additional insight into environmental variations within a block, and drew our attention to factors not well captured by the quantitative aspect of the tool (e.g., the apparent association between rat abundance and poverty), as well as inconsistencies in the relationship between certain environmental factors and relative rat abundance.

This study was able to identify a number of factors associated with relative rat abundance that have not been studied and/or well characterized previously (e.g., specific land use, residential density, and building disrepair). Several of our findings, however, are in agreement with previous research on the relationship between environmental factors and rat abundance. For example, studies have been able to show that the presence of exposed garbage is a strong predictor/promoter of rat infestations (121, 204). Our study also found that rats were relatively less common in blocks where the land use was predominantly industrial and single-family residential, and this is consistent with the findings of Cavia et al. (2009), who also found that Norway rats were uncommon in industrial and residential neighborhoods.

Other studies have also indicated that factors associated with poverty can promote rat infestations, likely due to a combination of factors including infrastructural
disrepair and decreased environmental hygiene, which combine to create ideal urban rat habitat (138, 147). Poverty might also impair an individual’s or community’s ability to prevent or eliminate infestations (e.g., through neighborhood rehabilitation or employing pest control professionals). In our study, there were several variables that, on first glance, are difficult to intuitively link to rat abundance, including proportion of block occupied by institutional parcels and housing over commercial. However, upon further examination, and in light of the qualitative data, it became clear that much of the housing over commercial was low-income housing, including single room occupancy hotel rooms (SROs). Similarly, institutional parcels in the area are primarily composed of outreach centers for impoverished members of the community. Observers specifically noted that rats were often trapped around SROs and that blocks with high trap success tended to have more SROs, low-income housing, and outreach centers. Observers also noted that far more rats were trapped in the impoverished DTES compared to the adjacent, more affluent Gastown district, and that blocks composed primarily of single-family residences were also more affluent than the rest of the DTES. Factors potentially associated with poverty were associated with trap success independent of variables accounting for infrastructural disrepair and accumulation of waste. This suggests that there are likely one or more environmental variables associated with poverty that were not accounted for in this study.

It is interesting to note that season did not appear to have a significant relationship with rat presence or abundance. Indeed, there has been considerable debate in the literature regarding the impact of season on rats in urban ecosystems (154). The absence of seasonal variation in urban rat population size might not be surprising given that urban ecosystems, being largely under anthropogenic control, would seem to be less prone to seasonal fluctuation in resource availability (i.e., the presence of suitable food and harborage) compared to more sylvatic settings. However, given that this study
only included one year’s worth of data, the true impact of season could not be definitively determined (183).

There were also a number of findings in our study that contrast those found in past research on the subject. For example, green space has been reported to be a strong promoter of rat infestations (121, 195). In particular, a study by Traweger et al. (2008) found that rats were most abundant in more natural areas with abundant vegetation, and that trap success was very low in areas with little vegetation, as well as heavily built-up areas, and areas frequented by humans. Our study found that there was no relationship between amount of green space and rat abundance, that rats were very common in built up areas, and that human activity (in the form of loitering) appeared to have a negligible to positive relationship with rat abundance. Increased use of an alley as a transport corridor did appear to have a negative association with the number of rats trapped. However, it is difficult to determine if this because rats avoid areas of increased activity or if alleys used for transport tended to be in better condition or in areas with other features that deter rat infestations. Overall, it is interesting to note that although Traweger et al. (2008) speculate that natural areas are essential to providing appropriate rat habitat, the results of our study indicate that Norway rats can thrive even in the most urbanized of settings.

Interestingly, the qualitative data also indicated the presence of conflicts within our own study. For example, although strewn garbage seemed to be strongly associated with rat abundance in general, there were some alleys and/or alley segments where rats were caught that appeared relatively clean. Similarly, although many rats were caught in alleys with unpaved areas, cracked pavement, and/or rat holes, some alleys with robust rat populations appeared to have relatively good pavement condition. These conflicts, in combination with the variety of environmental factors that were associated with trap success in our study, suggest that the relationship between rat abundance and the environment is a function of a complex interaction of factors. In other words, there
is not one single environmental factor that is a necessary determinant of rat abundance. Rather, a variety of different factors can combine to create suitable rat habitat.

Despite the success of the methodology, this study had several limitations. The relatively low number of experimental units (n = 43) limited the number of variables that could be included in the model. Future studies should seek to increase power (i.e., the number of blocks included) in order to better investigate the full suite of variables that might predict rat abundance. With regard to our trapping methodology, only rats accessing the back alley were accounted for in our measure of rat abundance. Although this ‘alley trapping’ approach is consistent with previous trapping studies of urban rats (143, 199), it is possible that some rats could reside entirely indoors and/or not access the alley. This may particularly be the case for black rats, as it has been suggested that these species prefer to reside in human structures (154). Indeed, in this study, it could not be determined if the low number of black rats was a result of the trapping methodology, or whether this species is truly rare within this urban area. Ideally, future studies should account for all indoor and outdoor rat populations within a block. However, as this would require intensive trapping on private property, it may not be a feasible proposition. Finally, it should be noted that while many factors have a clear direct relationship with rat abundance (e.g., presence of exposed garbage); other factors (e.g., land use) likely represent some underlying and unmeasured factor(s) that influence rat populations. Although these environmental ‘proxies’ may be valuable for predicting rat abundance, they are less useful for developing intervention strategies. After all, it is not reasonable to change the land use in a block to prevent rat infestations. It may therefore be useful for future studies to focus on and dissect the relationship between rat abundance and factors like land use and poverty.

Overall, this tool proved to be an efficient and effective way to examine the effect of urban environmental factors on relative rat abundance. In addition to their effects of rat abundance, environmental factors are also known to influence pathogen prevalence.
in rat populations (16). In the future, we hope to determine whether this tool could also be used to identify features of the urban environment associated with the prevalence of zoonotic microbes in rats.
Chapter 6: Ecology of *Leptospira interrogans* in Wild Urban Norway Rats

6.1 Synopsis

*Leptospira interrogans* is a bacterial zoonosis with a worldwide distribution for which rats (*Rattus* spp.) are the primary reservoir in urban settings. In order to assess, monitor, and mitigate the risk to humans, it is important to understand the ecology of this pathogen in rats. The objective of this study was to characterize the ecology of *L. interrogans* in Norway rats (*Rattus norvegicus*) in the DTES. Kidney samples from trapped rats were tested for the presence of *L. interrogans* using PCR and sequencing. A multivariable model was built to predict *L. interrogans* infection status in individual rats using season and demographic/morphometric data (e.g., mass, sex, maturity, condition, etc.) as independent variables. Spatial analysis was undertaken to identify clusters of high and low *L. interrogans* prevalence. The prevalence of *L. interrogans* varied remarkably among blocks (0 - 66.7%), and spatial clusters of both high and low *L. interrogans* prevalence were identified. In the final cluster-controlled model, characteristics associated with *L. interrogans*-infection in rats included mass (OR = 1.14, 95% CI = 1.07 – 1.20), increased internal fat (OR = 2.12, 95% CI = 1.06 – 4.25), and number of bite wounds (OR = 1.20, 95% CI = 0.96 – 1.49). Because *L. interrogans* prevalence varied with mass, body fat, and bite wounds, this study suggests that social structure and interactions among rats may influence transmission. The prevalence and distribution of *L. interrogans* in rats was also highly variable even over a short geographic distance. These factors should be considered in future risk management efforts.
6.2 Introduction

*Leptospira interrogans*, is among the most wide-spread of the urban rat-borne zoonoses, and is probably associated with the greatest human health burden, causing disease in both developing and developed nations (10, 26, 27). This bacterium asymptotically colonizes the rat kidney and is shed in the urine (10, 27), and direct or indirect contact with this urine can result in human infection (27). In people, infection can cause fever with progression to jaundice, renal failure, and pulmonary haemorrhage (10, 27).

As with other zoonotic diseases, it is important to characterize the ecology of *L. interrogans* in the animal reservoir in order to develop an in-depth understanding of disease risk in people, and to develop intervention strategies aimed at disease prevention (209). For example, by studying the ecology of *L. interrogans* in urban Norway rats, it may be possible to identify environmental and/or population characteristics that increase or decrease the prevalence of infection in rat populations, which will, in turn, influence the probability that people living in the same geographic area will be exposed to the bacterium. This information may facilitate the development of rat control strategies aimed at zoonotic disease prevention, specifically.

Previous studies have indicated that the prevalence of *L. interrogans* in rat populations is highly variable both among cities, and among different locations within the same city (107, 115-117, 158, 181, 210, 211). However, the factors influencing this variability in prevalence are unclear. Similarly, although some studies have found that the probability that a rat will be infected with *L. interrogans* increases with age (158, 181, 211), and with female sex (158), other studies have shown no association between *L. interrogans* infection and one or both of these variables (107, 116, 181, 211). Overall, there is a paucity of epidemiologic data regarding *L. interrogans* in urban rat populations.
This knowledge gap is a result of the fact that the complex ecology and biology of rats are seldom taken into account when studying the pathogens they carry. For example, many studies seek only to characterize *L. interrogans* prevalence and not to investigate the factors influencing prevalence (115, 117, 133, 165, 212). Frequently, the population to which the sampled rats belong is unclear or ignored altogether, making even these simple statistics of questionable value. Meanwhile those studies with a more epidemiologic focus often lack sufficient ecologic data on the rats under study to provide an in-depth analysis of *L. interrogans* dynamics in that population (123). Finally, some key studies have used the presence of anti-*L. interrogans* antibody as a proxy for infection (158); however, serostatus correlates poorly with infection status in rats (i.e., many infected rats do not develop antibodies against the infecting strain of *L. interrogans*) (117, 132, 165), likely because of the close evolutionary relationship between rats and *L. interrogans* (27).

The objectives of this study were: 1) to determine whether *L. interrogans* is present in Norway rats residing in the DTES; and 2) to use ecologic data on these rat populations to characterize the prevalence and distribution of *L. interrogans* within the DTES and the degree to which season and population characteristics influence the ecology of this bacterium.

6.3 Methods

**Sample selection**

Rats were trapped and processed as described previously (Chapter 2). During necropsy, ½ of one kidney was collected aseptically and stored at -80°C until DNA extraction.

A total of 630 rats were selected for *L. interrogans* testing. This number was arrived at by calculating the number of rats that would need to be tested within each block and the port site in order to accurately calculate the prevalence of *L. interrogans*.
within that location. This calculation was performed using the sample size for proportions function in the program Ecological Methodology (Exeter Software, Setauket, USA) with an expected proportion of 50.0%, a desired margin of error of 5%, and a fixed population correction for the size of the trapped population of the block in question. For each block, the rats to be tested were selected randomly.

**Leptospira interrogans PCR**

At the AHC, DNA was extracted from rat kidney tissues using the QiaAMP DNA Mini kit (Qiagen Inc., Canada). DNA extracts were then amplified using a real-time PCR (qPCR) assay which targets a 242 bp fragment of the *lipL32* gene of pathogenic *Leptospira* species (213). *Leptospira interrogans* serovar Copenhageni and nuclease-free water were used positive and negative controls, respectively.

A subsample of 21 randomly selected qPCR-positive kidney samples (approximately 1/3 of all qPCR-positive samples) underwent conventional PCR with DNA sequencing to confirm the presence of *L. interrogans* (214). Purified PCR products were sequenced using the ABI 3130 Genetic Analyzer and results were interpreted using DNASTAR Lasergene 10 SeqMan Pro program. All 21 samples were identified as *Leptospira interrogans*.

**Statistical analysis**

The primary outcome variable was *L. interrogans* infection status (positive vs. negative). Given that *L. interrogans* is the species of *Leptospira* spp. carried by rats, and given that all sequenced PCR products were identified as *L. interrogans* (vs. other *Leptospira* spp.), a rat was considered to be infected with *L. interrogans* if it was positive on the qPCR.

Explanatory variables that were considered included season, mass, length, sex, sexual maturity, body condition score, fat score, presence of cutaneous bite wounds,
and number of cutaneous bite wounds, as well as parity and pregnancy/lactation in females (see Table 6-1).

To identify characteristics associated with *L. interrogans* infection status, the distribution of the explanatory variables were examined among the sample as whole, as well as separately for *L. interrogans*-positive and -negative rats. Regression modeling was used to examine relationships between *L. interrogans* infection and each of the explanatory variables, first using simple logistic regression, and then using multiple logistic regression (MLR) and multiple logistic GLMMs (to control for the random effect of block). For each multivariate model, the final model was arrived at by manual stepwise regression using AIC to compare candidate models (the final model was that with the lowest AIC) (172). By creating both a MLR model and a GLMM, we were able to appreciate the effect of cluster-control. The dataset was then stratified and the final MLR and GLM models run in males vs. females to identify effect modification by sex.

All statistical analyses were conducted using R (R Development Core Team, Vienna, Austria). For multivariate models, individuals with missing data for one or more of the variables under study were excluded. Collinearity among explanatory variables was identified using Spearman’s rank correlation ($\rho$) (172). Highly correlated variables ($\rho > 0.8$) were modeled separately (172).

**Spatial analysis**

The location of each trap within the 43 block area of the DTES, and the number of rats caught in each trap that were *L. interrogans*-positive and -negative were mapped using ArcGIS 10.0 (ESRI, Redlands, USA). This information was imported into SaTScan (Boston, USA) for cluster analysis using a purely spatial Bernoulli model and scanning for areas with high and low rates of *L. interrogans* infection using a circular window with a maximum spatial cluster size of 50% of the population at risk. Clusters identified by SaTScan were visualized in ArcGIS. The port site was excluded from this analysis.
because trapping took place at multiple levels within a single geographic foot-print (which is difficult to represent in a two dimensional map) and because trapping was somewhat more opportunistic (vs. systematic) compared to the blocks.

### 6.4 Results

Among the 630 rats tested for *L. interrogans* by PCR, only 38 (6.0%) were black rats while the remainder were Norway rats. None of these 38 black rats were positive for *L. interrogans*, therefore black rats were removed from the analytic sample. The statistics in Table 6-1 pertain to Norway rats only (n = 592).
Table 6-1: Baseline characteristics and associations with *L. interrogans* PCR status among Norway rats (*Rattus norvegicus*).

<table>
<thead>
<tr>
<th>Category</th>
<th>Subcategory</th>
<th>Total (%)&lt;sup&gt;a&lt;/sup&gt; (n = 592)</th>
<th><em>L. interrogans</em> PCR status</th>
<th>p-value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Positive (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Negative (%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 66)</td>
<td>(n = 66)</td>
<td>(n = 526)</td>
</tr>
<tr>
<td>Season</td>
<td>Fall</td>
<td>191 (32.3)</td>
<td>44 (66.7)</td>
<td>147 (27.9)</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>113 (19.1)</td>
<td>14 (21.2)</td>
<td>99 (18.8)</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>209 (35.3)</td>
<td>8 (12.1)</td>
<td>201 (38.2)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>79 (13.3)</td>
<td>0 (0)</td>
<td>79 (15.0)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>325 (54.9)</td>
<td>39 (59.1)</td>
<td>286 (54.4)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>259 (43.8)</td>
<td>27 (40.9)</td>
<td>232 (44.1)</td>
</tr>
<tr>
<td>Sexual maturity</td>
<td>Mature</td>
<td>328 (55.4)</td>
<td>64 (97.0)</td>
<td>264 (50.2)</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>Median (IQR)</td>
<td>123.7 (63.6 – 252.8)</td>
<td>302.5 (252.0 – 344.9)</td>
<td>96.2 (59.2 – 221.2)</td>
</tr>
<tr>
<td>Nose-to-rump length (cm)</td>
<td>Median (IQR)</td>
<td>16.5 (13.0 – 21.0)</td>
<td>21.8 (21.0 – 23.0)</td>
<td>15.5 (13.0 – 20.0)</td>
</tr>
<tr>
<td>Total length (cm)</td>
<td>Median (IQR)</td>
<td>31.5 (25.2 – 39.0)</td>
<td>40.8 (39.0 – 42.7)</td>
<td>29.2 (25.0 – 37.9)</td>
</tr>
<tr>
<td>Body condition score</td>
<td>Median (IQR)</td>
<td>2.5 (2.0 – 3.0)</td>
<td>2.5 (2.0 – 3.0)</td>
<td>2.5 (2.0 – 3.0)</td>
</tr>
<tr>
<td>Fat score</td>
<td>Median (IQR)</td>
<td>1.0 (0.0 – 2.0)</td>
<td>2.0 (1.0 – 1.67)</td>
<td>1.0 (0.0 – 1.0)</td>
</tr>
<tr>
<td>Wound presence</td>
<td>No</td>
<td>477 (75.5)</td>
<td>29 (43.9)</td>
<td>418 (79.5)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>145 (24.5)</td>
<td>37 (56.1)</td>
<td>108 (20.5)</td>
</tr>
<tr>
<td>Wound number</td>
<td>Median (IQR)</td>
<td>0.0 (0.0 – 0.0)</td>
<td>1.0 (0.0 – 2.0)</td>
<td>0.0 (0.0 – 0.0)</td>
</tr>
<tr>
<td>Parous&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No</td>
<td>180 (69.5)</td>
<td>6 (22.2)</td>
<td>174 (75.0)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>73 (28.2)</td>
<td>20 (74.1)</td>
<td>53 (22.8)</td>
</tr>
<tr>
<td>Pregnant&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No</td>
<td>227 (87.6)</td>
<td>18 (66.7)</td>
<td>209 (90.1)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>26 (10.0)</td>
<td>8 (29.6)</td>
<td>18 (7.8)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Frequencies and percentages may not add to 100% because of exclusion of rats with missing data for the variable in question.

<sup>b</sup>Females only (n = 259).

<sup>c</sup>Determined using the Chi-squared test, Fisher’s exact test, or Welch’s t-test, where appropriate.

The overall prevalence of *L. interrogans* was 11.1% (66/592). However, there was marked variation in the prevalence of *L. interrogans* by block, with prevalence ranging from 0% to 66.7% (see Figure 6-1).
On bivariate analysis, the odds of being *L. interrogans*-positive was less in rats caught in the spring (OR = 0.14, 95% CI = 0.06 – 0.28) or winter (OR = 0.47, 95% CI = 0.24 – 0.89) compared to the fall (see Table 6-2). The odds of being *L. interrogans* positive was also less in sexually immature rats compared to mature rats (OR = 0.02, 95% CI = 0.001 – 0.09). The odds of being *L. interrogans*-positive increased with increasing mass (OR = 1.15, 95% CI = 1.12 – 1.19), nose-to-rump length (OR = 1.65, 95% CI = 1.46 – 1.90), and total length (OR = 1.32, 95% CI = 1.23 – 1.43). Rats with bite wounds had greater odds of being *L. interrogans*-positive compared to rats with no bite wounds (OR = 4.94, 95% CI = 2.91 – 8.44), and the odds of being *L. interrogans*-positive increased with increasing number of bite wounds (OR = 1.45, 95% CI = 1.25 – 1.69). Body condition score was not significantly associated with *L. interrogans* infection status (OR = 1.04, 95% CI = 0.73-1.49), however, the odds of being *L. interrogans*-positive was greater in rats with a greater volume of internal fat (OR = 5.12, 95% CI = 3.40 – 8.13).
In the final multivariable logistic regression model, season (OR = 0.19, 95% CI = 0.07 – 0.45 for spring vs. fall and OR = 0.31, 95% CI = 0.14 – 0.67 for winter vs. fall), wound number (OR = 1.19, 95% CI = 0.99 – 1.41), mass (OR = 1.12, 95% CI = 1.07 – 1.17), and fat score (OR = 2.10, 95% CI = 1.20 – 3.78) were retained (see Table 2). The relationship between these variables and *L. interrogans* positivity was in the same direction but of decreased magnitude compared to bivariate analysis. After controlling for clustering by block, only mass, wound number, and fat score were retained. In the final GLM model, the odds of being *L. interrogans*-positive increased with increasing mass (OR = 1.14, 95% CI = 1.07 – 1.20), volume of internal fat (OR = 2.12, 95% CI = 1.06 – 4.25), and number of bite wounds (OR = 1.20, 95% CI = 0.96 – 1.49), although the last relationship was only marginally significant.

Upon stratifying the models by sex, there was no apparent difference in the relationship between the explanatory variables and *L. interrogans* positivity in males vs. females. Among females, the odds of being *L. interrogans*-positive was higher in rats that were parous (vs. non-parous) and pregnant (vs. non-pregnant). However, neither pregnancy nor parity was significantly associated with *L. interrogans* positivity, or improved model fit, once incorporated into a multivariable model.
### Table 6-2: Unadjusted and adjusted odds ratios for being *L. interrogans* PCR-positive among Norway rats (*Rattus norvegicus*).

