An Assessment of Imported Malaria and the Risk of Autochthonous Transmission in Ontario, Canada

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Abstract

This thesis assesses the potential for autochthonous malaria transmission in Ontario, Canada. First, the spatial and temporal profile of imported malaria cases in Ontario is characterized. Next, the potential for interaction between the cases and mosquito vector is evaluated. Results of malaria tests that identified subjects as either positive (cases) or negative (controls) were overlaid with vector observation data. Case-control logistic regression showed that malaria cases were positively correlated with living in a neighbourhood with a high proportion of residents who are immigrants from malaria endemic areas. Cases were reported in the suburbs of the Greater Toronto Area, and only 9% were within a geographical area where autochthonous transmission would be possible. This research can identify points of inquiry into the potential for autochthonous malaria transmission in other areas, as well as highlight the importance of surveillance of a range of infectious diseases that are imported in Canada.

Résumé

Ce mémoire évalue le potentiel pour la transmission autochtone du paludisme en Ontario, Canada. Premièrement, le profil spatial et temporel des cas importés du paludisme est caractérisé. Ensuite, le potentiel de l'interaction entre les cas et le vecteur de moustique est évalué. Les résultats des tests du paludisme, qui identifie les sujets comme soit positif (les cas) ou négatif (les contrôles), sont superposés avec des données d'observation vectorielle. La régression logistique à cas témoins a montré que les cas du paludisme étaient positivement corrélés avec vivre dans un quartier avec une grande proportion des résidents qui sont des immigrants venants des lieux endémiques du paludisme. Les cas étaient rapportés dans les banlieues de la grande région de Toronto, et seulement 9% étaient au sein d'un milieu géographique où la transmission autochtone du paludisme était possible. Cette recherche identifie les points d'enquête dans le potentiel pour la transmission autochtone du paludisme dans autres régions, ainsi qu'illustre l'importance de surveiller la gamme de maladies infectieuses apportés au Canada.

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Chapter 1: Introduction

This thesis examines imported malaria cases in Ontario, Canada and assesses the risk of autochthonous (local) transmission. Malaria is a parasitic vector-borne disease that is responsible for approximately one million deaths a year, representing a significant global public health burden (WHO 2010b; Suh et al. 2004). The malaria parasite, *Plasmodium* species, is spread via the bite of an infected mosquito (*Anopheles* species) (Suh 2004). Malaria can be prevented through the use of chemoprophylaxis, or through methods designed to reduce mosquito bites, such bed nets (Griffith et al. 2007). Malaria is treatable, although access to appropriate medication remains an issue in many areas.

1.1. Climate change and malaria:

The relationship between climate change and malaria transmission is drawn through several paths. Climate can affect the life cycle and longevity of the malaria vector, the mosquito, as well as the reproduction of the malaria parasite within the vector (Gage et al. 2008). Climate can significantly enable or limit the potential geographic or temporal range of the malaria vector, inscribing boundaries of areas of endemicity (Martens et al. 1995a). When changes in climate occur, areas that were previously impervious to incursions by malaria vectors can become vulnerable to transmission.

The vast majority of current research on climate change and malaria focuses on how changes in climate could lead to changes in global malaria transmission patterns, more geographical than temporal (Kiszewski et al. 2004; Rogers and Randolph 2000; Martens et al. 1995a). Research tends to focus on the potential for expansion or contraction in the boundaries of areas where malaria transmission is currently endemic. As climate patterns shift, corresponding

movements in vector populations could spread the disease into new areas. However, little research has probed the possibility of the creation of new areas of transmission that are not adjacent to current transmission zones. Could the malaria parasite become established in a region outside of the current swath of global transmission? (See Figure 1.1). There are several areas around the world, including Europe and North America, where malaria transmission was endemic in the past. With projected changes in climate, could there be a return to malaria endemicity in the future?