<table>
<thead>
<tr>
<th>Category</th>
<th>Subcategory</th>
<th>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adjusted (MLR)&lt;sup&gt;f&lt;/sup&gt;</th>
<th>95% CI</th>
<th>Adjusted (GLMM)&lt;sup&gt;g&lt;/sup&gt;</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>Fall</td>
<td>Ref&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>Ref</td>
<td></td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.14</td>
<td>0.06 – 0.28</td>
<td>0.19</td>
<td>0.07 – 0.45</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>0.47</td>
<td>0.24 – 0.89</td>
<td>0.31</td>
<td>0.14 – 0.67</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Ref</td>
<td></td>
<td>--</td>
<td></td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.85</td>
<td>0.50 – 1.43</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Maturity</td>
<td>Mature</td>
<td>Ref</td>
<td></td>
<td>--</td>
<td></td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immature</td>
<td>0.02</td>
<td>0.001 – 0.09</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Wound presence</td>
<td>No</td>
<td>Ref</td>
<td></td>
<td>--</td>
<td></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4.94</td>
<td>2.91 – 8.44</td>
<td>1.19</td>
<td>0.99 – 1.41</td>
<td>1.20</td>
<td>0.96 – 1.49</td>
</tr>
<tr>
<td>Wound number</td>
<td>No</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.45</td>
<td>1.25 – 1.69</td>
<td>1.19</td>
<td>0.99 – 1.41</td>
<td>1.20</td>
<td>0.96 – 1.49</td>
</tr>
<tr>
<td>Mass (10g)</td>
<td>No</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<sup>a</sup> Odds ratio (OR) with 95% confidence interval (CI).

<sup>b</sup> Reference category.

<sup>c</sup> Insufficient power to provide a meaningful estimate for this category.

<sup>d</sup> Females only (n = 259).

<sup>e</sup> Results of bivariate modeling.

<sup>f</sup> MLR = Multiple logistic regression model

<sup>g</sup> GLMM = Generalized linear mixed model. *Note that both models are presented in order to demonstrate how results change after controlling for clustering by block. In the final GLMM, the estimated variance for the random effect of block was 4.34, indicating that block of origin had a significant impact on *L. interrogans* infection status.*
In the final GLMM, the estimated variance for the random effect of block was 4.34, indicating that block of origin had a significant impact on \( L. \) \textit{interrogans} infection status. Geographic clustering of cases was also evident on spatial analysis, which identified one cluster (very spatially compact) with greater than expected prevalence of \( L. \) \textit{interrogans} infection and two clusters with lower than expected prevalence of \( L. \) \textit{interrogans} spp. infection (see Figure 6-2).

**Figure 6-2:** Distribution of \( L. \) \textit{interrogans}-positive Norway rats (\textit{Rattus norvegicus}) and clusters of high and low \( L. \) \textit{interrogans} prevalence in an inner-city neighborhood of Vancouver, Canada. Observed vs. expected number of \( L. \) \textit{interrogans}-positive rats with relative risk and p values noted for each cluster. Inset = Map of Vancouver with location of study site.
6.5 Discussion

Overall, this study demonstrates that *L. interrogans* is present in Norway rats within this inner-city neighbourhood; however, the distribution of *L. interrogans* is not uniform. Previous studies have shown that the prevalence of *L. interrogans* in rats may vary among different locations within a city (107). However, this study showed that marked clustering can take place even over a very small geographic distance within a single neighbourhood.

The presence of clustering is consistent with what is known about the ecology of rats in urban centers. The size of a rat’s home range is determined by the availability of suitable harborage and food sources, social pressure from conspecifics or other rat species, and the presence of barriers to rat movement (138, 169). In urban centers, the ubiquity of resources and the barrier-effect of roadways combine to result in small home ranges that are often limited to a city block (138, 175). In the absence of drastic changes to the environment, long distance migration is uncommon (138). Additionally, rats are both colonial and territorial (164, 196); therefore, conspecifics residing in the same geographic area are likely to be members of the same colony and interact. For these reasons it is not surprising that the presence of numerous functionally distinct rat colonies would lead to heterogeneity in pathogen distribution within an urban ecosystem.

Clustering, however, has significant implications for analysis and interpretation of epidemiologic data in rats. It suggests that aggregated city-level pathogen prevalence, as is often reported (158, 165, 211), is not the best measure of *L. interrogans* frequency. Rather, it may be more valid to measure prevalence at the level of the colony or block. Clustering must also be taken into account when attempting to identify factors that influence the prevalence *L. interrogans* in order to avoid bias associated with variation in distribution of disease determinants among clusters. For example, in this study, season appeared to be a predictor of *L. interrogans* status in the MLR model. After
controlling for clustering, however, the effect of season was no longer significant. This suggests that the apparent effect of season may have been an artifact resulting from the fact that blocks with a high *L. interrogans* prevalence were trapped in one particular season.

In the final GLMM, body mass was positively associated with *L. interrogans* infection status. Specifically, a 10 g increase in the mass of a rat was associated with a 14% increase in the odds of that rat of being *L. interrogans*-positive. This association has been noted in previous studies of *L. interrogans* in urban rats (107, 158, 181), and was presumed to be a result of the fact that the older the animal (as mass is a good proxy for age (138)), the greater the likelihood that it will become exposed to and infected with *L. interrogans* (107, 181). However, given that *L. interrogans* is thought to be transmitted through urine (27), and given the colonial nature of rats and the opportunity for direct and indirect exposure to conspecifics from birth (164), it is surprising that young rats do not become exposed earlier in life. In this study, the median mass of *L. interrogans*-positive rats was 302.5 g, which is consistent with the mass of an adult rat, compared to 96.2 g for *L. interrogans*-negative rats, which is consistent with the mass of a juvenile (138). This suggests that the mechanism of *L. interrogans* infection in rats may be more complex than random environmental exposure, or else one would expect to see infection occur soon after young leave the nest.

This complexity is supported by the fact that the volume of internal fat, independent of mass, was also significantly associated with *L. interrogans* positivity, and number of bite wounds was marginally significant. Specifically, the odds of being *L. interrogans*-positive were more than twice as high for a rat with higher fat score compared to a rat with a lower fat score, and increased by 20% with each bite wound. This may suggest that *L. interrogans* transmission has a social or behavioural aspect. For example, dominant rats have greater access to food resources and are more likely to engage in aggressive behaviour compared to non-dominant rats (138, 163, 164). This
could result in dominant rats having greater internal fat stores and a higher incidence of bite wounds. Additionally, dominant rats are generally heavier than subordinate rats (138). It may therefore be the case that body fat, bite wounds, and/or mass are markers of social hierarchy, and the relationship between these variables and *L. interrogans* infection status is, at least in part, a result of behaviour. For example, dominant rats may exhibit more exploratory behaviour and have more contact with conspecifics compared to subordinates (164), both of which could increase the opportunity for exposure to *L. interrogans*.

Some studies, however, have found that high-ranking rats actually have fewer bite wounds compared to low-ranking rats (138), presumably because the low-ranking rats are more frequently on the ‘losing end’ of intraspecific conflicts. If this was the case then the association between number of bite wounds and *L. interrogans* positivity observed in this study might suggest that biting could be a method of *L. interrogans* transmission among rats. *Leptospira interrogans* has been transmitted from a rat to a human through biting (215), and biting is an important mode of rat-to-rat transmission of Seoul hantavirus, another rat-associated pathogen (69).

The potential role of maternal antibody transfer in mediating *L. interrogans* infection dynamics also deserves consideration. Maternal antibody has been shown to prevent persistent infection with Seoul hantavirus in rats, and may persist for up to 5 months of age (216). Maternal transfer of antibody could also delay infection with *L. interrogans*, and might partially explain why infection was uncommon in juveniles. That being said, *L. interrogans* are extremely well adapted to their reservoir hosts (27), and many rats infected with *L. interrogans* do not have detectable circulating antibody (117, 165). For this reason further study is needed to determine if maternal antibody plays a role in the ecology of *L. interrogans* in rats.

One limitation of this work is the fact that very few black rats were included in this study sample. This could be a result of the trapping methodology (i.e., trapping on
the ground in outdoor areas), which may bias towards trapping Norway vs. black rats. This is because Norway rats more commonly reside in outdoor underground burrows, while black rats are more commonly found in the upper levels of man-made structures (194). Indeed, previous studies have found that placing traps in elevated positions inside structures (e.g., in the roof) increased catch success for black rats (204). Alternatively, it could be the case that Norway rats are truly more ubiquitous in this urban environment compared to black rats. Norway rats, as a species, are larger and more aggressive compared to black rats, and tend to displace black rats where the two species coexist (138, 194, 196). In this study, none of the black rats were infected with *L. interrogans*, which may reflect either inadequate sampling or a low prevalence of disease in this species. Interestingly, other studies of *L. interrogans* in Norway and black rats have also found a comparatively low prevalence of infection in the black rats (123). This could be a result of the tendency of black rats to nest off the ground (as opposed to the ground-burrowing Norway rat) (194), which could decrease their exposure to urine-borne pathogens. Given the fact that Norway and black rats differ in many aspects of their ecology (163, 194), it is possible that the prevalence and epidemiology of *L. interrogans* differs between these two species. Future studies should seek to study *L. interrogans* in black rats, specifically.

In this study, we were able to show that the number of *L. interrogans*-infected rats in a block was not related to the size of the resident rat population. In other words, contrary to conventional wisdom, a larger rat infestation does not necessarily equal a larger *L. interrogans*-associated disease risk. Rather, it appeared that there was some block-level characteristic(s) that had a significant impact on *L. interrogans* prevalence. Although we could not identify these characteristics, it is our suspicion that they are likely features of the block environment.

Rats themselves are strongly influenced by the environment in which they reside (121, 204); therefore it seems likely that the environment would also influence *L.
*L. interrogans* ecology, either directly or indirectly through its effect on population ecology. The environment within our study area, however, is relatively uniform, being composed primarily of high-density residential and commercial properties with minimal green space. There were no obvious systematic differences between blocks with a high and low prevalence of *L. interrogans*. It is likely therefore, that small differences in block composition (e.g., availability of soil for burrowing and volume of exposed garbage), rather than high-level ecosystem differences, are influencing the distribution of *L. interrogans* in this environment. Future studies of *L. interrogans* in rats should seek to quantify these subtle differences and relate them to the *L. interrogans* ecology.

The findings from this study have potential public health significance as they suggest that the risk of a person being exposed to rat-associated *L. interrogans* is highly heterogeneous across the urban environment and not necessarily dependent on the number of rats infesting a particular area. Additionally, the association between *L. interrogans* and mass seems to suggest that established populations with a high proportion of adults pose a greater risk than populations with mostly juvenile rats. Given that large, dominant rats may be preferentially removed by trapping and poisoning campaigns, due to their propensity towards exploratory behaviour and competitive exclusion of subordinates (163, 217), it may be the case that trapping and poisoning may preferentially remove *L. interrogans*-infected rats. That being said, rodent control activities may also disrupt social structures, trigger long-distance rat migrations, and result in intraspecific antagonism (138, 217), any or all of which could have unpredictable effects on *L. interrogans* dynamics. Indeed in other situations, attempts to control disease in wild animals through culling have caused a paradoxical increase in disease prevalence by disrupting otherwise stable populations and thereby increasing disease transmission (112). For this reason future studies should aim to determine the impact of rat control strategies on *L. interrogans* dynamics in urban rats.
In conclusion, this study shows that the ecology of *L. interrogans* in Norway rats is inextricably intertwined with rat population ecology and that further study is needed in order to identify micro-environmental factors influencing *L. interrogans* prevalence in rats, to determine if the ecology of this bacterium varies among different rat species, and to determine how rat control strategies might impact *L. interrogans* dynamics in cities. Given the increasing incidence of urban rat-associated leptospirosis in people (16), and given that the ecology of *L. interrogans* and its rat reservoir hosts have a significant impact on the risk of transmission to people (16), it is clear that these further studies will be necessary if we are to proactively assess this potential public health threat.
Chapter 7: No evidence of infection with Seoul hantavirus, *Bartonella* spp., and *Rickettsia typhi* in Wild Urban Norway and Black Rats

7.1 Synopsis

Urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) are reservoirs for a variety of zoonotic pathogens responsible for significant human morbidity and mortality in cities around the world. Many of these pathogens, including *Rickettsia typhi*, *Bartonella* spp., and Seoul hantavirus, are thought to be endemic in rat populations worldwide, and are thus expected to be present wherever the reservoir host is found. Past field research, however, has suggested that the distribution of these organisms is likely less consistent than previously thought. No evidence of infection with Seoul hantavirus, *Bartonella* spp., or *Rickettsia typhi* was found on PCR or serology in DTES rats. The underlying reason(s) for the apparent absence of these pathogens is unclear, but could suggest that founding rat populations were not infected with these organisms. Further research is needed to fully describe the global distribution and ecology of rat-associated zoonoses. In particular, knowledge of disease absence, as well as presence, is needed to efficiently and effectively allocate resources for the monitoring and mitigation of rat-associated public health risks.

7.2 Introduction

Urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) are known to be the reservoir for a number of different zoonotic pathogens, including *Leptospira interrogans*, *Rickettsia typhi*, *Bartonella* spp., *Yersina pestis*, and Seoul hantavirus (SEOV) (16, 30). With the exception of plague (*Yersina pestis*), the majority of rat-associated zoonoses are thought to have a worldwide distribution, suggesting that they are likely to be endemic wherever Norway and black rats are found (16, 30).
That being said, studies that specifically seek to quantify the prevalence of these pathogens in urban rat populations have occasionally found them to be absent (51, 69, 134, 218-220). It many cases, it is unclear if absence is simply a result of undersampling (51, 69, 134, 219, 220) or variability in disease detection methods (134, 221). When this uncertainty is coupled with the relative paucity of research on urban rats, the true distribution of rat-associated zoonoses becomes unclear.

From a public health perspective, it is important to know which rat-associated zoonoses are present in a specific geographic area, as this information is essential to developing appropriate surveillance and intervention strategies. However, it is equally important to know which pathogens are absent in order to avoid wasting resources on programs that target pathogens that are not a risk to human health in that particular jurisdiction. From a research perspective, accurate data on the distribution of rat-associated zoonoses is crucial for understanding the ecology of these pathogens. Finally, it is important to continuously update data on pathogen distribution as it may be subject to significant change over time, particularly given the fact that rats are prone to long-distance migrations in association with human transport (222).

The objective of this study was to determine if rat populations in the DTES carry *Rickettsia typhi*, SEOV, and *Bartonella* spp.

### 7.3 Methods

**Sample selection**

Rats were trapped and processed as described previously (Chapter 2). During necropsy, ½ of one kidney and spleen was collected aseptically and stored at -80°C until DNA extraction.

As mentioned above (Chapter 4), it was determined that at least 630 samples should be included in each analysis in order to accurately calculate the prevalence of a pathogen within a city block. However, only 554 of 725 rats had serum samples
available for serologic testing. Adequate serum samples were not available from the remainder of the animals because of issues with small body size, trap-related mortality, and hemolysis.

For each block, the rats to be tested were selected randomly. Note that all serum samples available were included in serologic analyses.

**Serology for *Rickettsia typhi* and Seoul hantavirus**

Serum samples were tested for the presence of IgG against *R. typhi* using an in-house immunofluorescence assay at the National Microbiology Laboratory (NML), Winnipeg, Manitoba. Samples were applied to slides pre-coated with *R. typhi* antigen (CDC Viral and Rickettsial Zoonoses Branch) and incubated for 1 hour at 37°C. The slides were then washed for 15 minutes in FTA buffer (BD Biosciences) and dried. Goat anti-rat IgG–FITC conjugate (Sigma Aldrich) was applied to the slides, which were then incubated for 1 hour at 37°C, washed and dried. A reciprocal titer of $\geq 64$ was considered evidence of exposure to typhus group *Rickettsia* (TGR, which includes *R. typhi*).

Serum samples were also tested at the NML for the presence of IgG against SEOV using the hantavirus IgG DxSelect kit (Focus Diagnostics). The human conjugate provided in the kit was substituted with peroxidase-conjugated Protein G (Calbiochem) in order to detect antibodies produced in rats. The conjugate was diluted 1:3000 in sample buffer and all other steps were performed as per manufacturer’s instructions. Index values for the rat sera were calculated relative to the kit cut-off calibrator.

**PCR for *Rickettsia typhi*, *Bartonella spp.*, and Seoul hantavirus**

Spleen samples from a total of 680 rats were selected for testing. At the NML, genomic DNA was extracted from 20 mg of spleen using the NucleoSpin 96 Tissue kit
(Machery-Nagel) and the QIAxtractor (Qiagen). A multiplexed qPCR assay for the
detection of *R. typhi* and *R. felis* was performed as previously described (223), using *R.
typhi*, *R. felis* and rat glial cell positive controls. To confirm that the extractions were
successful and that there was no PCR inhibition, 10% of the samples were subjected to
qPCR for rat β-actin.

Splenic DNA extracts were also analyzed using a conventional PCR assay which
targets the 16S-23S ribosomal RNA intergenic spacer of *Bartonella* spp. at the AHC.
Briefly, PCR was performed using the Takara Premix Taq Kit (Fisher Scientific,
Canada). Each 25 µl reaction contained 12.5 µl of Ex Taq buffer, 1 µl each of forward
primer (5’- GGT TTT CCG GTT TAT CCC GGA GGG C -3’), reverse primer (5’- CTG
AGC TAC GGC CCC TAA ATC AGG A -3’), 5.5 µl nuclease-free water and 5 µl of
DNA template. Samples were run on a Tetrad2 thermocycler. The reaction was
incubated at 95°C for 5 minutes, and then amplified for 55 cycles at 95°C for 15 seconds,
68°C for 15 seconds, 72°C for 20 seconds and a final cycle at 72°C for 7 minutes. PCR
products were analyzed by gel electrophoresis. *Bartonella henselae* DNA and nuclease-
free water were used as the positive and negative controls, respectively.

Also at the AHC, kidney samples from a total of 633 rats were selected for
testing. Genomic RNA was extracted from homogenized kidney tissue using the
RNeasy Mini kit (Qiagen Inc., Canada) at the Animal Health Centre. A qPCR for the
detection of rodent borne hantaviruses was performed as previously described (224)
using SEOV RNA and nuclease-free water as positive and negative controls,
respectively.

At the NML, the RNA was extracted from the homogenized lung tissue of SEOV
seropositive rats (n = 8) and random selection of seronegative rats (n = 22) using the
RNeasy Mini kit (Qiagen Inc., Canada). Extracts were tested for rodent borne
hantaviruses using the same qPCR mentioned above (224).
7.4 Results

Two serum samples were reactive against TGR at a reciprocal titre of 64 and eight samples were reactive against SEOV at ELISA index values ranging from 0.98 to 8.51 (median = 1.99). All PCR testing for *R. typhi*, *Bartonella* spp., and SEOV was negative.

7.5 Discussion

Overall, the negative PCR testing in combination with the low prevalence of seropositivity suggests that *R. typhi*, *Bartonella* spp. and SEOV are absent from these rats.

There are several potential explanations for these results that should be considered. First, it is important to consider the validity of the test results. With regard to tissue selection, past research has successfully used spleen for PCR detection of *Bartonella* spp. (119, 225) and *R. typhi* (179) in rats, and both kidney and lung have been used for SEOV PCR (226).

Both *Bartonella* spp. and *R.* can also be isolated from fleas (and occasionally lice, mites, and ticks), which are vectors for these organisms (227-229). Ectoparasites (fleas and lice) were observed infesting rats collected for this study but have not yet been identified or tested (they will be part of a future study focused on vector ecology), which means that the presence of infected vectors cannot be completely ruled out. This is particularly in the case for *R. typhi*, as this bacterium can be maintained in flea populations through long-term infection and transovarial and transtadial transmission (227, 228). Additionally, the period of active rickettsemia (i.e., circulation of *Rickettsia* spp. in the peripheral blood), which is what PCR analysis of spleen would detect, can be transient (i.e., < 1 month) (228). However, *R. typhi* serologic testing was largely negative,
and infection with *R. typhi* in rats consistently elicits a long term IgG immune response (for to 40 weeks) (228), suggesting that seronegative animals were, indeed, uninfected.

In contrast to *Rickettsia* spp., *Bartonella* spp. are thought to cause long-term bacteremia in the reservoir host (47), therefore the total absence of positive PCR results for *Bartonella* spp. suggests the absence of this pathogen from these rats.

No vectors are involved in ecology of SEOV, which is transmitted among rats via urine and other body secretions (64, 65). In contrast to *Rickettsia* spp., rats demonstrate persistent SEOV infections in the organs even in the face of neutralizing antibody (230-232). Therefore negative PCR results suggest that SEOV is truly absent from the sampled rats.

The seroreactive samples are somewhat difficult to explain in the absence of PCR evidence for these pathogens, but most likely represent cross-reaction with related organisms or antigens, which is commonly encountered with many serologic tests. In the future, it may be useful to perform metagenomic analysis on urban rat populations to better characterize the full suite of bacteria and viruses that rats may carry.

Given that the prevalence of *R. typhi*, *Bartonella* spp., and SEOV can be low, and can sometimes vary with season and/or with rat population characteristics (43, 51, 64, 153, 233, 234), it is also important to consider whether our rat sampling protocol was adequate to detect these pathogens if they were present. We would argue that our systematic, intensive, and uniform trapping protocol in a series of contiguous city blocks allowed us to achieve a representative sample of animals with regard to both sample size and demographic composition of included animals. Additionally rats were trapped in all seasons and trap success was not significantly different among the seasons.

If both rat sampling and testing protocols were adequate to detect these pathogens, then it may be possible that they are truly absent from the rat populations included in the study. If this is the case then several different possibilities should be
considered. With regard to *R. typhi* and *Bartonella* spp. it could be the case that competent vectors were not present. The absence of *R. typhi* could also be a result of interspecific competition with another undetected *Rickettsia* spp., as studies have shown that infections of competent vectors with closely related *Rickettsia* spp. (e.g., *R. typhi* and *R. felis*) is rare (227). That being said, it is important to note that the multiplex PCR we used for the detected of *R. typhi* also screens for *R. felis*, which was not detected.

Alternatively, it could be that the physical and/or climactic environment is not conducive to the maintenance of these pathogens, particularly for those that require a vector for transmission. Although this possibility cannot be ruled out, it is interesting to note that all of these pathogens have areas with climates comparable to Vancouver (51, 69, 82, 235), which is mild compared to the rest of Canada. It is interesting that both *R. typhi* and hantaviruses have been shown to be most common in coastal cities, and particularly those with international ports, a phenomenon which is attributed to the increased frequency and/or probability of introduction of infected rats (222, 228, 236). Vancouver is a coastal city with a large international port, which was included in the study area, yet *R. typhi* and SEOV appear to be absent from rats inhabiting and surrounding the port.

Finally, it could be the case that *R. typhi*, *Bartonella* spp. and SEOV were never introduced into these rat populations in the first place. Both SEOV and *Bartonella* spp. demonstrate a degree of sequence diversity that suggests co-evolution with the rodent host (120, 237). This might suggest that the distribution of these pathogens is largely dependent on the dispersion and establishment of infected rats (120). Given that both Norway and black rats, which evolved in Asia, reached their current global distribution though numerous introduction events over time (154), and given that rats are not prone to long-distance migrations in the absence human transport (154), it could be the case the presence of a zoonotic pathogen in a particular location is largely dependent on the characteristics of the founding, or subsequently introduced rat population. There is very
little information regarding the genetic structure of Norway and black rat populations. Further study on the subject might help to elucidate patterns of disease distribution in these species.

It is also important to consider that if exclusion of a particular pathogen from a rat population is a random event, then that population could likely support the establishment of the pathogen if it is introduced in the future. This has been the case for *Yersinia pestis*, which has been introduced into previously plague-free locations in association with the importation of infected rats on ships (7). Therefore, although it is not worthwhile developing comprehensive strategies to monitor and mitigate the risks of RAZ that are not present in a particular jurisdiction, periodic surveillance of rat populations over time is warranted in order to detect introduced RAZ and to maintain an accurate and contemporary understanding of rat-associated public health threats.
8.1 Synopsis

Bites associated with wild and domestic Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) may have a variety of health consequences in people. Bite-related infections are among the most significant of these consequences; however, there is little data on the infectious agents that can be transmitted from rats to people through biting. This is problematic because without an accurate understanding of bite-related infection risks, it is difficult for health professionals to evaluate the adequacy of existing guidelines for evidence-based therapies. The objectives of this study were to increase our knowledge of the bacterial species associated with rat bites by studying bite wounds that wild rats inflict upon one another, and to review the literature regarding rat bites and bite wound management. Wild Norway and black rats trapped in the DTES were examined for bite wounds in the skin. All apparently infected wounds underwent aerobic and anaerobic culture, and isolated bacteria were identified. Thirty-six rats had bite wound-related infections, and approximately 22 different species of bacteria belonging to 18 genera were identified. *Staphylococcus aureus* was the most common isolate; however, the majority of infections (72.5%) were polymicrobial. Rat bites can result in infection with a number of aerobic and anaerobic, gram-positive and gram-negative bacteria. In humans, these wounds are best managed through early recognition and cleansing. The benefit of prophylactic antimicrobial treatment is debatable, but given the deep puncturing nature of rodent bites, we suggest that they should be considered a high risk for infection. Antibiotics selected should include coverage for a broad range of bacterial species.
8.2 Introduction

Animal bites are a significant public health issue in countries around the world. Although dog and cat bites have been studied extensively, there is relatively little information available about the consequences or management of bites from rodents, specifically rats (Rattus spp.).

Norway and black rats (Rattus norvegicus and Rattus rattus) have an almost worldwide distribution (154), and are found in both wild and domesticated settings. In the USA, it has been estimated that there are 1.39 – 10 rat bites per 100,000 people per year (238), and approximately 3,696 emergency department visits related to rat bites annually (239). The true incidence of rat bites is very difficult to estimate as rat bites are generally not reported to health authorities, and people may not seek health care subsequent to a bite.

Studies have, however, been able to identify risk factors for rat bites. Bites associated with wild rats occur most commonly in urban areas, in children or those with a mental or physical disability, and in impoverished communities (238-241). Most wild rat bites that are reported occur at night when an individual is asleep, and are therefore most likely to affect the uncovered face and upper extremities (238, 240, 241). In our experience, homelessness and scavenging in garbage containers may also be risk factors. It should be noted that rats are also found in domestic and laboratory settings, and in these environments, laboratory workers, pet store workers, and pet owners (particularly children) may be bitten (242).

Rat bites can result in a number of different health issues including physical injury (243-246), diabetic ulcers (240, 247), anaphylactic shock (248, 249), and even hypovolemic shock in infants (250). Rat bites can also cause infections (usually bacterial), ranging from cellulitis (251) and fasciitis (252), to life-threatening systemic disease (240, 242). Many sources suggest that 10% of rat bites become infected (242, 253); however, this figure appears to originate from a series of 22 ‘rodent’ bites for which
neither the species of rodent involved nor the circumstances surrounding the bite were disclosed (254). For this reason, the true incidence of infection subsequent to a rat bite is not clear.

The infectious agent most commonly associated with rat bites is *Streptobacillus monilliformis*, the causative agent of ‘rat bite fever’ (242). Infection with *S. monilliformis* is somewhat distinct in the arthralgia/arthritis and skin rash that it produces (242). Other infectious agents associated with rat bites include: *Corynebacterium kutscheri* (255), *Staphylococcus aureus* (256), *Staphylococcus epidermidis* (241), *Streptococcus* spp. (241, 256), *Leptospira interrogans* (257, 258), and *Bacillus subtilis* (241).

Information on rat bite-associated infections has largely been gleaned through case studies and small case series, therefore the true range of infectious agents associated with rat bites remains unknown. This knowledge gap stems from the fact that rat bites in humans, being relatively rare and/or rarely reported, are difficult to study in a comprehensive way. However, rat bites are very common among rats, as biting is a relatively frequent occurrence during a variety of social interactions among conspecifics (i.e., members of the same species) (164). For this reason it might be possible to learn more about bite-related infectious risks by studying bite-induced infections in rats themselves.

The objectives of this study were: 1) to identify the bacteria causing bite-related skin and soft tissue infections in DTES rats and thereby to supplement the existing knowledge base regarding bacteria that could be transmitted to people through rat bites, and 2) to review the literature regarding rat bites and bite wound management.

### 8.3 Materials and Methods

#### Sample collection

Rats were trapped and processed as described previously (Chapter 2). After euthanasia, each rat underwent a full physical examination, which included
examination for bite-wounds and bite wound-related skin infections, i.e., draining abscesses and open sores.

Open sores and draining abscesses were sampled in the field using a sterile BD CultureSwab™ with Liquid Amies medium (BD, Franklin Lakes, NJ). Rats were subsequently frozen and sent to the AHC for full post mortem examination, including sterile sampling of non-draining skin and superficial soft tissue infections.

It should be noted that conspecific bite wounds are common in rats, and can occur all over the body subsequent to a variety of different types of social interactions (187, 259). They are usually sharply-demarcated incision- or puncture-type wounds (260), although some can become secondarily infected and develop into open sores or abscesses (187). For this reason, any incision/puncture-type wound in the skin was considered a bite wound, and any open cutaneous sore or abscess associated with the skin was considered a bite wound-related infection. Ultimately, it was not possible to definitively determine that the aforementioned skin wounds and infections were due to rat bites. However, other causes of these types of skin lesions (e.g., injury from inert objects or other animal species) could not be identified in the literature.

**Bacterial culture and identification**

At the AHC, using aseptic technique, all specimens were inoculated onto Columbia Blood agar and MacConkey agar (Oxoid, Canada) and incubated at 35°C in 5-10% CO₂ and aerobic conditions, respectively, for 48 hours. Additionally, each specimen was set up for anaerobic culture on Columbia Blood agar and incubated at 35°C for 48 hours. Aerobic cultures were observed at 24 and 48 hours, and subcultures of all bacterial growth were made for further diagnostic evaluation. After 48 hours, all anaerobic cultures were observed, and all suspect anaerobic organisms were subsequently sub-cultured aerobically and anaerobically to confirm the organism as a
suspect anaerobic organism and to obtain a pure culture before proceeding with identification.

All sub-cultured organisms were observed for their growth characteristics and colony morphology on Columbia Blood agar and MacConkey agar, and microscopic morphology was determined using Gram stain. Based on these analyses, organisms were further screened by individual biochemical reactions, including the indole, oxidase, catalase, and coagulase tests. Where necessary, further biochemical testing was performed using the API 20E, API Coryne, API Strep (Biomerieux, Canada) and Biolog MicroLog system (Biolog Inc., USA).

Organisms that could not be identified using the aforementioned techniques (e.g. *Streptobacillus monilliformis*, *Pasteurella pneumotropica*, *Corynebacterium kutscheri*, etc.), underwent DNA extraction (QIAamp DNA Mini Kit, Qiagen, Canada), with PCR amplification and sequencing of the V1 to V3 regions (492 bp) of the 16S rRNA gene (261). Sequenced products were classified using BLAST against the NCBI Genbank database (http://blast.ncbi.nlm.nih.gov/), as well as using the Bio Informatic Bacteria Identification (leBIBI V5) system (http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi).

It should be noted that because samples were set up for routine aerobic and anaerobic cultures only, some fastidious organisms requiring enhanced culture techniques (e.g., chocolate agar, *Yersina* spp.-selective agars, etc.) may have been missed.

8.4 Results

All total of 725 trapped rats were included in the analysis (see Chapter 4 for a description of the trapped population).

Visible bite wounds were present in 177 rats (24.1%). Among the rats with bite wounds, the average number of wounds was 2.26 (range = 1 – 15) and there were 309
bite wounds in total. Thirty-six of 725 rats (5.0%) had active bite wound-related skin and soft tissue infections, therefore wound infections were present in 20.3% (36/177) of rats with bite wounds. Among these 36 rats there were a total of 40 bite wound associated infections, therefore 12.9% (40/309) of all bite wounds were infected.

The majority of bite wounds were polymicrobial, with 19/40 (47.5%) yielding two species of bacteria, 9/40 (22.5%) yielding three species, and 1/40 (2.5%) yielding four species; the remainder (11/40 or 27.5%) were monomicrobial (Fig. 8-1).

Figure 8-1: Bacterial species isolated from 40 bite wound-related skin and soft tissue infections in Norway and black rats (Rattus norvegicus and Rattus rattus).

A total of 80 isolates were obtained, which included approximately 22 different species of bacteria (Table 8-1, Fig. 8-1). Three isolates had identical 16S rRNA sequences and did not match to any known bacterial genera. Based on its 16S rRNA sequence, this bite-wound-associated bacterium fell within the Pasteurellaceae family and was most closely related to Haemophilus sp. and Aggregatibacter sp.

Of the 36 rats with bite wound associated infections, only one was a black rat. This rat had one wound from which Staphylococcus aureus and the aforementioned unidentified bacterial species were isolated. Although we were unable to find a
significant association between species and the presence of bite wounds (p > 0.05),
whether or not this is reflective of reality is to say due to the low number of black rats in
the study as a whole. However, we did find that the odds of having bite wounds was
greater in males (OR = 1.76, 95% CI = 1.24 – 2.51) and heavier rats (OR = 1.11, 95% CI =
1.09 – 1.13 per 10 g increase in mass). The odds of having an infected bite wounds was
also greater in heavier rats (OR = 1.08, 95% CI = 1.05 – 1.11 per 10 g increase in mass),
but was not associated with sex or species (p > 0.05).

Table 8-1: Bacteria isolated from 40 bite-wound-related skin and soft tissue infections in Norway and
black rats (Rattus rattus and Rattus norvegicus).

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter sp.</td>
<td>1</td>
</tr>
<tr>
<td>Aerococcus viridans</td>
<td>1</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>2</td>
</tr>
<tr>
<td>Clostridium sordelli</td>
<td>1</td>
</tr>
<tr>
<td>Corynebacterium kutscheri</td>
<td>2</td>
</tr>
<tr>
<td>E. coli (non-hemolytic)</td>
<td>14</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus fecalis</td>
<td>6</td>
</tr>
<tr>
<td>Enterococcus sp.</td>
<td>1</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>1</td>
</tr>
<tr>
<td>Pasteurellaceae (potentially novel genus)</td>
<td>3</td>
</tr>
<tr>
<td>Pasteurella pneumotropica</td>
<td>3</td>
</tr>
<tr>
<td>Pasteurella volantium</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>27</td>
</tr>
<tr>
<td>Staphylococcus cohnii</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus galinaceous</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>5</td>
</tr>
<tr>
<td>Streptobacillus monilliformis</td>
<td>4</td>
</tr>
<tr>
<td>Streptococcus gallinaceus</td>
<td>2</td>
</tr>
<tr>
<td>Streptococcus sp. (alpha hemolytic)</td>
<td>1</td>
</tr>
<tr>
<td>Truperella sp.</td>
<td>1</td>
</tr>
</tbody>
</table>
8.5 Discussion

Bacteria associated with rat bites

This study demonstrates the spectrum of bacterial species that may be associated with rat bite-related infections in the studied area. Many of these isolates clearly represent inoculation of rat oral flora, as they are commonly found colonizing mucus membranes in rats. These include *Corynebacterium kutscheri*, *Klebsiella pneumonia*, *Pasteurella pneumotropica*, as well as *Streptobacillus moniliformis* (262). The isolation of *Bacillus* spp. and *Clostridium sordellii*, on the other hand, might suggest inoculation of environmental bacteria (263). Similarly, although *Staphylococcus aureus* can be found colonizing the mucous membranes of rats (262), it is also found on the skin, and could have been translocated to deeper tissues through the act of biting.

Most interesting is the relative prevalence of these organisms. Within the literature, *Streptobacillus moniliformis* is represented as the bacterial species most commonly associated with rat bites (253, 264, 265). In our study, however, *S. monilliformis* was found in only 5% (4/80) of bite-related infections. By far, the most common isolate was *Staphylococcus aureus*, which represented 33.8% of all isolates. This raises the question as to whether *S. moniliformis* infection is really more common subsequent to rat bites in people (vs. rat bites in rats), or whether there is bias towards the recognition and/or reporting of *S. monilliformis* infections compared to other bacteria – perhaps related to the fact that *S. monilliformis* is associated with a distinct septicemic disease (242). Additionally, while anaerobes are commonly associated with dog and cat bites (Ball et al. 2007), very few anaerobes were isolated in this study. This could, however, be a result of sampling technique (i.e., use of transport media not specific for anaerobe recovery and/or prolonged time between sample collection and anaerobic culture).

Although we were able to identify a number of bacteria not previously associated with rat bites, we did not isolate several species that have been associated
with rat bites in the past. For example, one study of rat bites in humans found that *Staphylococcus epidermidis* represented 43% of bacteria cultured from non-infected wounds at presentation (241). However, since *Staphylococcus epidermidis* is commonly found on human skin, it may be the case that these isolates represent human skin flora inoculated into the wounds. Indeed, previous researchers have suggested that animal bite wound cultures obtained from non-infected wounds soon after the bite has occurred are rarely useful (265, 266), likely because of the scant number and deep location of inoculated bacteria in an early bite wound. Morgan (2005) goes as far as to suggest that cultures are only useful at the onset of clinical infection.

This is also worth noting that although *P. multocida* is the most likely organism to cause infection subsequent to cat bite (267), we did not isolate this bacterium in our study and rat bite-related *P. multocida* infections appear to be exceedingly rare (268).

**Risk of infection subsequent to a rat bite**

In our study, approximately 12% of bite wounds were associated with infection. This number is similar to the 10% figure often quoted by other sources (242, 269). However, we suggest that this likely underestimates the true prevalence of infection in this rat population because we could not account for infections that had resolved, caused systemic infections not visible to the naked eye (e.g., infection with *Leptospira* spp.), or resulted in significant morbidity or mortality. We suggest that the nature of the rat bite wound, i.e., deep punctures with small openings, could put these wounds at an elevated risk of infection similar to those of cats, where up to 80% of bites may become infected (270).

**Medical management of rat bites**

There is no literature specifically regarding the medical management of rat bites. However, a review of protocols for dealing with animal bites in general shows that
there is consensus regarding the importance of early recognition as well as thorough examination, debridement, irrigation, and disinfection (265, 266, 269, 270). Given the deep, puncture-type wounds inflicted by rats, assessing the depth of the wound and involvement of underlying structures, and providing adequate cleaning may be particularly challenging.

With regard to diagnostics, it has been suggested, as mentioned above, that early wound cultures are rarely useful (265). Rather, cultures should be performed if there is evidence of clinical infection (265, 269). Local wound cultures are most appropriate for cellulitis and localized infections, while blood cultures are needed where there are signs of systemic disease or septicemia (265). Because infection may not be apparent at the outset, the literature suggests that the patient should be re-examined in 24 to 48 h and taught to monitor themselves for signs of infection in the interim (265, 266, 269).

Given that rat bites are usually associated with small punctures, rather than large lacerations, the need for primary wound closure is probably minimal and should be avoided where possible in order to avoid infection (265, 266).

There is considerable debate regarding the need for prophylactic antibiotic treatment in bite wound management. One study withheld prophylactic antibiotics from 50 patients with rat bite wounds that were not infected at presentation and found that only one patient developed an infection (although these wounds were generally recognized early and appropriately cleansed and debrided) (241). Similarly, a Cochrane review of antibiotic prophylaxis for mammalian bites found that antibiotic prophylaxis was not effective at preventing most infections (271). This review, however, consisted almost entirely of dog-bite studies and included only one series of cat bites with puncture-type wounds that are more prone to infection (271). Antimicrobial prophylaxis did appear to be effective for bites involving the hands, where underlying structures, such as bones and joints, are more likely to be affected (271).
Other than the location and nature of the wound, the need for antimicrobial prophylaxis may be influenced by the time lapse between the bite and medical intervention and patient factors that may increase susceptibility to infection (e.g., immunodeficiency, prosthetic heart valves or joints, old age, etc.) (266).

With regard to the choice of antimicrobial, a number of different drugs have been used and/or recommended. However, given the polymicrobial nature of animal bites, there is some consensus that antimicrobials should be selected in a manner that ensures coverage for a variety of Gram-positive, and Gram-negative, aerobic and anaerobic bacteria (265, 269). Amoxicillin/clavulanic acid combinations have been suggested as a good first choice for this reason (265). Our findings suggest that this would remain a valid choice for empirical therapy of rat bite-related infections pending culture results.

An additional factor to consider is the need for rabies and/or tetanus prophylaxis. The spores of *Clostridium tetani* are ubiquitous in the environment, and therefore have the potential to be inoculated into an animal bite, as with any other penetrating injury (266). For this reason individuals with unknown or non-current vaccination status should be managed with tetanus human immunoglobulin and tetanus toxoid booster vaccination (266).

Although some sources have suggested that rabies prophylaxis should be considered for all rodent bites (266), to our knowledge there has never been a confirmed case of rabies in a Norway or black rat, or a case of human rabies associated with these species. For this reason we suggest that rabies prophylaxis is not warranted subsequent to most bites associated with apparently healthy rats. Similarly, although tularemia (infection with *Francisella tularensis*) has been suggested as a consequence of rodent exposure (266), we cannot find a confirmed case of tularemia in a Norway or black rat.

However, if the rat is ill or behaving abnormally, then diagnostics should be undertaken in order to rule out any unusual or unforeseen pathogens (e.g., rabies). It is important to note that biting can be a response to illness in an animal (266), therefore
due consideration of the health or behavioural status of the rat, if possible, would be prudent.

Overall, this study demonstrates that rat bites could result in the transmission of a wide variety of bacteria. *Staphylococcus aureus* was the most common isolate; however, the majority of infections (72.5%) were polymicrobial.