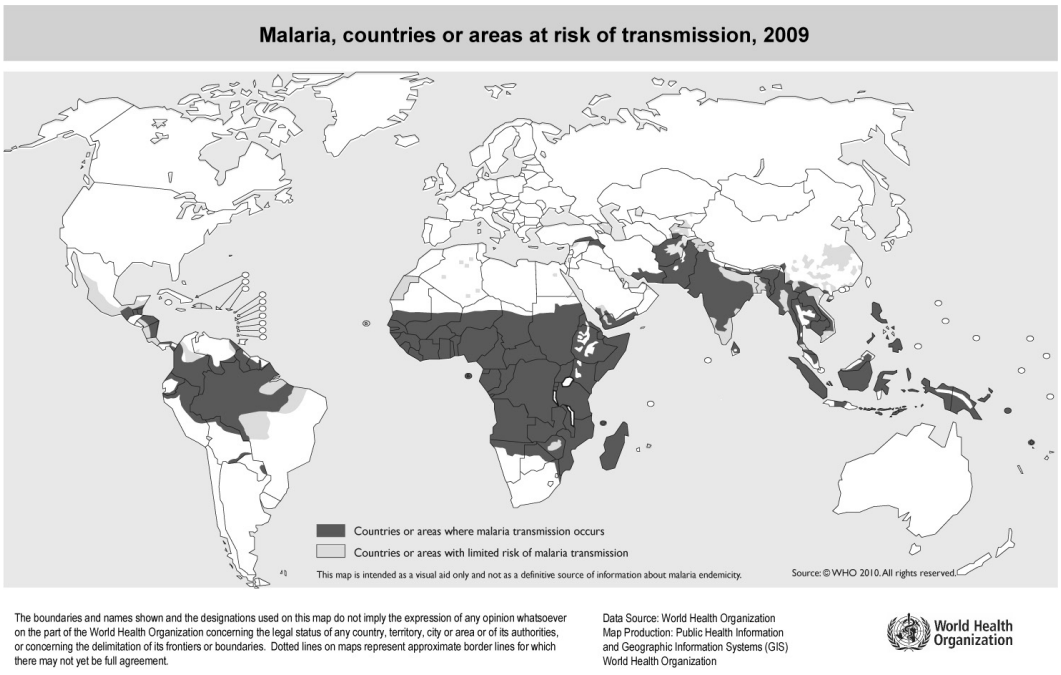

Figure 1.1: Current regions of global malaria endemicity, (WHO 2010a)

Although a return to endemicity may not be likely in most of Europe and North America, sporadic autochthonous cases do occur (Armengaud et al. 2008; Doudier et al. 2007; MacArthur et al. 2001; Sunstrum et al. 2001; Baqi et al. 1998; Zucker 1996; Layton et al. 1995). These locally-acquired mosquito-

transmitted cases of malaria highlight the presence of suitable climate and vectors that have the potential to transmit malaria. They also serve as a reminder that in many places, we may not well understand exactly which factors are holding malaria transmission in check. Factors related to imported malaria cases are a key determinant of autochthonous malaria transmission, and may be the missing piece to understanding how malaria patterns might change in the future. Examining the potential for autochthonous malaria transmission in an area of previous endemicity allows for a better understanding of the key populations that allow or constrain transmission: the mosquito vector and the human host.

1.2. Malaria in Canada:

Canada is a fitting example of a country that previously experienced endemic malaria transmission and has the potential for autochthonous transmission. Areas of southern Canada have the climate required for malaria transmission, and two species of mosquito (*Anopheles quadrimaculatus* in the east and *Anopheles freeborni* in the west) are competent malaria vectors. Significantly, Canada receives a reported 400 (approximately) cases of imported malaria a year, far more per capita than the United States (PHAC 2004; Kain et al. 1998). All of the conditions necessary for autochthonous malaria transmission exist in Canada. However, the potential for autochthonous transmission is not well understood. The province of Ontario in Canada was chosen as the study location due to both its important economic and political role within Canada and its history with endemic malaria. Ontario is host to a competent vector population (*Anopheles quadrimaculatus*), adequate summer temperatures for malaria transmission, and a large international airport that fuels the arrival of travellers who might host the malaria parasite.

1.3. Aim & Objectives:

Aim:

Characterize and evaluate the potential for autochthonous malaria transmission in Ontario, Canada, including factors related to international travel and immigration, imported malaria, and the competent vector population.

Objectives:

1. Characterize the spatial and temporal profile of imported malaria cases in Ontario.

This objective focuses on the importation of the malaria parasite into Ontario. A detailed description of imported malaria cases in Ontario allows for an assessment of how travel and housing patterns might affect the distribution of cases throughout the province. The locations of malaria cases are characterized in the context of neighbourhood-level variables related to immigration, and the individual travel histories of malaria cases are used to differentiate patterns specific to malaria species.