In humans, these wounds are best managed through early recognition and cleansing. The benefit of prophylactic antimicrobial treatment is debatable, but given the deep puncturing nature of rodent bites, we suggest that they should be considered a high risk for infection. Antibiotics selected should include coverage for a broad range of bacterial species.
Chapter 9: Carriage of Methicillin-Resistant *Staphylococcus aureus* by Wild Urban Norway Rats

9.1 Synopsis

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of multi-drug-resistant infections in people, particularly indigent populations. MRSA can be transmitted between people and domestic animals, but the potential for transmission between people and commensal pests, particularly rodents, has not been investigated. The objective of this study was to identify the presence and characterize the ecology of MRSA in rats (*Rattus* spp.) from the DTES. Oropharyngeal swabs were collected from rats trapped in 33 city blocks and one location within the adjacent port. Bacterial culture was performed and MRSA isolates were characterized using a variety of methods, including whole-genome sequencing (WGS). The ecology of MRSA in rats was described using phylogenetic analysis, geospatial analysis, and generalized linear mixed models. MRSA was identified in 22 of 637 (3.5%) rats tested, although prevalence varied from 0 – 50% among blocks. Isolates belonged to 4 clusters according to WGS, with the largest cluster (n=10) containing isolates that were genetically indistinguishable from community-acquired USA300 MRSA strains isolated from people within the study area. MRSA strains demonstrated both geographic clustering and dispersion. The odds of an individual rat carrying MRSA increased with increased body fat (OR = 2.53, 95% CI = 1.33 – 4.82), and in the winter (OR = 5.29, 95% CI = 1.04 – 26.85) and spring (OR = 5.50, 95% CI = 1.10 – 27.58) compared to the fall. The results show that urban rats carried the same MRSA lineages occurring in local human and/or animal populations, supporting recent transmission from external sources. MRSA carriage was influenced by season, most likely as a result of temporal variation in rat behaviour and rat-human interactions.
9.2 Introduction

*Staphylococcus aureus* is a gram-positive bacterium that colonizes epithelial surfaces and causes infections in humans (272). Methicillin-resistance is mediated by *mecA* and related genes, which are carried on mobile genetic elements and confer resistance to most beta-lactam antimicrobials, and frequently other antimicrobial classes (272-274).

Since its emergence, methicillin-resistance *S. aureus* (MRSA) has become a significant cause of hospital-associated infections worldwide (272). The early 2000s saw the emergence of community-associated MRSA (CA-MRSA), which, in contrast to hospital-associated MRSA (HA-MRSA), spreads and causes disease in the general population, outside of the healthcare setting and often in people without typical risk factors (272, 274). CA-MRSA is particularly prevalent in North America, where it is an important cause of skin and soft tissue infections (272, 274).

Although colonization with CA-MRSA is widespread, the incidence of disease is greater in homeless people and injection drug users (IDUs) compared to the general population (272, 275, 276). This is likely the result of a combination of factors including compromised health, crowding in shelters, poor skin integrity, and injecting in unhygienic environments (275, 276). Indeed, soft tissue infections, including those caused by MRSA, account for the majority of hospitalizations among IDUs in some settings (277).

Although transmission of MRSA is primarily person-to-person, there is evidence that MRSA can be spread between domestic animals and people (278, 279). Recently, questions have emerged regarding whether pest species might also be a source of MRSA (280). The potential for pest-to-human MRSA transmission is particularly concerning in impoverished, inner-city neighbourhoods, where factors associated with poverty may promote pest infestations and pest-human contact, and increase susceptibility to MRSA infection (16, 276).
Norway rats (*Rattus norvegicus*) are among the most common urban pest species and are known to be the source of a variety of zoonotic pathogens (16). However, very little is known about MRSA in rats. One study demonstrated carriage of livestock-associated (LA-) MRSA in rats living on pig farms in the Netherlands (281), while another identified antibiotic-resistant *S. aureus* in black rats trapped in downtown-Tokyo, although these isolates were not definitively identified or characterized as MRSA (282).

Interestingly, methicillin-resistant *Staphylococcus pseudintermedius* has been identified in rats in the DTES (283), and MRSA was isolated from bedbugs in the same area (280). The DTES is also home to a significant population of IDUs with a high prevalence of MRSA carriage (284) and infection (275), suggesting that this area should be a priority for the study of potential urban pest reservoirs of MRSA.

The objective of this study was to characterize the epidemiology of MRSA in rats from the DTES using culture, *spa* typing, antimicrobial susceptibility testing, and whole-genome sequencing, as well as data on rat distribution and demographic characteristics.

**9.3 Methods**

**Sample collection**

The study area was comprised of 33 city blocks within the DTES and the port site. Rats were trapped and processed as previously described (see Chapter 2). Immediately after euthanasia, the oropharynx and nares were sampled using a sterile BD CultureSwab™ with Liquid Amies medium (BD, Franklin Lakes, NJ).

**MRSA culture, *spa* typing, PVL PCR, and antibiotic susceptibility**

Swabs were sent to the Ontario Veterinary College (OVC), Guelph, Ontario, where they were placed in 2 mL of enrichment broth containing 10 g/L tryptone T, 75 g/L sodium chloride, 10 g/L mannitol, and 2.5 g/L yeast extract and incubated for 24 h at
35°C. Aliquots of 100 µL were streaked onto MRSA Chromogenic agar (BBL CHROMagar, Becton, Dickinson and Co., Sparks, MD, USA) and incubated at 35°C for 48 h. Tube coagulase-positive isolates were speciated by using a multiplex PCR (285). Methicillin resistance was confirmed by demonstrating the presence of penicillin-binding protein 2a antigen using a latex-agglutination test (Oxoid Ltd., Basingstoke, UK). MRSA isolates were characterized by spa typing (286) using the Ridom SpaServer (http://SpaServer.ridom.de). The lukF-PV gene encoding the Panton-Valentine leukocidin toxin was detected by qPCR (287). Antimicrobial drug susceptibility was performed by broth microdilution (Sensititre, Trek Diagnostics, Cleveland, OH, USA), and results were interpreted according to Clinical and Laboratory Standards Institute guidelines (288).

**MRSA whole-genome sequencing**

Total DNA was extracted from the rat MRSA isolates using the Qiagen DNeasy kit (Qiagen, Toronto, ON, Canada) sent to the British Columbia Centre for Disease Control where indexed DNA fragment libraries were prepared using the Nextera XT kit (Illumina, San Diego, CA, USA) as follows. After RNase treatment, DNA was quantitated in the QuBit (Life Technologies, Burlington, ON, Canada) and diluted to 0.2 ng/µl. For the Nextera XT kit, 5 µl of each sample were used as input for tagmentation followed by a low cycle number PCR reaction for indexing and adapter incorporation. Eight picomoles of each library was then quantified using a KAPA library quantification kit and loaded onto an Illumina MiSeq to generate 250 bp paired-end reads. Each library produced a mean of ~560,000 paired-end reads with 53-fold coverage of the MRSA genome.

To enable a comparison of genetic diversity, sequences from 33 human USA300 (ST8/spa t008) isolates were also included for analysis. These isolates were collected as part of previous studies and sequenced on the Illumina HiSeq 2000 at Canada’s Michael
Smith Genome Sciences Centre using methods described previously (289). These included 15 isolates from the DTES (275, 284) and 18 isolates from elsewhere within Vancouver (unpublished). Due to the large number of reads produced by the HiSeq 2000, a subsample of 10% of reads was randomly selected from all human MRSA samples for use in downstream analysis, resulting in 2,200,000 base paired-end reads (2 x 75 bp) with 89-fold coverage of the MRSA genome for the human MRSA isolates.

Sequence reads were assembled using a pipeline specifically developed for bacterial genomes (290), modified for *S. aureus* by mapping to the USA300-FPR3757 reference genome (291), and estimated to have a false positive rate of 2.5x10^-9 per nucleotide, i.e. 0.0075 per genome (292). A mean of 92% and 93% of reads were mapped, resulting in 92% and 99% coverage of the USA300 reference genome for rat and human samples respectively before quality filtering. After filtering, calls made for a mean of 90% and 94% of the USA300 reference for the rat and human samples, respectively. One rat sample was excluded since all positions failed the filter due to poor sequence quality.

**Bioinformatics**

To determine phylogenetic relationships among isolates, maximum-likelihood trees were generated from the entire genome sequence using PhyML (293) with the HKY85 substitution model, assuming a homogeneous mutation rate, with 500 bootstrap replicates.

In order to estimate the relatedness of the rat and human MRSA isolates, the average pairwise diversity ($\Pi$) was determined for all samples within ($\Pi_x$) and between ($\Pi_{xy}$) groups by calculating the sum of the total number of variant positions among samples in or between each group under consideration and then dividing that figure by the total number of samples.
To investigate the presence of mobile genetic elements potentially acquired from other bacterial species, the accessory genome was searched for all non-*S. aureus* elements using Velvet (294) in conjunction with the VelvetOptimiser (http://bioinformatics.net.au/software.velvetoptimiser.shtml). Reads that did not map to the USA300-FPR3757 reference were assembled and all contigs >1 kb were matched to the NCBI nr database using BLASTn. Any contig for which all matches with a BLAST score >95% of the maximum BLAST score for that contig were not *S. aureus* was considered potential non-*S. aureus*.

For in silico detection of the PVL gene and determination of the *spa* type from the genome sequence data, the VelvetOptimiser was used to assemble all the reads, after which the PVL and *spa* typing laboratory primers were used as queries against the contigs. Contigs containing any of the primers were selected for further analysis. PVL was deemed present if both PVL primers matched with no mismatches to the same contig, or to two contigs with an overlapping region that could be joined. *spa* type was determined by extracting the region between the two *spa* primers from a contig (after manual joining of two contigs if necessary) and entering the resulting sequence into the Ridom SpaServer.

**Statistical analysis**

For the statistical analysis, the primary outcome variable was MRSA status (positive vs. negative). Explanatory variables that were considered included season of capture, mass, length, sex, sexual maturity, and fat score.

To identify characteristics associated with MRSA status, the distribution of the explanatory variables were examined among the sample as whole, as well as separately for MRSA-positive and -negative rats. Regression modeling was then used to examine relationships between MRSA status and each of the explanatory variables, first using simple logistic regression, and then using multiple logistic GLMMs (to control for the
random effect of block). The final multivariate model was arrived at by manual stepwise regression using AIC to compare candidate models (the final model was that with the lowest AIC) (172).

All statistical analyses were conducted using R (R Development Core Team, Vienna, Austria). For multivariate models, individuals with missing data for one or more of the variables under study were excluded. Collinearity among explanatory variables was identified using Spearman’s rank correlation (ρ) (172). Highly correlated variables (ρ > 0.8) were modeled separately (172).

Spatial analysis

The location of each trap, the number of rats caught in each trap, and the number of rats that were MRSA-positive (including WGS group) were mapped using ArcGIS 10.0 (ESRI, Redlands, CA). This information was imported into SaTScan (Boston, USA) for cluster analysis using a purely spatial Bernoulli model and scanning for areas with high rates of MRSA using a circular window with a maximum spatial cluster size of 50% of the population at risk. Separate analyses were conducted for each MRSA WGS group vs. rats negative for that group (i.e., MRSA-negative rats and rats carrying other strains of MRSA). Clusters identified by SaTScan were visualized in ArcGIS.

9.4 Results

MRSA culture and Statistical analysis

A total of 637 rats were trapped and tested for MRSA carriage. Of these, MRSA was isolated from 22 rats (3.5%) (Table 9-1). The prevalence of MRSA varied by block (Figure 9-1).

Of the 637 rats tested, 601 (94.3%) were Norway rats and 36 (5.7%) were black rats. None of the black rats were positive for MRSA. Given the low number of black rats
trapped and differences in the ecology of brown and black rats, black rats were excluded from further statistical and spatial analyses.

Figure 9-1: Number of MRSA-positive vs. -negative Norway rats (*Rattus norvegicus*) in each study location. Prevalence of MRSA is noted above each bar.
Table 9-1: Characteristics of 22 MRSA isolated obtained from wild Norway rats (*Rattus norvegicus*).

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Block</th>
<th>Cluster</th>
<th>spa type</th>
<th>MLST</th>
<th>PV L</th>
<th>Ampicillin</th>
<th>Cefoxitin</th>
<th>Chloramphenicol</th>
<th>Ciprofloxacin</th>
<th>Clindamycin</th>
<th>Daptomycin</th>
<th>Erythromycin</th>
<th>Gentamicin</th>
<th>Levofloxacin</th>
<th>Linezolid</th>
<th>Moxifloxacin</th>
<th>Nitrofurantoin</th>
<th>Oxacillin + 2% NaCl</th>
<th>Penicillin</th>
<th>Quinupristin/dalfopristin</th>
<th>Rifampin</th>
<th>Streptomycin</th>
<th>Tetracycline</th>
<th>Tigecycline</th>
<th>Trimeprin/Sulphamethoxazole</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
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<td>t008</td>
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<td>R</td>
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<sup>a</sup>Not available. <sup>b</sup>Three repeat insertion from t008. <sup>c</sup>One variant position different from sequence type specified.

### Statistical analysis

On bivariate analysis, rats caught in the spring and winter had greater odds of being MRSA-positive compared to rats caught in the fall (Table 9-2). The odds of being MRSA-positive also increased with increasing mass and volume of internal fat. There was no significant association between MRSA-status and length, sex, or sexual maturity. The final model included season and body fat (Table 9-2).

#### Table 9-2: Relationship between MRSA-status, season, and morphometric characteristics among a population of wild Norway rats (*Rattus norvegicus*).

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<th>Category</th>
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<td>Median (IQR)</td>
<td>20.9 (6.4 – 33.0)</td>
<td>15.7 (6.6 – 26.1)</td>
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<td>Median (IQR)</td>
<td>20.0 (13.1 – 22.0)</td>
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<td>1.0 (0.0 – 2.0)</td>
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*a Frequencies and percentages may not add to 100% because of exclusion of rats with missing data for the variable in question.
*b Reference category.
*c Insufficient power for accurate estimation.
*d Odds ratio (OR) with 95% confidence interval (CI) generated using a logistic generalized linear mixed model to control for clustering by city block of origin.
*e Results of bivariate modeling.
*f Results of the final multivariate model building procedure.
**MRSA spa typing and antibiotic susceptibility**

Six different spa types were observed, the most common of which was t008 (n=7), followed by t267 (n=5), t034 (n=5), t002 (n=2), and t818 (n=1, a four repeat truncation of t008). Two isolates belonged to a spa type (‘tNew’) that had never been observed before and represented an insertion of three repeats into t008. Characteristics of the 22 isolates are summarized in Table 9-1.

**Whole-genome sequencing**

Whole-genome sequencing of the rat MRSA samples showed that they grouped into 4 clusters in accordance with the spa typing and multi-locus sequence type (MLST) data (Figure 9-2), the latter of which was generated from the WGS data. Cluster one (n=10) isolates belonged to sequence type (ST8) and consisted of three spa types: spa t008, t818, and ‘tnew’. All but one of these isolates was positive for PVL and consistent with the USA300 clone. Cluster two (n=5) isolates belonged to ST398/t034, typically referred to as LA-MRSA. Cluster three (n=4) was ST97/t267. Cluster four (n=2) consisted of ST105/t002, a clonal complex 5 strain that is classified by PFGE as USA100 or Canadian epidemic MRSA 2 (CMRSA-2). Isolates from clusters 2-4 were PVL negative. There was 100% concordance between in silico and lab based results for PVL and spa type.
Figure 9-2: Maximum likelihood phylogenetic tree of all MRSA samples isolated from Norway rats (*Rattus norvegicus*). Branch color represents cluster: Green=Cluster 1; Pink=Cluster 2; Yellow=Cluster 3; Orange=Cluster 4. Labels give isolate ID and date. MLST, *spa* type, and presence of PVL shown in right columns. Abbreviations: ST=Sequence Type; tNew=*New spa* type with 3 repeat insertion from t008. Scale bar represents the average number of substitutions per site.

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<td>M12-121</td>
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</table>

**Bioinformatics**

In general, within 3 of the clusters the isolates were genetically diverse (Figure 9-2). Isolates in cluster one differed from each other by a mean of 93 variant positions, while those in clusters two and four differed by an average of 53 and 100 variant positions. In contrast, isolates within cluster three had no variant positions between them. However, there were also several similar isolates in clusters one and two. In
cluster one, isolates M12-85 and M12-117 had 7 variant positions between them, and isolates M12-121 and M12-118 had 2 variant positions between them. In cluster two, isolates M12-473, M12-474 and M12-475 had 9-10 variant positions between them.

When DTES-origin human samples were compared to the rat samples in cluster one, the genome sequence results could not distinguish between the two (Figure 9-4). The average pairwise distance among the rat samples and DTES human samples (within group comparison ($\Pi_x$)) was 119 and 104 variant positions respectively, and the average pairwise distance between the rat and DTES human samples (between group comparison ($\Pi_{xy}$)) was 114 variant positions.

Investigation of non-\textit{S. aureus} regions within the accessory genome revealed a 4 kb region of interest present in all ST97 strains/cluster three with 89% similarity to \textit{Staphylococcus pseudintermedius}, and no matches to \textit{S. aureus} strains in the NCBI Nr database. However, further investigation revealed that this region has been identified in a ST395 \textit{S. aureus} isolate recently sequenced from Italy (295).

---

**Figure 9-3:** Number of variant nucleotide positions between every MRSA sample isolated from Norway rats (\textit{Rattus norvegicus}) calculated from reference based assembly.
Figure 9-4: Maximum likelihood phylogenetic tree of all ST8 Norway rat (Rattus norvegicus) and human MRSA samples. Labels give isolate ID, sample location and date. Text color represents sample isolation location: Red=Rat; Dark blue=Human DTES; Pale blue=Human other. MLST, spa type and presence of PVL shown in right columns. Abbreviations: DTES=Downtown East Side; CA=Community-acquired; HA=Hospital-acquired; ST=Sequence Type; tNew=New spa type with 3 repeat insertion from t008. Scale bar represents the average number of substitutions per site.

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</table>
Spatial analysis

Figure 9-5 illustrates the distribution of MRSA-positive rats, including WGS-based clustering for positive rats. Two distinct clusters of higher than expected MRSA prevalence were identified, 1 for cluster one and 1 for cluster 3.

Interestingly, isolates M12-561, M12-562, M12-563, M12-564, which had no variant positions between them, all originated from the same city block (block 30). Isolates M12-85 and M12-117, which had 7 variant positions between them, and isolates M12-121 and M12-118, which had 2 variant positions between them, originated from block 19. Isolates, M12-473 and M12-475, which had 10 variant positions between them, originated from block 27. In contrast, other samples originating from the same block showed relatively high genetic diversity.

Figure 9-5: Geographic distribution of MRSA-positive Norway rats (*Rattus norvegicus*) from an inner-city neighborhood of Vancouver, Canada, and clusters of high and low MRSA prevalence by whole genome sequence (WGS) group. Observed vs. expected number of MRSA-positive rats with relative risk and p values noted for each cluster. Inset = Map of Vancouver with location of study site.
9.5 Discussion

This study demonstrates that urban rats carry a variety of MRSA lineages previously described in humans and livestock. More specifically, four distinct genetic clusters were identified in the rat population under study.

Four of the isolates were assigned to cluster three, which corresponds to ST97, an MRSA clone that has been reported in both people and livestock (296-298). Five isolates were assigned to cluster two, which corresponds to the LA-MRSA strain ST398 (279). Interestingly, both ST97 and ST398 have been previously identified in rats collected from livestock farms (281). The origin of these strains in our study area, however, is unclear. ST398 is known to colonize and infect humans, but is much more prevalent in production animals (279), suggesting that the most likely source of this ST was contaminated animal products (299). Groceries, restaurants, and other premises that process, prepare, and sell animal products are common throughout the study area, so exposure through infestation of these premises or through waste scavenging is possible. Although ST97 originated in livestock, it is now recognized to be an emerging pandemic clone of CA-MRSA (298), making it difficult to speculate as to the reservoir of this ST.

Other isolates were more clearly of human origin. For example, 2 isolates were assigned to cluster four, which corresponds to ST105. This ST belongs to clonal complex 5 (CC5), the leading cause of HA-MRSA infection in Canada (300), as well as CA-MRSA colonization in Canada and the United States (300, 301). MRSA CC5 has also been reported in various domestic animals in Canada (302).

The most common genetic cluster in our study corresponded to the CA-MRSA strain USA300 (291), the most common strain of MRSA circulating in the DTES human population (284). Rat isolates from this cluster were genetically indistinguishable from DTES human isolates and had the same degree of genetic diversity. These findings are
supportive of multiple transmissions of MRSA between humans and rats, although the direction is unclear and common-source exposure cannot be excluded.

Although spatial clustering is a common characteristic of urban rat populations (154, 164) and the microbes that circulate within them (178), clustering was not a strong feature of MRSA in rats with the exception of ST97. Indeed all MRSA isolates from block 30 that were characterized by WGS were genetically identical and identified as ST97. At this point it cannot be determined whether geographic clustering of ST97 represents rat-rat MRSA transmission or exposure to a common ‘non-rat’ source of MRSA within the block.

The statistical analysis suggests that MRSA status (positive vs. negative) in rats is associated with both body fat and season. Body fat is likely a good marker of dominance within a rat colony, as dominant rats have the greatest access to food resources (154, 178). Dominant rats are also more likely to explore new objects and territories and to engage in social interactions (e.g., fighting and mating) (154), which may increase their likelihood of being exposed to MRSA in other rats or the environment. The association with MRSA carriage and season could reflect temporal variation in rat-rat or rat-human interactions (17, 154).

In addition to their ability to carry MRSA, rats might also act as a ‘mixing vessel’, facilitating transfer of genetic elements between \textit{S. aureus} and other bacterial species. Interestingly, all of the ST97 isolates contained a genetic element identical to that found in \textit{Staphylococcus pseudintermedius}, which has also been identified in this rat population (283). Unfortunately, the rats from block 30, where the ST97 was found, were not among those tested for \textit{S. pseudintermedius}. Given that this element has been identified in \textit{S. aureus} ST97 from another study (295), it is difficult to confirm its true origin. Regardless, it is important to consider that rats actively explore a number of different human environments, and therefore have a unique opportunity to become exposed
to colonized with a variety of human pathogens and to facilitate gene transfer among these organisms.

Although these results show that rats can carry MRSA, the pathways through which MRSA is acquired by rats, the degree to which MRSA is maintained in rat populations, and the potential significance of rats as a reservoir of MRSA for humans, animals, and the environment remain unclear. One limitation of the present study was the focus on MRSA versus *S. aureus* in general. Inclusion of methicillin-sensitive *S. aureus* in future studies may help to elucidate the ecology of *S. aureus*, including MRSA, in rats. Additionally, we chose to sample the rat nares and oropharynx only, as this was the methodology used in a past study of MRSA in rats [11]. However, given that MRSA can be shed in the feces of other animal species (303), it would be prudent to sample the rectum or feces in future studies to investigate if the same is true for rats, particularly since fecal shedding of MRSA in rats could increase their potential to spread the bacterium.

Previously, studies of disease risks associated with rats have largely focused on pathogens for which rats are the primary reservoir (e.g., *Leptospira* spp.). However, this study shows that rats can also carry pathogens more commonly associated with humans and other animal species. This is particularly significant with regard to MRSA because: a) MRSA infection is a substantial problem in impoverished urban communities; and b) these communities are also most likely to experience rat infestations and rat-to-human disease transmission. Overall, this study signals the need for additional research regarding the ecology and significance of MRSA carriage in urban rats.
Chapter 10: Carriage of Clostridium difficile by Wild Urban Norway and Black Rats

10.1 Synopsis

*Clostridium difficile* is an important cause of enteric infections in people. Recently, concerns have been raised regarding whether animals could be a source of *C. difficile* spores. Although colonization has been identified in a number of domestic species, the ability of commensal pests to serve as a reservoir for *C. difficile* has not been well investigated. The objective of this study was to determine if DTES rats carry *C. difficile*. *Clostridium difficile* was isolated from the colon content of trapped rats and characterized using ribotyping, toxinotyping, and toxin gene identification. Generalized linear mixed models and spatial analysis were used to characterize the ecology of *C. difficile* in rats. *Clostridium difficile* was isolated from 95 of 724 (13.1%) rats, although prevalence varied from 0% to 46.7% among city blocks. The odds of being *C. difficile*-positive decreased with increasing mass (OR = 0.67, 95% CI = 0.53 – 0.87), suggesting that carriage is more common in younger animals. Strains isolated included 9 ribotypes that matched recognized international designations, 5 identified by our laboratory in previous studies, and 21 ‘novel’ ribotypes. Some strains were clustered geographically; however, the majority were dispersed throughout the study area, supporting environmental sources of exposure and widespread environmental contamination with a variety of *C. difficile* strains. Given that urban rats are the source of a number of other pathogens responsible for human morbidity and mortality, the potential for rats to be a source of *C. difficile* for people deserves further consideration.
10.2 Introduction

_Clostridium difficile_ is an obligate anaerobic bacterium and important human pathogen (304, 305). It forms highly resistant spores, which can persist in the environment for long periods of time, facilitating transmission (306, 307). Clinical manifestations of _C. difficile_ infection (CDI) in humans can range from asymptomatic carriage to mild diarrhea to fatal colitis (304, 307). Disease is a result of the proliferation of toxigenic strains of _C. difficile_ and the production of toxin A and B (TcdA and TcdB), with unknown contribution from a third toxin, binary toxin (CDT) (308).

The ability of _C. difficile_ to colonize the intestine is dependent on disruption of the normal colonic microbiota (306, 309). For this reason, disease in humans is often precipitated by the administration of antibiotics (306, 309). Indeed, CDI is most common in hospitals and long-term care facilities, particularly in the elderly, and those with co-morbidities and receiving antibiotics (304, 306, 309).

Over the past few decades there has been a marked increase in the incidence and severity of CDI (309). This is, in part, due to the emergence of ‘hypervirulent’ and epidemic strains, particularly ribotypes 027 and 078, which are often more pathogenic, transmissible, and difficult to treat (304, 305, 309, 310). There has also been an increase in community-associated CDI, which occurs in people with no history of hospitalization or antimicrobial therapy, and in individuals previously thought to be low risk (e.g., perinatal women) (304, 305, 309, 310).

A variety of animal species may also become colonized with _C. difficile_ and/or develop clinical disease secondary to infection (304, 307, 311). There is significant overlap of strains known to infect animals and people, and in many cases human and animal isolates are indistinguishable (304, 305, 307). This has raised the possibility of zoonotic transmission of _C. difficile_ between animals and people, either through direct contact, through the food chain (i.e., contamination of meat products), or through the environment (304, 309).
Although both companion and food-producing animals have been considered as potential sources of *C. difficile*, the potential for commensal pests, particularly rodents, to be a reservoir for this bacterium has received little attention. A high prevalence of *C. difficile* carriage (66%), including carriage of ribotype 078, was detected in house mice (*Mus musculus*) infesting a pig farm in the Netherlands, where the researchers postulated that pests could play a role in the maintenance and transmission of *C. difficile* (312). Carriage of *C. difficile* in urban rodents, however, has not been investigated. Urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) are known to be a significant source of disease for people, and can transmit a variety of different pathogens through direct contact, through contamination of food stuffs, or through the environment (16). This raises the question of whether urban rats could also be a source of *C. difficile*.

The objectives of this study were to determine if Norway and black rats from the DTES carry *C. difficile*, to characterize isolated strains, and to begin to describe the ecology of *C. difficile* in urban rat populations.

### 10.3 Methods

#### Sample selection

Rats were trapped and processed as described previously (Chapter 2). During necropsy, the colon was collected aseptically and stored at -80°C. Colon samples from 724 of 725 rats were included in the analysis (one rat was excluded because of excessive autolysis).

*Clostridium difficile* isolation, toxin gene identification, ribotyping, and toxinotyping

Colonic contents were shipped to the OVC where they were inoculated into 2 ml of *C. difficile* moxalactam norfloxacin (CDMN) enrichment broth (Oxoid Ltd, Nepean, Ontario, Canada) containing 0.1% sodium taurocholate. Samples were incubated
anaerobically at 37°C for 7 days. A 1 ml aliquot of broth was mixed with an equal amount of anhydrous alcohol and incubated a room temperature for 60 minutes. After centrifugation (3800 g for 10 min), the pellet was inoculated onto CDMN agar (Oxoid Ltd, Nepean, Ontario, Canada) and incubated anaerobically at 37°C for 48-96 hours. Suspicious colonies were subcultured onto Columbia blood agar and incubated for 48h at 37°C, with subsequent identification based characteristic morphology and odour of the colonies, Gram stain, and the presence of the L-proline aminopeptidase activity (Remel Inc, Lenexa, Kansas, USA). A single colony for each isolate was subcultured, stored at -80°C, and re-cultured prior to molecular analysis.

All isolates identified as *C. difficile* were investigated for the presence of toxin A (*tcdA*) (313), toxin B (*tcdB*) (314), and binary toxin (*cdtA*) (315) genes using PCR and characterized using ribotyping (316). When a ribotype pattern was identified as an international ribotype, based on comparison to the reference strains from the Cardiff-ECDC Collection, the appropriate numerical designation (e.g., 078) was assigned. Alternatively, an internal laboratory designation was assigned. Toxinotyping was also performed on all isolates (317).

**Statistical analysis**

The primary outcome variable was *C. difficile* presence (positive vs. negative). Explanatory variables that were considered included season of capture, mass, sex, sexual maturity, fat score, presence of cutaneous bite wounds, number of cutaneous bite wounds, and pregnancy and lactation in sexually mature females.

The distribution of each explanatory variable was examined among the sample as whole, as well as separately for *C. difficile*-positive and -negative rats. Regression modeling was then used to examine relationships between *C. difficile* status and each of the explanatory variables, first using simple logistic regression, and then using multiple logistic GLMMs (to control for the random effect of block). The final multivariate model
was arrived at by manual stepwise regression using AIC to compare candidate models (the final model was that with the lowest AIC) (172).

All statistical analyses were conducted using R (R Development Core Team, Vienna, Austria). For multivariate models, individuals with missing data for one or more of the variables under study were excluded. Collinearity among explanatory variables was identified using Spearman’s rank correlation ($\rho$) (172). Highly correlated variables ($\rho > 0.8$) were modeled separately (172).

To determine whether the epidemiology of \textit{C. difficile} varied by ribotype, the multivariate model building process was repeated for rats with ‘novel strains’ (i.e., those not consistent with an international designation and not previously identified in our laboratory) vs. \textit{C. difficile}-negative rats (rats with previously identified strains were excluded), and for previously identified strains vs. \textit{C. difficile}-negative (rats with ‘novel’ strains were excluded).

Finally, city block was entered into a simple logistic regression model as a fixed effect to determine if block of origin was significantly associated with the odds of an individual rat being infected with \textit{C. difficile}.

\textbf{Spatial analysis}

The location of each trap within the study area, and the number of rats caught in each trap that were tested for \textit{C. difficile} and that were \textit{C. difficile}-positive were mapped using ArcGIS 10.0 (ESRI, Redlands, USA). For \textit{C. difficile}-positive rats, the ribotype that each rat was carrying was also mapped. This information was imported into SaTScan 9.1.1 (Boston, USA) for cluster analysis using a purely spatial Bernoulli model and scanning for areas with high and low rates of \textit{C. difficile} carriage using a circular window with a maximum spatial cluster size of 50% of the population at risk. SaTScan uses a scanning window statistic to identify clustering of observed events (‘cases’).
compared against the distribution of expected events (‘controls’) if spatial locations of all events were independent.

For the first analysis, any \textit{C. difficile}-positive rat was considered a ‘case’ and any \textit{C. difficile}-negative rat was considered a ‘control.’ Subsequently, this analysis was repeated separately for each ribotype with more than one isolate to identify any areas of clustering by ribotype. For these analyses, any rat with the ribotype of interest was considered a ‘case’ and all \textit{C. difficile}-positive rats with other ribotypes were considered ‘controls.’

For all spatial analyses the port site was excluded because of privacy concerns (trapping occurred in private property), because trapping took place at multiple levels within a single geographic footprint (which is difficult to represent in a two dimensional map), and because trapping was somewhat more opportunistic (vs. systematic) compared to the blocks.

10.4 Results

\textit{Clostridium difficile} was detected in 95 of 724 (13.1%) rats trapped, although the prevalence varied markedly by city block, from 0% to 46.7% (Figure 10-1). Block of origin was significantly associated with the odds of being \textit{C. difficile}-positive (data not shown).
Of the 95 isolates, 1 (1.1%) had \textit{tcdB} but not \textit{tcdA} or \textit{cdtA}, 78 (82%) had \textit{tcdA} and \textit{tcdB} but not \textit{cdtA}, and 16 (16.9%) had all three toxin genes. A total of 35 different ribotypes were identified (Table 10-1). These included 9 ribotypes that matched recognized international designations, 5 ribotypes identified by our laboratory in human samples from previous studies but not matching international designations, and 21 ribotypes not previously identified by our laboratory in humans or animals (and not matching international designations). There was no evidence of geographic clustering for \textit{C. difficile}-positive rats in general (Figure 10-2a). However, clusters were identified for ribotypes 078, A, and VR6 (Figure 10-2b and 10-2c). Ribotypes identified at the port included 001 (n = 7), 014 (n = 1), and VR11 (n = 1).
Table 10-1: Relative frequency of *Clostridium difficile* ribotypes (with toxin profile and toxinotype) isolated from urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*).

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<tr>
<td>VR7</td>
<td>tcdA, tcdB</td>
<td>XII</td>
<td>4 (0.6)</td>
</tr>
<tr>
<td>VR8</td>
<td>tcdA, tcdB, cdtA</td>
<td>III</td>
<td>5 (0.7)</td>
</tr>
<tr>
<td>VR9</td>
<td>tcdA, tcdB</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR10</td>
<td>tcdA, tcdB, cdtA</td>
<td>IV</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR11</td>
<td>tcdA, tcdB</td>
<td>0</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>VR12</td>
<td>tcdA, tcdB</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR13</td>
<td>tcdA, tcdB</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR14</td>
<td>tcdA, tcdB</td>
<td>0</td>
<td>4 (0.6)</td>
</tr>
<tr>
<td>VR15</td>
<td>tcdA, tcdB</td>
<td>XII</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR16</td>
<td>tcdA, tcdB</td>
<td>XXI</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>VR17</td>
<td>tcdA, tcdB</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR18</td>
<td>tcdA, tcdB</td>
<td>XXI</td>
<td>4 (0.6)</td>
</tr>
<tr>
<td>VR19</td>
<td>tcdA, tcdB</td>
<td>XII</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR20</td>
<td>tcdA, tcdB</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>VR21</td>
<td>tcdA, tcdB, cdtA</td>
<td>V</td>
<td>1 (0.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Numeric identifiers (e.g., 001) indicate ribotypes matching international designations. Letter identifiers (e.g., A) indicate ribotypes not matching international designations but previously identified in our laboratory. ‘VR’ designations (e.g., VR1) indicate ribotypes not matching international designations and not previously identified in our laboratory.
Figure 10-2: A) Geographic distribution of Clostridium difficile-positive Norway and black rats (Rattus norvegicus and Rattus rattus). B) Distribution of C. difficile ribotypes with international designations or previously identified in our laboratory and clusters of high prevalence. C) Distribution of ‘novel’ C. difficile ribotypes and clusters of high prevalence. Inset = Location of study site.
The distribution of the explanatory variables in the sample as a whole, and in C. difficile-positive and -negative rats is detailed in Table 10-2.

### Table 10-2: Baseline characteristics and associations with *Clostridium difficile* status among urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*).

<table>
<thead>
<tr>
<th>Category</th>
<th>Subcategory</th>
<th>Total (%)&lt;sup&gt;a&lt;/sup&gt; (n = 724)</th>
<th>Positive (%)&lt;sup&gt;a&lt;/sup&gt; (n = 95)</th>
<th>Negative (%)&lt;sup&gt;a&lt;/sup&gt; (n = 629)</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Norway</td>
<td>684 (94.5)</td>
<td>89 (93.7)</td>
<td>595 (94.6)</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>40 (5.5)</td>
<td>6 (6.3)</td>
<td>34 (5.4)</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>Fall</td>
<td>239 (33.0)</td>
<td>23 (24.2)</td>
<td>216 (34.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>135 (18.6)</td>
<td>23 (24.2)</td>
<td>112 (17.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>259 (35.8)</td>
<td>45 (47.4)</td>
<td>214 (34.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>91 (12.6)</td>
<td>4 (4.2)</td>
<td>87 (13.8)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>400 (55.2)</td>
<td>48 (50.5)</td>
<td>352 (56.0)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>316 (43.6)</td>
<td>44 (46.3)</td>
<td>272 (43.2)</td>
<td></td>
</tr>
<tr>
<td>Sexual maturity</td>
<td>Mature</td>
<td>417 (57.6)</td>
<td>47 (49.5)</td>
<td>370 (58.8)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Immature</td>
<td>237 (32.7)</td>
<td>40 (42.1)</td>
<td>197 (31.3)</td>
<td></td>
</tr>
<tr>
<td>Mass (g)</td>
<td>Median (IQR)</td>
<td>134.0 (63.8 – 251.6)</td>
<td>90.6 (56.7 – 215.2)</td>
<td>147.7 (65.8 – 257.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fat score</td>
<td>Poor</td>
<td>307 (42.4)</td>
<td>47 (49.5)</td>
<td>260 (41.3)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>195 (26.9)</td>
<td>25 (26.3)</td>
<td>170 (27.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>202 (27.9)</td>
<td>19 (20.0)</td>
<td>183 (29.1)</td>
<td></td>
</tr>
<tr>
<td>Wound presence</td>
<td>Yes</td>
<td>176 (24.3)</td>
<td>20 (21.1)</td>
<td>156 (24.8)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>547 (75.6)</td>
<td>75 (78.9)</td>
<td>472 (75.0)</td>
<td></td>
</tr>
<tr>
<td>Wound number</td>
<td>Median (IQR)</td>
<td>0.0 (0.0 – 0.0)</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0 – 0.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>Pregnant&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Yes</td>
<td>33 (20.0)</td>
<td>2 (7.4)</td>
<td>31 (22.5)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>132 (80.0)</td>
<td>25 (92.6)</td>
<td>107 (77.5)</td>
<td></td>
</tr>
<tr>
<td>Lactating&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Yes</td>
<td>79 (47.9)</td>
<td>12 (44.4)</td>
<td>67 (48.6)</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>86 (52.1)</td>
<td>15 (55.6)</td>
<td>71 (51.4)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Frequencies and percentages may not add to 100% because of exclusion of rats with missing data for the variable in question.

<sup>b</sup> Sexually mature females only (n = 165).

<sup>c</sup> Determined using the Chi-squared test or Welch’s t-test, where appropriate.

After controlling for clustering by block, only mass was a significant predictor of *C. difficile* status in either bivariate or multivariate models (Table 10-3), with a mass increase of 10g being associated with a 33% decreased odds of being *C. difficile*-positive (OR = 0.67, 95% CI = 0.53 – 0.87). In this model, the variance associated with the random effect of block of origin was 0.45. The final model did not change significantly when
‘novel’ or previously recognized ribotypes were used as the outcome of interest (data not presented).

Table 10-3: Relationship between rat characteristics and *Clostridium difficile* status among Norway and black rats (*Rattus norvegicus* and *Rattus rattus*).

<table>
<thead>
<tr>
<th>Category</th>
<th>Subcategory</th>
<th>Unadjusted&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Adjusted&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95% CI</td>
</tr>
<tr>
<td>Species</td>
<td>Black</td>
<td>2.05</td>
<td>0.57 – 7.34</td>
</tr>
<tr>
<td></td>
<td>Norway</td>
<td>REF&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>Winter</td>
<td>1.64</td>
<td>0.71 – 3.82</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>1.83</td>
<td>0.84 – 3.96</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>0.45</td>
<td>0.12 – 1.74</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>0.86</td>
<td>0.55 – 1.36</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Maturity</td>
<td>Immature</td>
<td>1.59</td>
<td>0.95 – 2.66</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Wound presence</td>
<td>Yes</td>
<td>0.69</td>
<td>0.40 – 1.20</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Wound number</td>
<td>Yes</td>
<td>0.69</td>
<td>0.40 – 1.20</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Mass (10 g)</td>
<td>Good</td>
<td>0.67</td>
<td>0.53 – 0.87</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0.84</td>
<td>0.47 – 1.48</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Fat score</td>
<td>Good</td>
<td>0.55</td>
<td>0.28 – 1.04</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0.84</td>
<td>0.47 – 1.48</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Pregnant&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes</td>
<td>0.30</td>
<td>0.06 – 1.41</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Lactating&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes</td>
<td>0.95</td>
<td>0.39 – 2.35</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>REF</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Odds ratio (OR) with 95% confidence interval (CI) generated using a generalized linear mixed model to control for clustering by city block of origin.

<sup>b</sup> Reference category.

<sup>c</sup> Sexually mature females only (n = 165).

<sup>d</sup> Results of bivariate modeling.

<sup>e</sup> Results of the final multivariate model building procedure.

### 10.5 Discussion:

Canada is one of the many countries experiencing a significant and growing health burden associated with CDI. Indeed, within the province of British Columbia (BC), where Vancouver is situated, *C. difficile* has overtaken methicillin-resistant *Staphylococcus aureus*, as the most common cause of healthcare-associated (HCA)
infection in acute care facilities (318). In 2012/2013, there were 3,246 cases of CDI reported in BC acute care facilities, of which 72.6% were classified as HCA and 27.4% were classified at community-associated (CA) or of unknown association (318). Within the Vancouver Costal Health Authority, specifically, the annual incidence rate of CDI in 2012/2013 was 8.4 cases per 10,000 inpatient days (318). Of particular concern was a 69.8% increase in the number of cases of CA CDI cases in BC from 2009/2010 to 2012/2013 (318). The source of *C. difficile* in cases of CA CDI is not well understood, but could potentially include those of animal origin.

To our knowledge, this is the first study to demonstrate that wild urban Norway and black rats can carry *C. difficile*. Ribotypes identified in these rats included 9 internationally recognized ribotypes known to colonize and/or cause disease in people humans and domestic animals (319, 320), 5 ribotypes without international designations but previously identified in human samples at our laboratory, and 21 ribotypes without international designations and not previously identified by our laboratory amongst a collection of over 5000 isolates from humans and animals.