2. Evaluate the potential interactions between the vector population and imported malaria cases.

This objective builds upon the first, using the geographical and temporal patterns of the malaria cases to assess the potential for interaction between the malaria parasite and the vector population. The geographical range of the vector population is compared to the distribution of the malaria parasite, and a potential for interaction is deduced.

1.4. Justification and implications:

This thesis assessing the risk of autochthonous malaria transmission in Ontario, Canada highlights the importance of both the human host and the mosquito vector in the cycle of malaria transmission. This research can identify potential points of inquiry into the potential for autochthonous malaria transmission in other areas where the disease might re-emerge, such as the United States or Europe. Additionally, this assessment of potential local transmission as a result of imported cases can be used to highlight the importance of surveillance of a range of infectious diseases that are imported into Canada.

Chapter 2: Literature review

This thesis is broadly informed by concepts in medical geography and spatial epidemiology, including the importance of scale in spatial analyses of disease risk. The research methods draw upon several bodies of work, including mixed methods research, integrated risk assessment, and quantitative modelling. The research aim is situated within the field of climate change and emerging infectious disease research, specifically focusing on climate change and the re-emergence of malaria transmission.

2.1. Concepts, methods and context:

2.1.1. Concepts

2.1.1.1. Medical geography and spatial epidemiology

Although the disciplines of geography and epidemiology have developed through different histories, the particular subfields of medical geography and spatial epidemiology share common themes. Medical geography has developed through a long history reaching back more than 200 years and began as describing "the spatial distribution of disease" (Koch 2009, page 100). The discussion of the relative roles of geographers and physicians in shaping the history of medical geography points to the close relationship between this unique hybrid of social and medical science (Brown and Moon 2004). The field of medical geography continues to evolve, and the term "health geography" is now often used to encompass a wider range of research related to health and place (Brown and Duncan 2002). The methods of medical geography and spatial epidemiology sometimes differ due to their split disciplinary roots, but contemporary research often draws from both and finds itself comfortably within both spheres. In fact, current definitions of spatial epidemiology identify it as combining methods from many disciplines (Beale et al. 2008). Publications in medical journals emphasize the importance of place in health and serve to

draw the attention of medical and public health professionals to the potential utility of geographical methods and ways of inquiry (Dummer 2008). Many terms are often used interchangeably to reference research related to health and place: medical geography, health geography, spatial epidemiology, and geographical epidemiology. In order to help clarify these terms for researchers, summary publications are often found in epidemiology journals (Rezaeian et al. 2007). Within the field of infectious disease research, there is a special interest in the use of spatial epidemiology to better understand the relationships between vector, pathogen and host (Ostfeld et al. 2005). The common themes and current development of multi-disciplinary methods in medical geography and spatial epidemiology provide a rich conceptual basis for research concerned with health and space.

2.1.1.2. Issues of scale in geography

The importance of scale in geographical research is often unarticulated in research publications in medical geography or spatial epidemiology. Too often the scale of geographical research may not seem to have been selected with purpose and in fact might be quite arbitrary (Meentemeyer 1989). However there are many issues related to selecting the appropriate scale for geographical analysis. Scale is not simply the level at which the analysis is performed. Scale includes both grain and extent (Pereira 2002). Therefore there are many different levels of potential scales of analysis. Disciplines other than geography have historically also had an interest in scale (Sayre 2005). The importance of scale in ecological research provides a natural bridge to thinking about how scale could impact analysis in spatial epidemiology. In practice, subfields within geography have not communicated well their similar struggles with scale (Ruddell and Wentz 2009). However, the subfields within the discipline have much to gain from sharing their insights into how to work with issues of scale, just as spatial epidemiology can learn from the work of medical geography.

2.1.2. Methods:

1.2.1. Mixed methods research

Mixed methods research includes research that does not confine its methods to only quantitative or qualitative approaches. Both quantitative and qualitative work have their merits and mixed methods research often benefits from this diversity of approach (Johnson and Onwuegbuzie 2004). Quantitative research can include computer based modelling and mapping of quantitative data. Qualitative work can include key informant interviews and literature-based research into broader research themes. Often quantitative work can help develop concrete, detail-based knowledge whereas qualitative research can fill in the gaps and holes that the quantitative work can't cover, or help explain why certain quantitative results were found. Both types of research are important within the mixed methods framework and serve to increase the breadth and depth of the research output (Sechrest and Sidani 1995).