Ribotype 001, a North American pulsetype 2 (NAP2) strain, is an important cause of CDI in humans in Canada (321), and was one of the most common ribotypes identified in this study. This ribotype has also been identified in various animal species in Canada, including dogs, cats and horses (322, 323). Ribotype 078, which is an emerging cause of CA CDI in people (310), was also relatively common among these rats. This ribotype is frequently isolated from livestock (324, 325), leading to concerns that zoonotic transmission may be a significance source of this pathogen for people (310, 326). While only one rat harbored ribotype 027/NAP1, this is noteworthy because of its clinical importance in people (305, 309). This ribotype, which is toxinotype III and posses CDT, is considered to be an epidemic hypervirulent strain as it accounts for a significant proportion of CDI outbreaks in people and is associated with increased disease severity and rate of relapse (327, 328). One novel strain, VR8, detected in 5 rats
in this study was also toxinotype III and CDT positive. Whether this strain carries the same human health concerns as ribotype 027 is unknown but should be considered. Ribotype 014, found in 3 rats in this study, is another common pathogenic strain in humans and has been found in various animal species (329, 330). It was reported to be the most common ribotype in a study of river water in Slovenia (331), which could suggest water as a source of exposure for rats and/or that rats may be one source of water contamination with this strain.

Twenty-one ‘novel’ ribotypes were identified in this study and accounted for 52% of isolates. Lack of harmonization of ribotyping methodology and the absence of a comprehensive comparative database hinder accurate epidemiologic analysis of C. difficile strains, therefore it cannot be definitively determined whether or not these ribotypes have ever been identified in humans or other species. However, the fact that these strains have not been found in the authors’ large and diverse isolate collection suggests that at least some might be rat- or wildlife-associated, or at least rare in other species. Ultimately, the risk posed to humans or other animal species by these ‘novel’ strains is unclear at this time.

The only factor significantly associated with C. difficile infection in these rats was mass. Increased body mass was significantly associated with decreased odds of being C. difficile- positive, suggesting that carriage is more common in younger rats. Indeed the median mass for C. difficile-positive rats was 90.6 g vs. 147.7 g for C. difficile-negative rats. Mass is often used as a proxy for chronologic age in rats, but can also be influenced by a variety of other factors including sex, nutritional condition, species, and even population of origin (138, 154, 160, 161, 184). In this study, species, sex, internal fat stores, and block of origin were controlled for in the analysis, leading us to believe that the association between C. difficile and mass likely suggests that carriage is, indeed, a function of age. This is similar to humans and other animal species where colonization is most common in the young (325, 332), likely because an immature gut microbiome is
conducive to the establishment of *C. difficile* (333). It should be noted that the rats included in this study, by virtue of them being in the ‘trappable’ population, were weaned and had left the nest (170). It would be interesting to know whether the prevalence of *C. difficile* is even greater in pre-weaned rats, as it is in infant humans (332, 334, 335).

Although within the study site as a whole there was no clear clustering of *C. difficile*-positive rats, the prevalence of *C. difficile* did vary significantly by city block (which was also reflected by the variance associated with the random effect of block in the GLMM). This suggests that while no one area was particularly conducive to *C. difficile* carriage, there may be block-level variations in environmental or population characteristics that impact the probability of *C. difficile* carriage in rats. Similarly, the majority of the ribotypes appeared to be geographically dispersed. Given that urban rats exist in tight-knit colonies with small home ranges (usually limited to a city block) and minimal inter-colony contact (154, 175), the overall paucity of clustering by ribotype seems to suggest that transmission of *C. difficile* among rats is minimal. Rather, it appears more likely that rats acquire *C. difficile* from the environment, and that environmental contamination with numerous different *C. difficile* ribotypes is ubiquitous within the study area. Environmental exposure is thought to be among the most important routes of *C. difficile* infection in humans and other species (304, 306, 332). Significant geographic clusters of ribotypes 078, A, and VR6 (and likely 001 at the port) might suggest that some transmission among rats is possible, but might also indicate a common environmental source of exposure.

The exact source of exposure, however, is more difficult to determine. Within the urban environment, rats have the potential to come into contact with spores in a number of different circumstances, for example while foraging in refuse or even while simply traversing the alley surface. It is interesting to note that there is a significant amount of human excrement in the alleys within our study area, which could serve as a
source of exposure for rats. Rats might also be exposed to spores in sewage, although it cannot be determined if the rats included in this study access the sewer systems. Within the study area, there are a number of facilities that prepare and sell animal products, making these facilities a potential source of livestock-origin strains. There are also a number of companion animals, such as cats and dogs, which reside in the neighbourhood. Finally, it is also possible that rats might become exposed to spores in the feces of other rats either through contamination of the burrow system or through coprophagic behaviours in rat pups (336, 337).

It is interesting to note that there was no clinical or post-mortem evidence of diarrhea or enteritis in any of the C. difficile-positive rats. This is consistent with the literature, which suggests that rats are relatively refractory to CDI (311). However, all of the strains identified carried at least one toxin gene, with 82% carrying both tcdA and tcdB genes, and 16.9% carrying all three genes. Additionally, although most (n=28) C. difficile strains identified in this study were toxinotype 0, five other toxinotypes were found, including toxinotypes III and V, which receive much attention because they include ribotypes 027 and 078, respectively (317). This suggests that the C. difficile carried by these rats could be pathogenic for humans and other species (304, 306, 311). The potential for rats to be a significant source of C. difficile is amplified by their capacity to contaminate the environment, particularly foodstuffs, with their feces (154). Indeed, fecal contamination of food is a known route of exposure for other rat-associated zoonoses (16), and there is evidence that zoonotic or food borne transmission may be an important component of the epidemiology of certain C. difficile strains, such as ribotype 078 (338).

One limitation of the current study was its cross-sectional nature, which prevented us from being able to determine to what degree C. difficile positivity represents true colonization versus transient passage of spores. Future studies ought to include longitudinal sampling, if possible, in order to determine the degree to which C.
*difficile* can be maintained and propagated in rat populations. Additionally, we chose to characterize only one *C. difficile* isolate per rat, and therefore could not address the possibility that a single rat could carry more than one ribotype. It may be worthwhile to characterize multiple isolates per individual in the future in order to better characterize the ecology *C. difficile* in rats.

Past research on rat-associated zoonotic risks has largely focused on pathogens for which the rat is the natural host (e.g., *Leptospira interrogans*, *Streptobacillus moniliformis*, *Yersinia pestis*, etc.). However, this study shows that urban rats can become colonized with other pathogens present in their environment, and could subsequently server as a reservoir for these organisms. Given the exploratory nature of rats (164) and their propensity to inhabit and/or exploit every aspect of the urban ecosystem (154), the capacity for rats to accumulate pathogenic microbes should not be underestimated. Overall, the frequency and diversity of *C. difficile* strains identified in this study, including novel strains as well as those commonly found in humans and domestic animals, suggest that the ecology of *C. difficile* in urban rats is complex and warrants further study.
Chapter 11: Conclusion

11.1 Summary of Findings

The goal of this dissertation was to begin to characterize rat-associated health risks in Vancouver’s DTES by describing the characteristics of urban rat populations, identifying the zoonotic pathogens circulating within the rat populations under study, and studying the ecology of those pathogens in rats.

The literature review in Chapter 2 identified which zoonotic pathogens have been associated with rats in the past and characterized what is known about the biology and ecology of each pathogen. By comparing and contrasting the published literature on each RAZ, a number of common themes were identified, which informed the development of this project. For example, the evidence that urbanization increases the incidence of RAZ in people, in combination with the paucity of literature regarding rats and RAZ in Canada, supported the idea that a Canadian field study was necessary. Additionally, evidence that impoverished inner-city neighbourhoods are at an increased risk of rat infestations, rat-human contact, and zoonotic disease transmission allowed us to narrow in on the DTES as the most appropriate study area. The apparent influence of rat population characteristics and environmental factors on RAZ ecology highlighted the importance of developing an understanding of DTES rat populations and the DTES environment before attempting to identify and understand zoonotic pathogen dynamics in rats. It also highlighted the importance of developing an informed and intensive trapping protocol in order to ensure that the data on rats and RAZ was accurate and that we were able to interpret those data.

Given the lack of data on rats in Canada, specifically, knowledge gained from the literature review was augmented by surveying Canadian PCPs. The information collected from PCPs supported that supplied by the review and identified additional
areas for consideration during the design and execution of the field-work. For example, PCPs highlighted the fact that rat infestations are particularly problematic in the DTES compared to other areas of the Vancouver. They also identified that rat infestations appear to be highly influenced by features of the urban environment, and their responses informed the development of the environmental observation tool (Chapter 5). It should also be noted that informal conversations and collaborations with local PCPs stemming from the survey were critical to the design and subsequent success of the trapping study.

Chapter 4 describes this trapping study and the characteristics of the trapped rat population. In characterizing that population, the focus was on factors that might influence the ecology of RAZ in rat populations. For example, it was found that rat populations were unevenly distributed across the urban landscape. The identification of this clustering was critical to sampling and analysis framework developed for the RAZ studies (Chapter 6-10). Additionally, a variety of relationships among rat demographic and morphometric characteristics were identified. For example, body mass has long been used as a proxy for age in rats but this study showed that mass is actually influenced by a number of factors, including length, body fat, and block of origin. This highlighted the importance of using a multivariate regression in studies of RAZ ecology in order to avoid confounding. It also facilitated a better understanding of the relationships between specific rat characteristics and pathogen presence.

In Chapter 5, the design and implementation of a systematic environmental observation tool is described. This tool was used to develop a better understanding of the relationship between features of the urban environment and rat abundance. The results of this study supported, occasionally contradicted, and augmented the information gained from the literature review and from the PCP survey. The success of the tool and associated methodology suggested that it could be used in the future for
developing a better understanding of the relationship between RAZ and the urban environment.

Chapters 6 – 8 describe testing for zoonotic pathogens that had previously been identified in rats including *Leptospira interrogans*, *Rickettsia typhi*, *Bartonella* spp., Seoul hantavirus, and bacteria associated with rat bites. Chapter 6 describes the presence of *L. interrogans* in DTES rats, and the fact that infected rats were clustered by block. It was also found that the odds of an individual rat being infected varied with mass, body fat, and bite wounds, suggesting that social structures may play a role in *L. interrogans* dynamics. Interestingly, there was no evidence of infection with *R. typhi*, *Bartonella* spp., and SEOV in DTES rats (Chapter 7), despite the fact that a review of the literature suggested that these pathogens should be endemic in rat populations worldwide. In Chapter 8 intra-specific bite wound infections in rats were used to identify the bacteria that could be transmitted from rats to people through biting. These data augment the body of knowledge regarding rat bite-related infectious risks by highlighting the variety of bacteria that can be transmitted through biting, as well as the polymicrobial nature of most bite-related infections. This chapter also presents a review of literature regarding rat-bites and bite wound management in people.

The final chapters (9 and 10) describe carriage of MRSA and *C. difficile*, two organisms with zoonotic potential that have not previously been associated with urban rats. Additionally, several features of the ecology of these organisms were identified. For example, *C. difficile* appeared to be most common in young rats. More significantly, rats were found to carry strains of MRSA commonly found in DTES residents, implying transmission of MRSA from people to rats and suggesting that it might be possible for pathogens to flow in the reverse direction.
11.2 Synthesis and Implications

After careful consideration of the results presented above, the following themes were identified (see Table 11-1):

**Rats in Vancouver's DTES carry a number of different zoonotic or potentially zoonotic organisms**

The rats included in this study were found to carry a number of different bacterial species with the potential to cause human illness should they be transmitted from rats to people. These included *Leptospira interrogans*, *Streptobacillus monilliformis* and other oral bacteria, MRSA, and *C. difficile*.

With regard to the RAZ identified in this study, some of these organisms (e.g., the bacteria associated with rat bites) require direct contact between rats and humans for transmission, while others (e.g., *L. interrogans* and likely *C. difficile* and MRSA) can likely be transmitted through the environment. Research in other jurisdictions has shown that people can become indirectly infected with RAZ such as *L. interrogans* through contamination of the environment, food, or water sources. In this project, however, the most compelling evidence for this mode of transmission came from the MRSA study, as it is likely that USA300 strains were passed from humans to rats through the environment, raising concerns that pathogens could flow in the reverse direction. The possibility that current levels of ‘co-habitation’ among rats and people might be sufficient to allow pathogen transmission increases the potential public health significance of rat infestations in the DTES and elsewhere.

**Urban rats have the potential to be a source of pathogens for which they are not the ‘usual’ reservoir**

As mentioned above, DTES rat populations can carry a variety of zoonotic or potentially zoonotic pathogens and these pathogens can be broadly classified into two
categories: 1) pathogens for which rats are recognized to be the natural reservoir (e.g., *L. interrogans*), and 2) pathogens normally associated with humans, other animal species, or the environment (e.g., MRSA and *C. difficile*), but not normally associated with rats. To date, the majority of the research has focused on the first category. However, this study suggests that the scope of research into rat-associated health risks ought to be expanded to include pathogens that might not traditionally be associated with rats.

Given that urban rats live in close association with people and given that rats explore/exploit numerous aspects of the urban ecosystem (e.g., dwellings, alleys, sewers etc.), it is not surprising that rats can become colonized with a broad range of organisms found in people, domestic animals, food stuffs, refuse, etc.

Along with their reservoir potential, the possibility that rats could be a ‘mixing vessel’ deserves consideration. In other words, it is possible that rats could bring together organisms from a variety of different sources, and thus facilitate horizontal transfer of mobile genetic elements among these organisms. These mobile genetic elements could include genes conferring antimicrobial resistance or increased virulence, resulting in the development of more virulent organisms than were there to begin with. The potential for gene transfer was suggested in the MRSA study. We found that one strain of MRSA appeared to carry a gene insert from methicillin-resistant *Staphylococcus pseudintermedius*, which was also identified in DTES rats. Although we cannot be sure that this represents horizontal transfer of genetic material between bacteria found within the rat host, it suggests that this should be an avenue for further study.

**Rat-associated zoonoses thought to be endemic in rats worldwide might be absent from certain populations**

With regard to zoonoses for which rats are the natural reservoir, the absence of certain pathogens from our study population, specifically *R. typhi*, SEOV, and *Bartonella* spp., was unexpected and conspicuous. The published literature seems to suggest that
all of these pathogens are effectively part of the ‘normal’ microbial flora of urban rats, and are therefore endemic in rat populations worldwide. It is not clear why *R. typhi*, SEOV, and *Bartonella* spp. are not present in DTES rats. It could indicate the lack of appropriate vectors and/or a physical or climactic environment not conducive to pathogen maintenance. Alternatively, it could be the case that these pathogens are absent because they were never introduced into DTES rat populations in the first place. Specifically, the ‘pathogen profile’ of existing urban rat populations may be a reflection of what the founding animals were carrying, particularly given the fact that once a rat colony is established, spontaneous long-distance movements are rare, especially where natural or anthropogenic barriers are in place.

It should also be noted that, from a public health perspective, a thorough understanding of which pathogens are absent from rat populations of interest is just as important as knowing which pathogens are present, as it may prevent unnecessary expenditure of surveillance and research resources on non-existent zoonotic risks. That being said, if absence of certain zoonoses from DTES rats can be explained by random pathogen exclusion subsequent to a founder event, then it is important to consider the fact that these rat populations would likely be able to transmit and maintain those zoonoses should they be introduced. Human transport remains a method for long distance movement of rats and their pathogens; therefore as long as people move in and out of cities, there is potential for pathogen introduction.

**Rat population ecology is intimately related to the ecology of zoonotic pathogens in rat populations**

The results of this project clearly demonstrate that rat populations and RAZ are heterogeneously distributed across the urban landscapes, even over very short geographic distances (i.e., one city block to the next). This clustering supports the idea that urban rats exist in tight colonies with small home ranges and minimal movement of
animals among colonies under ‘normal’ conditions. Clustering of rats and their pathogens also has a number of important implications. From a public health perspective, it means that the risk of exposure to zoonotic pathogens is extremely site specific, and that the number of rats is not necessarily proportional to the disease risk associated with them. For example, we found that some city blocks with very large rat populations had fewer *L. interrogans*-infected animals than blocks with very small populations. The fact that colonies with a high prevalence of *L. interrogans* can co-exist in close geographic proximity to those that were *L. interrogans*-free suggests that rat colonies act like self-quarantining units. This latter point is particularly interesting because it suggests that any disruption that causes migration of rats among colonies could facilitate disease spread. These disruptions could include things such as building demolition, property re-development, or even intensive rodent control campaigns.

Clustering of rats and RAZ also has significant implications for future research design and interpretation. For example, it suggests that aggregate prevalence of a pathogen across a city or over several study sites, which is what is commonly reported for RAZ, is not a meaningful measure of pathogen abundance. Rather, disease prevalence should be reported at whatever level best approximates the colony, as colonies appear to function as relatively distinct populations. It also highlights the importance of developing a thoughtful sampling scheme, since sampling that is opportunistic or haphazard (i.e., that does not take into account urban rat ecology) is likely to produce inaccurate results with regard to pathogen prevalence. Controlling for clustering by block (or whatever geographic unit best represents the size of a colony) is also important during the analysis, as lack of cluster control could mask true associations or create erroneous ones. This was seen in the *L. interrogans* study (Chapter 6) where season appeared to be an important predictor prior to hierarchical modeling, but was shown to have a negligible effect after controlling for clustering by block. As far
as we can tell, this appears to be the first study on urban rats or their diseases to control for clustering.

Clustering of rats and their diseases by block is just one example of how the ecology of rats influences the ecology of infectious pathogens that circulate among them. Indeed, for every pathogen considered in this study, the odds of infection were influenced by one or more demographic or morphometric factors. Leptospira interrogans, for example, was more common in heavier rats, fatter rats, and rats with cutaneous bite wounds, suggesting that transmission might be associated with dominance-related social contacts. The odds of carrying MRSA, on the other hand, were influenced by season, potentially because of seasonal differences in rat-rat or rat-human contact. Finally, the odds of C. difficile carriage were greatest in young rats, suggesting that, as in humans, an immature gut microbiota might be more permissive to the establishment of C. difficile.

Although it seems intuitive that rat physiology, behaviour, etc., would influence pathogen dynamics in rat populations, rat ecology is seldom taken into account when studying rat-associated zoonoses. Neglecting the ecological context of RAZ not only prevents comparability among studies, it also hinders the development of a deeper understanding of these pathogens and can lead to erroneous conclusions regarding RAZ ecology.

As mentioned above, disruptions that trigger long distance migrations could facilitate disease spread. However, even in the absence of rat-dispersal, colony instability could result in changes within the colony itself (e.g., increased aggression) that might affect disease transmission patterns.

11.3 Strengths and Limitations

The major strength of this project was the approach, which sought to first develop an understanding of DTES rat population ecology before endeavoring to characterize
the ecology of RAZ in those populations. Specifically, the fact that rat sampling protocols, data collection, statistical analysis, and interpretation of study results were conducted with careful consideration of ecological data (gathered from the literature and generated over the course of the project), meant that we were able to develop a better understanding of RAZ ecology than past researchers.

The project was also strong in terms of innovation in methodological approaches. For example, a new tool to evaluate the urban microenvironment was developed by borrowing research methodologies from the social sciences. This tool was then used to better understand the relationship between rat abundance and features of the urban landscape. Additionally, by studying bite-related infection in rats, which has never been done before, it was possible to develop a better understanding of infectious risks posed to humans by rat bites. Finally, by testing our rat populations for MRSA and \textit{C. difficile}, the existing body of knowledge regarding zoonotic risks associated with rats was expanded. It is known that these two organisms can be transmitted from domestic animals to people, but potential for urban rats to carry these bacteria had not been previously investigated.

One of the most significant achievements of the project was the development of a comprehensive knowledge translation program. This program centered on the development and maintenance of the Vancouver Rat Project website (www.vancouverratproject.com). The website includes information about the project and project team, as well as about rats and RAZ. Most importantly, the Resources page includes lay language summaries of each paper published through the study, as well as a video that was created to explain the results of the \textit{L. interrogans} study (Chapter 6). The website, project e-mail (vancouverratproject@gmail.com), and social media, specifically Twitter (@VanRatProject), were used to engage in an active discourse with the public. Project results were also communicated through frequent interactions with the popular media (including television, print, radio, and online news agencies). This
approach allowed the Vancouver Rat Project to have a more significant impact than if knowledge translation efforts had been limited to peer-reviewed scientific journals.

With regard to limitations, one of the most significant was the cross-sectional nature of this study; specifically, the two-week study period for each block and the removal (versus release) of rats after trapping. This methodology made it difficult to accurately study seasonal variations in rat and RAZ ecology and precluded the detection of interannual changes within each block or within the study area as a whole. Additionally, because each rat was sampled only once, it could not be determined whether the presence of organisms like *C. difficile* and MRSA represented transient carriage or more long-term colonization. This distinction is important, as the ability of rats to maintain and propagate *C. difficile* and MRSA would increase the public health significance of these organisms in rats.

Another limitation was the fact that the project was limited to a relatively small geographic area. Although this focused approach allowed the development of an in-depth understanding of rats and RAZ in the DTES, it is difficult to determine whether these results can be extrapolated to other areas of Vancouver or to other cities. Certain findings related to underlying ecology of rats and RAZ are likely applicable wherever rats are found. For example, the factors influencing body weight and intra-specific wounding in rats, the relationship between demographic/morphometric characteristics and RAZ carriage, the infectious risks associated with rat bites, etc. However, other results may be more strongly contextual in nature, particularly those related to organisms acquired for humans, other animals, or the environment. For example, although all rats likely have the ability to carry MRSA and *C. difficile*, the prevalence and specific strains of MRSA and *C. difficile* in rat populations probably vary by study location. Additionally, the presence versus absence of certain ‘rat-origin’ RAZ, such as *R. typhi*, SEOV, and *Bartonella* spp., appears to be location-dependent as these organisms have been found in other North American cities but seem to be absent in the DTES.
Finally, given that the trapping was limited to outdoor public property, it is possible rats residing primarily indoors were under-represented or missed altogether. This might explain the low numbers of black rats in the study sample, as black rats are more likely to reside entirely in man-made structures compared to Norway rats. The differing ecology of Norway and black rats suggests that the ecology of RAZ might differ between the two species. ‘Indoor’ rat populations are important to account for as they might have more contact with humans compared to rats that live partially or entirely outdoors.