2.1.2.2. Risk assessment and integrated assessment modelling

This research project is situated within the research area of integrated assessment modelling and risk assessment. Risk assessment can take on a variety of forms; health risk assessments can include the integration of social and environmental factors into epidemiological data (Aagaard-Hansen et al. 2009). Integrated assessment models aim to assess the risk of changes to environmental and human systems with an emphasis on including the uncertainties and dependencies inherent in the system (Briggs 2008). There is significant support for the use of this type of research related to emerging infectious diseases and related risk factors. Integrated assessment models can be an important tool for research into the complex relationships between disease risk factors (Martens and McMichael 2002). Of greatest importance are models that incorporate many types of factors at multiple scales (Ebi 2008). To better understand dynamic disease processes, models need to be process-based

and be able to incorporate feedback mechanisms. These types of models allow information and factors from many different disciplines to be combined into one systematic framework. (Martens and McMichael 2002).

There is understanding and acknowledgement in the risk assessment community that models are not perfect and that as a methodology, modelling is not without significant limitations (Ebi 2008). However, models remain important tools for public health research. "Models facilitate understanding of what is known and what still needs to be understood of how systems actually function" (Ebi 2008, page 6). A model of a "real-world" system can never completely replicate the system it is representing, but models allow for the improved conceptualization of dynamic disease processes and outcomes (Martens and McMichael 2002). These models are not without limits, however, especially when there is insufficient quantification available of key relationships in the model (Martens and McMichael 2002). However, the creation of the model and the identification of key relationships can be an important tool for identifying important future areas of research, and future studies can attempt to more precisely quantify the key relationships. In the case of models focused on improved understanding of disease risk in advance of an outbreak or changing incidence, the heuristic and process-based value of such models is feasible and appropriate.

2.1.3. Context

2.1.3.1. Malaria and its associated risk factors

Malaria is a common infectious disease that is transmitted across the globe, mainly in developing countries (Suh et al. 2004). The malaria parasite (*Plasmodium falciparum*, *vivax*, and other species) is transmitted by the mosquito vector (*Anopheles* species) to the human host (Suh et al. 2004). Malaria can cause serious medical complications and death (Griffith et al. 2007).

Malaria is a serious health problem in many areas of the world, causing approximately one million deaths a year (WHO 2010b). Malaria is also a concern for travellers to areas where the disease is commonly found in the population, or where transmission is endemic (PHAC 2004). Malaria can often be prevented with the use of chemoprophylaxis, although drug-resistant strains of the parasite exist and continue to emerge (Griffith et al. 2007). Other preventative measures for malaria transmission include sleeping with bed nets and trying to avoid mosquito bites by changing ones clothing or behaviour. Malaria is also often treatable, although many people do not have access to either chemoprophylaxis or treatment.

There are several key factors that can affect the rate or possibility of malaria transmission (see Figure 2.1). The prevalence of infection within the human host population determines the rate at which the mosquito vector can become infected. The bite rate of mosquitoes to humans also affects the rate at which mosquitoes become infected. Certain minimum temperatures are required for the parasite to replicate within the mosquito vector so the temperature can affect the rate at which the mosquito becomes infectious. Four weeks is approximately the period of time it takes the malaria parasite to replicate within the mosquito at 18-20 degrees C (Berrang-Ford et al. 2009). The prevalence of chemoprophylaxis or other preventative measures within the population will change the rate of infection from the mosquito vector to the uninfected and non-immune human population, and the time to treatment will determine the prevalence of infection within the human population.