11.4 Avenues for Further Research

Although this project was able shed considerable light on the ecology of rats and RAZ, it also resulted in the identification of a number of new questions requiring address (Table 11-1). Several priority areas for future research are detailed below.

Metagenomics analysis

Although trapped rats were screened for a variety of ‘known’ zoonotic and potentially zoonotic organisms, it is possible that they may be also carrying other, previously unrecognized, bacteria and viruses. The ‘traditional’ diagnostic techniques (i.e., PCR and culture) used in this project require some *a priori* knowledge of what microbes could be present in a sample, and thus limit the ability to detect unexpected or ‘novel’ pathogens. One solution to this issue would be the use of metagnomic technologies (339). Through deep genomic sequencing and bioinformatics, these technologies are able to better characterize the full suite of microbes present in a tissue and to identify previously undiscovered pathogens. It would be helpful to use metagenomics in future investigations of RAZ to make sure that we have a complete picture of potential rat-associated infectious disease risks.
Factors influencing RAZ prevalence and distribution in rats

Although we know that RAZ are often clustered by block, the precise reasons behind block-level variations in RAZ presence and/or prevalence remain unclear. Some of this variation can be accounted for by controlling for season and demographic/morphometric characteristics; however, this does not explain why some blocks are totally disease free and why the random effect of block was significant in many of the hierarchical regression analyses.

It is possible that some of this residual variation is a result of the microenvironment in the block itself (i.e., that some blocks are more conducive to maintaining certain pathogens than others). Indeed, this project showed that microenvironmental factors influence rat abundance (Chapter 5), and future studies should seek to determine if there is any relationship between these factors and disease abundance (potentially using our environmental observational tool).

Another factor deserving of consideration is the genetic structure of rat populations. Past observational research has suggested that rat colonies have a small home range, often limited to a city block, and that there is minimal movement of rats among colonies. If this is the case then colonies may, in effect, act as ‘self-quarantining’ units, which could explain the heterogeneous distribution of disease across the urban landscape. By using molecular ecology methods (i.e., looking at genetic relationships among rats within and among colonies) we could better investigate the degree to which colonies are truly segregated (340).

Molecular ecology might also help us to understand why certain RAZ are present/absent in specific urban centres. As discussed above, the presence or absence of a pathogen within a city might be a reflection of the characteristics of the founding rat populations. This hypothesis could be investigated by comparing the genetic structure of rat populations in different cities (340). By using population genetics to understand
how rats are distributed, we may be able to shed some light on the true global
distribution and ecology of RAZ.

Genetics might also impact pathogen prevalence through phenotypic variations that
influence the ability of rats to maintain or transmit RAZ (341). For this reason it might
be helpful to examine the relationship between RAZ infection in rats and specific
genetic markers.

In other wildlife species, chronic stress (as measured by increased tissue cortisol
levels) has been linked to decreased fitness and increased susceptibility to infection and
disease (342, 343). Stress in wild animals is often the results of anthropogenically-
induced external pressures, particularly habitat change. Given that habitat change is
common in the urban environment, stress could also be playing a role in RAZ
dynamics. Future studies of RAZ ecology should seek to examine the relationship
between tissue cortisol levels and RAZ infection in rats.

Exposure to toxins and pollutants has also been shown to induce
immunosuppression and increased susceptibility to infections in wildlife species (343). Urban rats are likely exposed to a number of different chemicals either incidentally
(e.g., urban environmental contaminants) or on purpose (e.g., rodenticides), and it
would be interesting to know if and how RAZ ecology is influenced by exposure to
these substances. Future studies of RAZ should seek to incorporate toxicology testing in
order to address these possibilities.

**Detecting RAZ transmission from rats to people**

Although a number of potential zoonotic risks associated with rats were identified
over the course of this project, what remains to be determined is whether pathogens are
actually being transmitted from rats to people in the DTES. This question is most easily
answered for RAZ such as *L. interrogans*, for which the rat is the most common reservoir
in the urban environment. Antibody against *L. interrogans* in city dwellers, particularly
if it can be shown to be antibody against rat-associated serovars such as Copenhageni, is highly indicative of rat-to-human pathogen transmission. Future studies should seek to identify evidence of RAZ exposure in people and to identify specific risk factors associated with exposure. In particular, it will be important to identify the degree to which rats and humans are in contact, as well as the nature and circumstances associated with that contact. As noted in the conceptual framework (Figure 1-1), rat-human contact, whether direct or indirect, is essential for zoonotic disease emergence, therefore understanding this contact is important for accurately assessing the public health risks associated with rats.

However, it should be noted that, for organisms like MRSA and C. difficile where the reservoir can be humans or other domestic animal species, proving that rats are the source of infection is much more difficult. Indeed, longitudinal intensive surveillance of sympatric rat and human populations, in combination with detailed examination of the organism of interest (i.e., using WGS), would be required to even have the potential to detect transmission of rat-origin MRSA or C. difficile strains to people. Even this sort of surveillance would not guarantee that transmission would be detected.

**Rats as a ‘mixing vessel’ for virulence or resistance genes**

In Chapter 9, the fact that one strain of MRSA appeared to carry a gene insert from methicillin-resistant *Staphylococcus pseudintermedius*, which was also identified in DTES rats, is reported. Although it is difficult to definitively determine that this insert represents transfer of genetic material within the rat host, it suggests the possibility that rats could bring together pathogens from different origins/ecosystems and facilitate the transfer of virulence or resistance genes among those organisms. This hypothesis could be investigated in the future by using antimicrobial susceptibility testing and whole genome sequencing of bacteria carried by rats.
Impact of environmental change and rat control strategies on rat and RAZ

One of the limitations of this project was its cross-sectional nature, which impeded detection of temporal changes in rat or RAZ ecology. In particular, it was not possible to determine how anthropogenic changes in the urban environment impact rats and the pathogens they carry. It is well established that environmental shifts, such as climate change, are having, and will continue to have, significant impacts on disease ecology in animals and people (106). But what environment changes more quickly or dramatically than that of the city? Interestingly, the impact of environmental modifications (e.g., re-development, infrastructural changes, etc.) and rodent control methodologies on RAZ has yet to receive any attention.

The effects of these external pressures are important to study as they could trigger rat dispersal and facilitate RAZ spread, and/or cause social instability within the colony that might alter RAZ transmission dynamics. Changes in the urban landscape might also alter the frequency and nature of rat-human contact. For this reason, future studies should aim to use longitudinal methodologies to better address temporal changes in rat and RAZ ecology. Temporal changes might be best detected using mark-recapture studies (168) taking place in the same locations over prolonged periods of time (i.e., during each season and for multiple years) with concomitant collection of environmental data. These studies might specifically focus on areas undergoing significant environmental change or might even impose certain conditions on an area of interest (e.g., specific rodent control techniques such as trapping and poisoning).

Given that certain demographic and morphometric data (e.g., weight, sex, sexual maturity, etc.) can be easily collected without harming the animal, mark-recapture is effective for detecting long-term variations in rodent ecology (168, 170). However, it is more difficult to obtain biologic samples (e.g., blood, urine, and feces) of sufficient quantity and quality using non-lethal methods, and certain samples (e.g., internal organs) require that the animal be euthanized. For this reason it will likely be necessary
to combine trap-removal and trap-mark-recapture studies to develop a thorough understanding of rats and RAZ in the future.

**Rat and RAZ ecology in different locations**

In the future, it would be useful to expand this research to other areas of Vancouver and to other cities in Canada in order to determine the degree to which our findings are site-specific vs. applicable to other geographic areas. It might also be useful to focus on transportation hubs, particularly shipping ports, in order to determine the degree to which rats and their pathogens are moved among locales in association with human transport. Finally, it would be helpful to conduct trapping studies within structures in order to ascertain the presence and significance of ‘indoor-only’ rat populations, and to compare the population and disease ecology of these rats with that of rats residing partially or completely outdoors.

**11.5 Recommendations**

Given that the results presented here show that rats could pose a health risk to people, in addition to the ongoing research suggested above, it may be prudent to begin developing more immediate strategies to monitor and mitigate potential rat-associated health threats. Currently, within the city of Vancouver there are no formalized platforms or programs for education on rats and RAZ, there are no tracking systems for rat infestations, rat-human contacts, or rat-associated zoonotic disease in people. Most importantly, there is no clear governmental responsibility for dealing with rat-related problems, except for those occurring on public property (responsibility of the City of Vancouver) or in venues dealing with food (responsibility of the Vancouver Coastal Health Authority). Therefore, there are a number of potential programs that could be implemented to better deal with urban rat issues in the city.
To date, there have been no reported cases of wild rat-associated zoonotic disease in Vancouver residents. However, it is difficult to know if this truly reflects and absence of rat-to-human pathogen transmission, or if it is a result of misdiagnosis, underdiagnosis, and underreporting. Additionally, as mentioned above, rats could be a source of pathogens which are difficult or impossible to trace back to them (e.g., MRSA and \textit{C. difficile}). Currently, there appears to be little awareness within the health care community regarding RAZ, and it is challenging for health professionals to identify and access suitable diagnostic tests. For this reason it would be prudent to educate health professionals regarding rat-associated health risks and to ensure that appropriate diagnostics are easily available should a physician suspect a rat-associated disease. This could be partly accomplished by producing a database for physicians that details known and potential RAZ, risk factors and routes of transmission, symptoms, diagnostic tests (including how they can be accessed), and appropriate management. A similar database could be made available to the public so that they could easily access quality information on rats and rat-associated health risks. Additionally, it would be wise to develop a reporting system for rat-associated diseases in people, incidents of rat-human contact, both direct (i.e., biting) and indirect (i.e., exposure to rat excreta), and even rat infestations. This sort of reporting system could be one tool that health officials could use to gauge the public health significance of rats and to initiate appropriate responses. In conjunction with the development of a reporting system, it would also be helpful to generate a communication platform through which individuals could pose questions about rats and rat-related health risks to those best equipped to answer them. This might take the form of a website and/or hotline manned by a designated coordinator. It would also require the identification of a network of individuals and agencies with different areas of expertise or responsibility regarding rats that would be able to respond to submitted queries. Finally, it would be prudent to develop a formalized response policy or protocol for assessing and dealing with rat
infestations and rat-human contacts wherever they occur. Although rat infestations occurring on private property in the city of Vancouver are the responsibility of the property owner, they may not be appropriately dealt with due to lack of information, interest, or financial resources. These infestations may pose an ongoing health threat to those residing on or coming into contact with the property. It is important to note, however, that for any of these suggestions to be successfully developed and implemented, one or more branches of municipal government and/or public health will need to step up to the plate and agree to take responsibility for Vancouver’s rat issues.

The idea of a concerted and coordinated effort to deal with urban rats is not new. Indeed, other jurisdictions have already developed effective systems to deal with rats and rat-associated health risks. Perhaps the best example of such a system is in the New York City, were rat infestations are the responsibility of the Department of Health and Mental Hygiene (http://www.nyc.gov/html/doh/html/environmental/rats-mice.shtml). The department proactively takes responsibility for ensuring that rat infestations occurring anywhere in the city are detected, monitored, and eliminated. It also provides a hotline and website that residents can use to make complaints about rats or mice, as well as any conditions that might attract rodents. The department has created the Rat Information Portal (http://www.nyc.gov/html/doh/html/environmental/rat-info-portal.shtml), a website that maps rat infestations and rat inspection data throughout the city and provides the public with information about how to prevent and control rat infestations. It also requires that all rat bites be reported within 24 hours through telephone or online reporting services, and hosts a ‘Rodent Academy’ that offers courses on rat management for the public and for PCPs.

The Rat Information Portal makes a point of noting that rats are a significant urban issue that requires “…a coordinated response: property owners, tenants, businesses and government need to work together.” Given the potential health risks associated with urban rats, this is a mantra that most certainly ought to be adopted in
Vancouver, as well as other cities. Given how little we actually understand about rats and RAZ, a case can be made that any team involved in a coordinated effort to respond to the potential health risks posed by rats should also include a diverse set of interdisciplinary health professionals and researchers (e.g., a veterinarians, physicians, epidemiologists, microbiologists, social scientists, etc.).

11.6 Conclusions

Past research and historical experience has suggested that urban rats can be the source of a number of zoonotic pathogens responsible for significant human morbidity and mortality. However, there is little contemporary data on urban rat ecology or rat-associated health risks, particularly in Canada, where there had only ever been one study of rats and the pathogens they carry. This is problematic because without a contemporary and comprehensive understanding of RAZ, it is virtually impossible to determine if rats pose a health risk to people or to develop informed and effective strategies to monitor and mitigate that risk.

This project showed that rats in Vancouver’s DTES carry a number of zoonotic or potentially zoonotic organisms and therefore could be a public health threat. It was also able to provide insight into urban rat ecology and the ecology of RAZ, both of which influence the risk of pathogen transmission to people. The information presented here may serve as a platform from which to launch additional research on rats and RAZ. It may also be a tool for more immediate risk assessment and risk mitigation strategies.
Table 11-1: Conclusions from the research described in this dissertation and avenues for further study.

| Knowledge gained from this study |  
|----------------------------------|---------------------------------------------------------------|
| Rats in Vancouver’s DTES carry a number of different zoonotic or potentially zoonotic organisms.  
| Many of these organisms can likely be transmitted through the environment (e.g., *L. interrogans*), making rat infestations a potential health risk even in the absence of direct human-rat contact.  
| Pathogens carried by rats in the DTES included those for which the rat is a natural reservoir (e.g., *L. interrogans*), as well as pathogens normally associated with humans, other animal species, or the environment (e.g., MRSA and *C. difficile*).  
| Certain RAZ thought to be endemic in rat populations worldwide (e.g., *Rickettsia typhi*, Seoul hantavirus, and *Bartonella* spp.) were not found in DTES rat populations, but there is ongoing potential for foreign rats and their diseases to be introduced into the DTES through the international shipping port.  
| Rats and RAZ are heterogeneously distributed across the urban landscape, even over very short geographic distances. This clustering has significant implications for RAZ ecology and public health, well as research design and interpretation.  
| The ecology of rat populations and the pathogens that circulate among them are inextricably intertwined. Failure to account for rat population ecology when studying RAZ can limit our understanding of these pathogens, prevent comparability among studies, and lead to erroneous conclusions regarding RAZ ecology.  
| The full spectrum of factors that determine disease presence and prevalence within a city block remain unclear. Even after accounting for season and measured population characteristics, there was residual variation in disease abundance that could not be explained.  
| Some of the results of this project are specific to the DTES (e.g., the presence and prevalence of specific RAZ), while others can likely be extrapolated to other geographic areas (e.g., the underlying principles influencing the ecology of rats and RAZ).  
| Ongoing research and surveillance is needed in order to address new questions (see below) and to monitor changes in rat and RAZ ecology over time.  

182
Remaining knowledge gaps

- What is the full range of zoonotic or potentially zoonotic organisms carried by urban rats? Can metagenomics technology help us to answer this question?
- Are pathogens actually being transmitted from rats to people in the DTES? Are we capable of detecting rat-to-human transmission of pathogens like MRSA and *C. difficile* for which rats are not the only reservoir?
- Could rats serve as a ‘mixing vessel’, facilitating transfer of resistance or virulence genes among pathogens?
- Why are certain RAZ thought to be endemic in rat populations worldwide (e.g., *Rickettsia typhi*, Seoul hantavirus, and *Bartonella* spp.) absent from DTES rat populations? Can a better understanding of global rat population structure (through molecular ecologic methods) help us to understand how RAZ are distributed among different urban centers?
- To what degree do human transport hubs facilitate the movement of RAZ among different geographic locations? Are there physical or functional barriers in place that prevent establishment of foreign rats and RAZ in a new area?
- What is the impact of rat population genetics, chronic stress, or environmental pollution on the ecology of rats and RAZ?
- Is the ecology of rats and RAZ changing over time? What are the factors driving these changes?
REFERENCES


APPENDICES
Appendix A : Pest control professional rat survey.

Pest Control Professional Rat Survey

1. What is the name of your current company? (please include the branch for companies with multiple locations) ________________________

2. What area(s) do you service? (city/municipality and province) ________________________

3. What is your current role within the pest control profession? (please check one)
   □ Technician  □ Manager  □ Company owner  □ Other: ________________________

   Please answer the following questions 4-7 based on your experience with your current company (or one that you worked for in the past 5 years).

4. Over the past year (or most recent year that you worked for the company), what proportion of this company’s business involved rat control (vs. other pests)? (please check one)
   □ <25%  □ 25-50%  □ 50-75%  □ >75%  □ N/A  □ Don’t know

5. Does the number of rat infestations this company responds to change with the season? (please check one)
   □ Yes  □ No  □ Don’t know

   If you answered ‘yes’ to the previous question please continue to question 6, otherwise skip to question 7.
6. Please rank the seasons (from 1 to 4) in terms of how busy your company is with rat-related jobs, with 1 being the busiest season and 4 being the least busy (use each ranking only once):

____ Summer ______ Fall _______ Winter _______ Spring

What do you think is the reason for this difference? Comment: ________________________________

__________________________________________________________________________________

__________________________________________________________________________________

__________________________________________________________________________________

Please answer the following based on your general experiences with regard to rat infestations and rat control.

7. In general, how frequently do you deal with rat infestations in each of the following locations? Please check one box for each location or circle N/A if you do not service a particular location.

<table>
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<th>Location</th>
<th>Daily</th>
<th>Several times per week</th>
<th>Weekly</th>
<th>Several times per month</th>
<th>Monthly</th>
<th>Less than once per month</th>
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<td>Commercial areas selling/storing food</td>
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<td>Commercial areas not associated with food</td>
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Comment: 

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__________________________________________________________________________________

__________________________________________________________________________________

4
8. What is the average number of rats per infestation in each of the following locations?

*Please check one box for each location or circle N/A if you do not service a particular location.*

<table>
<thead>
<tr>
<th>Location</th>
<th>Less than 10 rats</th>
<th>10 – 20 rats</th>
<th>20 – 50 rats</th>
<th>50 – 100 rats</th>
<th>More than 100 rats</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residential</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Commercial areas selling/storing food</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Commercial areas not associated with food</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Industrial</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Other:</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Comment: __________________________________________________________

________________________________________________________________

________________________________________________________________

________________________________________________________________
9. How important are each of the following factors in promoting rat infestations?

<table>
<thead>
<tr>
<th>Factor</th>
<th>(NOT important)</th>
<th>(VERY important)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Exposed garbage</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>b. Abandoned buildings/structures</td>
<td>1 2 3 5 5</td>
<td></td>
</tr>
<tr>
<td>c. Building disrepair</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>d. Parks and green spaces</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>e. Gardens</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>f. Compost</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>g. High population density (lots of people living/working in an area)</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>h. Low socioeconomic status (poor neighbourhoods)</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>i. Freshwater (ponds, lakes or rivers)</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>j. Saltwater (seaside areas)</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>k. Cracked pavement</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>l. Presence of perishable food on the premise</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>k. Other</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>l. Other</td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
</tbody>
</table>

Comment: __________________________

_______________________________

_______________________________

_______________________________
10. How much of a health risk do you think rats pose to pest control professionals? (please check one)

☐ No health risk  ☐ Minimal health risk  ☐ Moderate health risk  ☐ Severe health risk

Comment: ________________________________________________________________

11. How much of a health risk do you think rats pose to the general public? (please check one)

☐ No health risk  ☐ Minimal health risk  ☐ Moderate health risk  ☐ Severe health risk

Comment: ________________________________________________________________

12. In your opinion, what is the main reason why your clients are concerned about rat infestations?

☐ Personal/public health (i.e., concern about disease)

☐ Property damage

☐ Aesthetic reasons (e.g., because rats are distasteful)

☐ Because of government regulations

☐ Don’t know

☐ Other: ______________________________________________________________

Comment: ________________________________________________________________
13. In your opinion, how knowledgeable is the general public about rats? *(please circle one)*

(Not knowledgeable) 1 2 3 4 5 (Very knowledgeable)

Comment: __________________________________________________________

14. What is the **MOST effective** way to eliminate rat infestations **in the long term**?

☐ Poison baits  ☐ Trapping and removal

☐ Environmental modification  ☐ Other: ___________________________

Comment: _________________________________________________________

15. What is the **LEAST effective** way to eliminate rat infestations **in the long term**?

☐ Poison baits  ☐ Trapping and removal

☐ Environmental modification  ☐ Other: ___________________________

Comment: _________________________________________________________

16. In your opinion, what are **BEST** methods to **PREVENT** rat infestations?

_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
16. Do you have anything else that you would like to say about rat infestations or rat control?