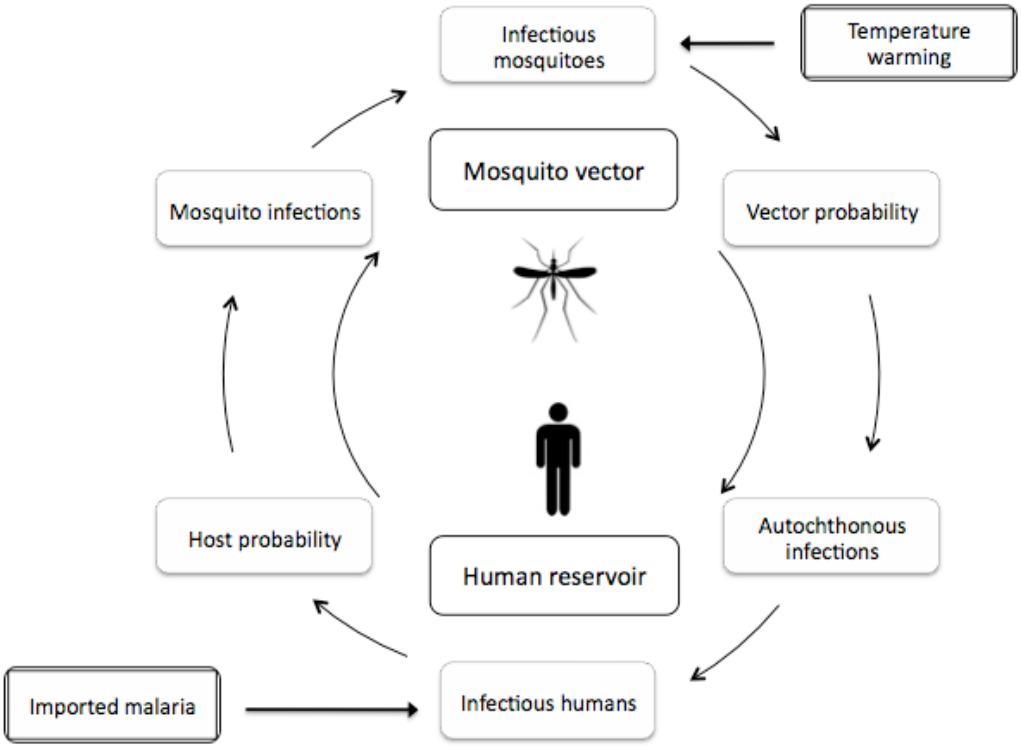

Figure 2.1: The malaria transmission cycle

2.1.3.2. Climate change and health

In the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, a chapter was devoted to the ways in which climate change could impact health outcomes (Confalonieri et al. 2007). These included temperature effects, natural disasters, food security, air quality issues, and changes in infectious disease incidence (Confalonieri et al. 2007). Another significant report on the health effects of climate change was recently released by the Lancet and University College London (Costello et al. 2009). The report identified climate change as "the biggest global health threat of the 21st century" (Costello et al. 2009). When examining the effect that climate change might have on health, it is important to incorporate the differential burdens of various diseases.

Although a disease might have a small proportional increase due to climate change, it could have a large burden (e.g., diarrhea or malnutrition) (Campbell-Lendrum and Woodruff 2006). Socioeconomic factors also play a significant role in health outcomes and analysis of the effects of the contributing burden of climate change in exclusion of these factors will lead to inaccurate assessments of real disease risk (Campbell-Lendrum and Woodruff 2006).

2.1.3.3. Climate change and emerging infectious diseases

Are we entering an "age of emerging infectious diseases?" This question has been raised within the research community and current trends of disease emergence seem to support this suggestion (Ebi 2008). There are several ways in which a disease can be interpreted to be "emerging" or "re-emerging" (Aron and Patz 2001). A disease can increase in both geographical range (area in which disease transmission is found) and temporal range (time of year during which disease transmission is seen). Different types of pathogens can become resistant to current methods of treatment or prevention (i.e., antibiotic resistance). Pathogens can also undergo genetic changes that can result in new variants (Aron and Patz 2001). Climate change can have a special effect on emerging infectious diseases. Climate change can alter the physical environments and vector populations in which infectious pathogens live, breed and die, thus affecting their survival (Patz et al. 2008). Climate can also have effects across multiple systems, including human and vector populations, contributing to emerging infectious diseases (Patz et al. 2008).

Climate has a significant effect on vector-borne diseases, including malaria (Confalonieri et al. 2007). There are several ways in which vector-borne diseases are sensitive to climate change. The amount of area containing suitable habitat for the vector can be altered, affecting the spatial distribution of the vector (Gage et al. 2008). The temporal distribution of the vector can also change

depending on temperature, precipitation and humidity patterns (Gage et al. 2008). The reproduction and survival of both the pathogen and the vector can also be influenced by climate change (Gage et al. 2008). Vector-borne diseases are most affected by climate change on the edges of areas of endemicity, especially at very high altitudes (Martens et al. 1995b). Because the greatest changes could come at the borders of endemic areas, changes will be the most significant in areas where transmission is currently absent (Martens et al. 1995b). The more densely populated parts of Canada fall on the borders of endemic areas for certain vector-borne diseases, such as West Nile virus and Lyme disease (Charron et al. 2008). Thus, Canada could be in a position to see significant relative impacts of climate change on vector-borne diseases.

Although there is potential for changes in vector-borne disease transmission due to climate change, it is difficult to ascertain the effects of climate without taking into account other mitigating factors, such as human response (Gubler et al. 2001). If current public health infrastructure is maintained in developed countries, it is not likely that there would be a dramatic increase in vector-borne disease burden (Gubler et al. 2001). In addition, not all research on climate change and vector-borne disease has found that there are significant impacts. Climate may have a relatively small effect on vector-borne disease transmission; human influences on land use may be more important determinants (Reiter 2001).

It is not yet completely understood how projected changes in climate will affect global malaria transmission (Confalonieri et al. 2007). The Intergovernmental Panel on Climate Change (IPCC) has identified this as an area that requires additional research (Confalonieri et al. 2007). Climate change and increased climate variability can affect the transmission of malaria in several ways, related to both the vector and the parasite (Gage et al. 2008). Climate can affect the

vector life cycle and reproduction, as well as the reproduction of the parasite within the vector. This in turn contributes to vector density, which affects the mosquito-human bite rate. Precipitation changes also contribute to changes in humidity levels, which are related to adult mosquito survival (Gage et al. 2008).

Looking specifically at climate change and malaria, similar results are found regarding the potential for increased risk due to changes in transmission patterns at the borders of endemic areas (Martens et al. 1995a). In addition to geographical changes in areas with transmission, the specific temporal transmission patterns of malaria could be affected by climate change (Martin and Lefebvre 1995). There is potential for an increase in seasonal malaria transmission, which could be more serious among people who do not have immunity from living in an endemic area (Martin and Lefebvre 1995).

Current research remains in debate regarding evidence for the potential impact of climate change on malaria transmission. Different methodologies have revealed conflicting predictions. An example of this debate played out in the pages of two prominent journals in 2004 and 2005. An analysis of historical data from the highlands of East Africa revealed that climate variability had a significant effect on malaria transmission in the region (Zhou et al. 2004). However, the methods used were criticized as not being appropriate for the hypothesis (Hay et al. 2005). The original research team rebutted the criticism in an update and stated again that there was a significant effect on malaria transmission due to climate (Zhou et al. 2005).

An earlier critique of several studies looking at malaria transmission in high altitude areas cited a lack of sufficient climate change in the recent past to compare to increases in malaria transmission (Hay et al. 2002). Researchers hypothesize that the observed increase in malaria transmission may be due to

the emergence of drug-resistance in the parasite. A more general critique of research looking to link climate change and malaria transmission claims that the studies don't include enough non-climatic factors related to humans and vectors (Reiter 2008).

2.1.3.4. Human movement and emerging infectious diseases

One of the most important non-climatic factors in the transmission of infectious diseases and the emergence of infectious diseases is human movement. Transportation patterns can be very illuminating when trying to better understand spatial and temporal infectious disease patterns. The importance of global air transit patterns in the spread of influenza A (H1N1) is a recent example of how the process of human movement and transportation can affect emerging infectious diseases (Khan et al. 2009b). Information regarding air transportation routes can even be used in modelling the potential future spread of emerging infectious diseases from a myriad of potential origins (Khan et al. 2009a). Several types of human movement are known to have an impact on infectious disease transmission; the process of migration and subsequent travel is especially significant (Gushulak and MacPherson 2004).

Human movement is a key factor in vector-borne infectious disease transmission as well (Stoddard et al. 2009). Human movement has a significant effect on the degree to which humans and vectors interact (Stoddard et al. 2009). Small-scale movements around a neighbourhood or town can be very important factors determining if there will be transmission between a vector and a human (Stoddard et al. 2009). These patterns must be considered in tandem with the spatial and temporal behaviour of the vector (Stoddard et al. 2009). The specific role of human movement in malaria transmission is an important aspect of malaria re-emergence (Martens and Hall 2000). Several types of human movement are potentially a key part of the transmission process: daily, periodic,

seasonal, long-term and migration (Martens and Hall 2000). Humans can transport the parasite from endemic areas to non-endemic areas, either in their own bodies or through the accidental movement of infectious mosquitoes (Martens and Hall 2000). It is clear that both human movement patterns and climate change need to be considered when assessing malaria as an emerging infectious disease.