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

Would you like this company to be acknowledged in publications resulting from this survey? (please circle one)

YES    NO

If you indicate 'yes' your company's name will be included in the 'acknowledgement' section of any resulting publications. Due to practical constraints, we (the researchers) will not be contacting anyone individually prior to publication.

Finally, please indicate your e-mail address if you would like to be entered in the draw to win a $100 gift certificate: ____________________________________________
Appendix B: A systematic environmental observation tool to predict relative rat abundance in urban centres.

VANCOUVER RAT PROJECT
ENVIRONMENTAL OBSERVATION TOOL

Date: ____________________________
Block Number: ____________________
Block Location: ____________________

All block evaluations are to take place during working hours (8:00 am to 4:00 pm, Monday – Friday). For features showing temporal variation (e.g., human activity) night-time and weekend characteristics will not be captured.

Block evaluations should take place as close to the time of active trapping as possible. This will ensure that the environmental characteristics recorded reflect those present at the time of trapping.

Unless otherwise stated, consider the block as a whole (including any and all features of that block visible from any block or alley or face that can be derived from aerial maps).

Land Use Characteristics

Parcel Allotment

The following questions ask you to classify parcels as a whole according to their predominant tenure.

Residential parcels include any parcel dedicated to be a permanent residence (e.g., houses, townhomes, etc.). Commercial parcels include those dedicated to retail or business (e.g., stores, restaurants, etc.). Industrial parcels include those dedicated to the production or processing of products (i.e., factories, warehouses, etc.). Institutional parcels include those dedicated to not-for-profit services (i.e., churches, outreach centers, etc.). Green space parcels include those dedicated solely to green space (i.e., gardens, parks, etc.). Parcels that include green space as well as structures should be assigned the land use of the structure. Vacant/undeveloped parcels refer to plots of land which are clearly not in use as indicated by lack of a structure on the property or lack of land development. Parcels under demolition include those containing buildings that are in the process of being demolished (parcels containing the remains of long demolished buildings should be considered vacant/undeveloped). Parcels that are under construction include those with buildings undergoing construction or major renovation; that do not contain any occupants. Abandoned parcels include those with structures that were previously used but are now vacant and not undergoing construction or demolition (do not include undeveloped lots, which have no structures). Open parcels include those that do not contain any buildings but are not vacant/undeveloped, demolished, or green spaces (e.g., parking lots). Code mixed only if a predominant form cannot be determined (e.g., a street has 3 residences and 3 commercial stores). Code other if none of the above categories apply.

1. How would you characterize the land use on this block? (Code the predominant form)

   Primarily residential: 1
   Primarily commercial: 2
   Primarily industrial: 3
   Primarily institutional: 4
   Primarily green space: 5
   Primarily vacant/undeveloped lots: 6
   Primarily under demolition: 7
   Primarily under construction: 8
   Primarily abandoned: 9
   Primarily open spaces: 10

   Mixed: 11
   Other: 12

   If you stated 'other' please specify:

   Answer q. 7-12 according to the total block area occupied by parcels corresponding to each of the following land uses.

2. Approximately what proportion of the block is occupied by residential parcels?

   None: 0
   Less than 1/4: 1
   Between 1/4 and 1/2: 2
   Between 1/2 and 3/4: 3
   More than 3/4: 4
3. Approximately what proportion of the block is occupied by commercial parcels?
   None ................................................... 0
   Less than ¼ ........................................... 1
   Between ¼ and ½ ..................................... 2
   Between ½ and ¾ ..................................... 3
   More than ¾ .......................................... 4

4. Approximately what proportion of the block is occupied by industrial parcels?
   None ................................................... 0
   Less than ¼ ........................................... 1
   Between ¼ and ½ ..................................... 2
   Between ½ and ¾ ..................................... 3
   More than ¾ .......................................... 4

5. Approximately what proportion of the block is occupied by institutional parcels?
   None ................................................... 0
   Less than ¼ ........................................... 1
   Between ¼ and ½ ..................................... 2
   Between ½ and ¾ ..................................... 3
   More than ¾ .......................................... 4

6. Approximately what proportion of the block is occupied by green space parcels?
   None ................................................... 0
   Less than ¼ ........................................... 1
   Between ¼ and ½ ..................................... 2
   Between ½ and ¾ ..................................... 3
   More than ¾ .......................................... 4

7. Approximately what proportion of the block is occupied by vacant/undeveloped parcels?
   None ................................................... 0
   Less than ¼ ........................................... 1
   Between ¼ and ½ ..................................... 2
   Between ½ and ¾ ..................................... 3
   More than ¾ .......................................... 4

8. Approximately what proportion of the block is occupied by parcels under demolition?
   None ................................................... 0
   Less than ¼ ........................................... 1
   Between ¼ and ½ ..................................... 2
   Between ½ and ¾ ..................................... 3
   More than ¾ .......................................... 4

9. Approximately what proportion of the block is occupied by parcels under construction?
   None ................................................... 0
   Less than ¼ ........................................... 1
   Between ¼ and ½ ..................................... 2
   Between ½ and ¾ ..................................... 3
   More than ¾ .......................................... 4

10. Approximately what proportion of the block is occupied by abandoned parcels?
    None ................................................... 0
    Less than ¼ ........................................... 1
    Between ¼ and ½ ..................................... 2
    Between ½ and ¾ ..................................... 3
    More than ¾ .......................................... 4

11. Approximately what proportion of the block is occupied by open parcels?
    None ................................................... 0
    Less than ¼ ........................................... 1
    Between ¼ and ½ ..................................... 2
    Between ½ and ¾ ..................................... 3
    More than ¾ .......................................... 4

12. Approximately what proportion of the block is occupied by other land uses?
    None ................................................... 0
    Less than ¼ ........................................... 1
    Between ¼ and ½ ..................................... 2
    Between ½ and ¾ ..................................... 3
    More than ¾ .......................................... 4

Residential Density

Questions 13-18 seek to quantify residential density.

13. How would you characterize the predominant housing type on this block?
    No residential units .................................. 0
    Single-family houses ................................ 1
    Duplex/Rowhouses .................................. 2
    Low-rise apartment or condominium buildings (1-4 floors) ................. 3
    Mid-rise apartment or condominium buildings (4-10 floors) .............. 5
    High-rise apartment or condominium buildings (> 10 floors) .......... 6
    Housing units over commercial stores .................................. 7
    Mixed ................................................. 8
Code mixed only if a predominant form cannot be determined (e.g. a street has 3 single family units and 3 duplexes).

If you stated ‘mixed’ please specify:


Answer the following questions according to the total area occupied by the structures of interest (vs. the parcels as a whole).

14. Approximately what proportion of the block is occupied by single family houses?
   None .............................................. 0
   Less than $\frac{1}{4}$ ..................................... 1
   Between $\frac{1}{4}$ and $\frac{1}{2}$ .............................. 2
   Between $\frac{1}{2}$ and $\frac{3}{4}$ ............................ 3
   More than $\frac{3}{4}$ ..................................... 4

15. Approximately what proportion of the block is occupied by duplexes or rowhouses?
   None .............................................. 0
   Less than $\frac{1}{4}$ ..................................... 1
   Between $\frac{1}{4}$ and $\frac{1}{2}$ .............................. 2
   Between $\frac{1}{2}$ and $\frac{3}{4}$ ............................ 3
   More than $\frac{3}{4}$ ..................................... 4

16. Approximately what proportion of the block is occupied by low-rise apartment or condominium buildings (1-4 floors)?
   None .............................................. 0
   Less than $\frac{1}{4}$ ..................................... 1
   Between $\frac{1}{4}$ and $\frac{1}{2}$ .............................. 2
   Between $\frac{1}{2}$ and $\frac{3}{4}$ ............................ 3
   More than $\frac{3}{4}$ ..................................... 4

17. Approximately what proportion of the block is occupied by mid-rise apartment or condominium buildings (5-10 floors)?
   None .............................................. 0
   Less than $\frac{1}{4}$ ..................................... 1
   Between $\frac{1}{4}$ and $\frac{1}{2}$ .............................. 2
   Between $\frac{1}{2}$ and $\frac{3}{4}$ ............................ 3
   More than $\frac{3}{4}$ ..................................... 4

18. Approximately what proportion of the block is occupied by high-rise apartment or condominium buildings (>10 floors)?
   None .............................................. 0
   Less than $\frac{1}{4}$ ..................................... 1
   Between $\frac{1}{4}$ and $\frac{1}{2}$ .............................. 2
   Between $\frac{1}{2}$ and $\frac{3}{4}$ ............................ 3
   More than $\frac{3}{4}$ ..................................... 4

19. Approximately proportion of the block is occupied by housing units over commercial storefronts?
   None .............................................. 0
   Less than $\frac{1}{4}$ ..................................... 1
   Between $\frac{1}{4}$ and $\frac{1}{2}$ .............................. 2
   Between $\frac{1}{2}$ and $\frac{3}{4}$ ............................ 3
   More than $\frac{3}{4}$ ..................................... 4

**Food-Associated Non-Residential Land Use**

The following questions (q. 20-24) seek to identify and quantify the number of establishments producing, storing, processing, selling, and/or providing food (not including residential buildings). “Other” buildings providing food include those not covered by the other categories that can be reasonably expected to provide food to patrons (i.e., outreach centers, benevolence societies, etc.)

For buildings with different usages on different levels (i.e., non-residential units with housing above), the area of that building should be included in the following estimates of non-residential land use.

Answer the following questions according to the total area occupied by the structures of interest (vs. the parcels as a whole).

20. Approximately proportion of the block is occupied by non-residential buildings not associated with food?
   None .............................................. 0
   Less than $\frac{1}{4}$ ..................................... 1
   Between $\frac{1}{4}$ and $\frac{1}{2}$ .............................. 2
   Between $\frac{1}{2}$ and $\frac{3}{4}$ ............................ 3
   More than $\frac{3}{4}$ ..................................... 4
21. Approximately proportion of the block is occupied by restaurants?
None ...........................................0
Less than \(\frac{1}{4}\) ..................................1
Between \(\frac{1}{4}\) and \(\frac{1}{2}\) .............................2
Between \(\frac{1}{2}\) and \(\frac{3}{4}\) .............................3
More than \(\frac{3}{4}\) ...................................4

22. Approximately proportion of the block is occupied by groceries or other commercial venues selling food?
None ...........................................0
Less than \(\frac{1}{4}\) ..................................1
Between \(\frac{1}{4}\) and \(\frac{1}{2}\) .............................2
Between \(\frac{1}{2}\) and \(\frac{3}{4}\) .............................3
More than \(\frac{3}{4}\) ...................................4

23. Approximately proportion of the block is occupied by industrial establishments producing, processing, and/or storing food?
None ...........................................0
Less than \(\frac{1}{4}\) ..................................1
Between \(\frac{1}{4}\) and \(\frac{1}{2}\) .............................2
Between \(\frac{1}{2}\) and \(\frac{3}{4}\) .............................3
More than \(\frac{3}{4}\) ...................................4

24. Approximately proportion of the block is occupied by "other" establishments associated with food?
None ...........................................0
Less than \(\frac{1}{4}\) ..................................1
Between \(\frac{1}{4}\) and \(\frac{1}{2}\) .............................2
Between \(\frac{1}{2}\) and \(\frac{3}{4}\) .............................3
More than \(\frac{3}{4}\) ...................................4

If you stated "other" buildings providing food please specify:

Property Condition

Use the following guide to code for building and property conditions:

Extremely poor: Structure looks unfit/unsafe for humans. Significant amount of broken glass, peeling paint, damaged structures (e.g. stairs, walls, etc.). Dirty and unkempt property with little or no apparent regard for upkeep of property. Major overhaul needed to improve property. Abundant refuse and/or severe overgrowth. May include abandoned structures and properties. Poor: Adequate living/working conditions (albeit poor environment). Moderate amount of broken glass, peeling paint, damaged structures, etc. Considerable amount of work needed to improve property. Moderate amounts of refuse and/or overgrowth. Fair: Passable conditions with some attempt to keep property but moderate success. Some broken windows, peeling paint, damaged structures, etc. Would recommend a moderate amount of repair. Some refuse/overgrowth. Good: In decent working/living condition. Some repairs could be done (mainly for aesthetic reasons). Looks clean and well kept. Excellent: Immaculate near perfect condition. No repair needed. Shows that extra care and effort has been directed towards upkeep. Mixed condition: Only code for two-grade differences wherein at least 25% of the properties are at one grade and 25% are two grades higher.

Answer q.24-36 based on your general impression of the block as a whole. For building condition, discount any buildings undergoing demolition, construction, or major renovation.

25. What is the general condition of the buildings?
None/not applicable ..........................0
Extremely poor ................................1
Poor ..............................................2
Fair ..............................................3
Good ............................................4
Excellent ......................................5
Mixed (extreme difference) .................6

26. Approximately what proportion of the block is occupied by buildings in extremely poor condition?
None ...........................................0
Less than \(\frac{1}{4}\) ..................................1
Between \(\frac{1}{4}\) and \(\frac{1}{2}\) .............................2
Between \(\frac{1}{2}\) and \(\frac{3}{4}\) .............................3
More than \(\frac{3}{4}\) ...................................4

27. Approximately what proportion of the block is occupied by buildings in poor condition?
None ...........................................0
Less than \(\frac{1}{4}\) ..................................1
Between \(\frac{1}{4}\) and \(\frac{1}{2}\) .............................2
Between \(\frac{1}{2}\) and \(\frac{3}{4}\) .............................3
More than \(\frac{3}{4}\) ...................................4
28. Approximately what proportion of the block is occupied by buildings in fair condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

29. Approximately what proportion of the block is occupied by buildings in good condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

30. Approximately what proportion of the block is occupied by buildings in excellent condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

Grounds include any space or parcel within a block that is not occupied by a building. May include open/undeveloped/green space parcels, as well as open spaces in a parcel with structures.

31. What is the general condition of the grounds?
   None/not applicable .......................... 0
   Poor/deteriorated ............................ 1
   Fair ............................................. 2
   Good .......................................... 3
   Excellent ..................................... 4
   Mixed (extreme difference) ............... 5

32. Approximately what proportion of the block is occupied by grounds in extremely poor condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

33. Approximately what proportion of the block is occupied by grounds in poor condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

34. Approximately what proportion of the block is occupied by grounds in fair condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

35. Approximately what proportion of the block is occupied by grounds in good condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

36. Approximately what proportion of the block is occupied by grounds in excellent condition?
   None ........................................ 0
   Less than ¼ .................................. 1
   Between ¼ and ½ .............................. 2
   Between ½ and ¾ ............................. 3
   More than ¾ .................................. 4

Green Space Characteristics
For an area to be considered a green space it must: 1) show significant plant growth (i.e., areas with sparse grass or weeds should not be included) and 2) occupy a significant proportion of the property parcel (i.e., very small areas of plant growth in an otherwise build-up parcel should not be included).

Answer the following questions according to the total area occupied by the green spaces of interest (vs. parcels as a whole).
37. Approximately what proportion of the block is occupied by green space?

<table>
<thead>
<tr>
<th>None</th>
<th>Less than 1/4</th>
<th>Between 1/4 and 1/2</th>
<th>Between 1/2 and 3/4</th>
<th>More than 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Unkempt green space is an area that is overgrown by vegetation and/or does not appear to receive regular maintenance.*

38. Approximately what proportion of the block is occupied by unkempt green space?

<table>
<thead>
<tr>
<th>None</th>
<th>Less than 1/4</th>
<th>Between 1/4 and 1/2</th>
<th>Between 1/2 and 3/4</th>
<th>More than 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Well kept green space is considered to be green space that receives regular maintenance. It may include lawns, non-food gardens, or other landscaped areas.*

39. Approximately what proportion of the block is occupied by well kept green space?

<table>
<thead>
<tr>
<th>None</th>
<th>Less than 1/4</th>
<th>Between 1/4 and 1/2</th>
<th>Between 1/2 and 3/4</th>
<th>More than 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Green space that is in any way geared toward the production of produce should be considered to be a food garden.*

40. Approximately what proportion of the block is occupied by food gardens?

<table>
<thead>
<tr>
<th>None</th>
<th>Less than 1/4</th>
<th>Between 1/4 and 1/2</th>
<th>Between 1/2 and 3/4</th>
<th>More than 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

### Alley Surface Characteristics

For the following questions consider only the alley surface at the alley face. The alley face considered should include the 1 m of land on either side of the alley border.

Use the following definitions for questions 41-44. Only paved alley surfaces should be considered for this question.

**Poor**: Frequent/severe deep cracking of pavement (cracking sufficient to permit rat burrowing). Alley surface is broken up and in need of serious repair.

**Fair**: Moderate amount of cracked pavement. Cracking is mainly superficial (unlikely to promote rat harborage).

**Good**: Generally even surface with minimal cracking.

41. How would you rate the general condition of the paved alley surfaces at the alley faces?

<table>
<thead>
<tr>
<th>Poor</th>
<th>Fair</th>
<th>Good</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

42. Approximately what proportion of the alley face is in poor condition?

<table>
<thead>
<tr>
<th>None</th>
<th>Less than 1/4</th>
<th>Between 1/4 and 1/2</th>
<th>Between 1/2 and 3/4</th>
<th>More than 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

43. Approximately what proportion of the alley face is in fair condition?

<table>
<thead>
<tr>
<th>None</th>
<th>Less than 1/4</th>
<th>Between 1/4 and 1/2</th>
<th>Between 1/2 and 3/4</th>
<th>More than 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

44. Approximately what proportion of the alley face is in good condition?

<table>
<thead>
<tr>
<th>None</th>
<th>Less than 1/4</th>
<th>Between 1/4 and 1/2</th>
<th>Between 1/2 and 3/4</th>
<th>More than 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Alley surface not covered in concrete or asphalt is considered unpaved. If there is any unpaved surface within 1 m of the alley border (regardless of total*
depth) that part of the alley face should be considered unpaved. Deeply cracked pavement should still be considered to be paved.

45. Approximately what proportion of the alley face is bordered by some sort of non-paved surface?
None ........................................ 0
Less than ¼ ................................... 1
Between ¼ and ½ ........................... 2
Between ½ and ¾ ........................... 3
More than ¾ .................................. 4

A rat hole is considered to be any hole at the alley face that appears to have been created by a rat or that would reasonably permit the passage of an adult rat.

46. How many rat holes are there at the alley face: __________

Rat corridors are spaces between buildings that may allow rats to access the alley face from deeper within the property.

47. How many rat corridors are there at the alley face: __________

Presence of Waste

For the following questions consider only waste present near the alley face on an average day. The alley face considered should include the 5m of land on either side of the alley border.

A little waste is when there is a very low volume of waste or the waste is confined to a small area. A lot of waste is when there is a high volume of waste and/or waste is present throughout a large proportion of the alley. Some waste is somewhere between a little and a lot.

For question 48, garbage, litter, and junk include any organic or inorganic materials that have been discarded in places other than a designated receptacle. Open compost piles may be included.

48. Is there garbage, trash, junk, or litter?
None ........................................ 0
A little ....................................... 1
Some ........................................ 2
A lot .......................................... 3

49. Is there garbage overflowing from receptacles in the alley?
None ........................................ 0
A little ....................................... 1
Some ........................................ 2
A lot .......................................... 3

How many of each of the following types of receptacles are there?
50. Commercial garbage ______
51. Private garbage ______
52. Commercial recycling ______
53. Private recycling ______
54. Commercial organic ______
55. Private organic ______

56. Are there any obvious strong odors anywhere in the alley face (urine stench, rotting garbage, etc.)?
No ........................................... 0
Yes ............................................ 1

Alley Usage

Alleys may be used as transportation corridors (e.g., for foot or vehicular traffic, for moving goods into and out of buildings, etc.). Note that transportation may include transport of people, vehicles, or goods. Alleys may also be used for loitering. Loitering is considered to be any non-transportation-related activity (i.e., socializing, resting, using drugs, etc.).

Use should be judged holistically through several morning and afternoon visitations during the trapping period. Use may also be inferred through signs of human presence (e.g., left over belongings or refuse, drug paraphernalia, etc.).

An alleyway is lightly used if very few people are seen and/or if people are seen infrequently. An alleyway is heavily used if groups of people are seen using the alley and/or if people are very frequently seen in the alley. A moderately used alley is somewhere between a lightly and a heavily used one.

57. How heavily is this alleyway used by people loitering?
Not used .................................... 0
Lightly used .................................. 1
Moderately used ............................ 2
Heavily used ................................ 3
58. How heavily is this alleyway used as a transportation corridor?

- Not used: 0
- Lightly used: 1
- Moderately used: 2
- Heavily used: 3

General Comments
Appendix C: Guidance document for the systematic environmental observation tool.
Building Condition

- Grounds are defined as any space or parcel within a block that is not occupied by a building.
- Includes open, undeveloped or green space parcels, as well as open spaces in a parcel with structures.
- Rated for the general state of upkeep of a property (not including building structures).
Paved Surfaces
- Defined as any surface in a block that is paved.
- Rated for its general state of repair/disrepair.

Non-Paved Surfaces
- Defined as any surface that is not paved in a block.

Rat Holes
- Features that are considered hazards for rat entry and infestation.