2.2. Specific approaches to risk assessment of autochthonous malaria

2.2.1 Autochthonous malaria in non-endemic areas

This project assesses the risk of autochthonous malaria transmission in a region of a non-endemic country, Canada. Autochthonous transmission is characterized as sporadic, locally-acquired mosquito-transmitted cases of malaria. Autochthonous malaria cases are reported through a variety of avenues. Types of publications documenting autochthonous malaria cases can include case reports published in journals or government public health reports such as the Morbidity and Mortality Weekly Report in the United States, which documented an autochthonous case in Florida in 2003 (CDC 2004). Informal and incomplete worldwide surveillance of autochthonous malaria cases is provided in part by ProMED-mail, a service of the International Society for Infectious Diseases. ProMED-mail serves as an international clearinghouse for reports of emerging infectious diseases. In 2009, ProMED-mail reported autochthonous cases of malaria in Georgia, Italy, and Singapore (ProMED-mail 2009a, 2009b, 2009c).

Autochthonous malaria cases in non-endemic areas around the world have been documented, in many locations including several states in the United States (Michigan, California, Georgia, New Jersey, New York), Brazil, France, Madagascar and Canada (Armengaud et al. 2008; Doudier et al. 2007; Cerutti et al. 2007; Rabarijaona et al. 2006; Limongi et al. 2008; Sunstrum et al. 2001; MacArthur et al. 2001; Baqi et al. 1998; Zucker 1996; Layton et al. 1995). Case

reports can include a wide range of variables associated with the malaria case, including geographical factors such as whether a competent vector was found near the case and the location of the case's home in relation to potential human hosts of the parasite (MacArthur et al. 2001; Sunstrum et al. 2001). A review of multiple autochthonous cases in the United States included cases in California, New Jersey and New York City (Zucker 1996). Case reports often emphasize the need for continued or improved surveillance of malaria cases that don't present with the typical risk factor of international travel in areas where malaria is not endemic (Layton et al. 1995; Limongi et al. 2008). The potential role of international travel in transporting the pathogen from one non-endemic area to other non-endemic areas is also noted (Doudier et al. 2007). In areas that have endemic transmission in rural areas but have urban areas of non-endemicity, sporadic urban autochthonous cases are documented (Rabarijaona et al. 2006). The appearance of some autochthonous cases has even been used to postulate the presence of a simian reservoir for malaria (Cerutti et al. 2007).

In areas where malaria transmission has never been endemic, it is categorized as emergent malaria. Examples of this can be areas adjacent to endemic transmission zones, where malaria transmission is expanding into a new area. In areas where malaria transmission has previously been endemic, it is categorized as re-emergent malaria. An example of this is in Afghanistan and Tajikistan, where malaria has re-emerged due in part to instability in the area, which has contributed to a breakdown of vector control capabilities in addition to a high degree of human movement (Faulde et al. 2007; Pitt et al. 1998). Sporadic autochthonous cases can be indicative of a potential for malaria emergence or re-emergence, but in no way can be considered evidence for significant risk of such. Autochthonous cases are potentially more significant as an infectious disease event demonstrating the risks of imported pathogens into non-endemic zones.

Often part of surveillance activities, documentation of imported malaria cases can be found for areas across the globe, including many areas outside of North America such as Reunion, the United Kingdom, Barcelona, France, Indonesia, Colombia and Switzerland (D'Ortenzio et al. 2009; Rodger et al. 2008; Millet et al. 2008; D'Ortenzio et al. 2008; Legros et al. 2007; Lederman et al. 2006; Jennings et al. 2006; Osorio et al. 2007; Lehky Hagen et al. 2005; Osorio et al. 2004). Imported malaria studies often include an assessment of the common characteristics of patients, such as demographic information and travel history (D'Ortenzio et al. 2009; Osorio et al. 2004). The potential for clustering of malaria near airports has been assessed (Rodger et al. 2008). Another prominent focus of studies examining imported malaria is potential risk factors for prolonged infection or fatal outcomes (D'Ortenzio et al. 2008; Legros et al. 2007; Jennings et al. 2006). A study in Colombia identified the need to investigate the role of migration in imported malaria at larger scales (Osorio et al. 2007). Researchers also highlighted the need for analysis that includes interactions between factors that can influence the risk of imported malaria (Lehky Hagen et al. 2005). Finally, a European group of researchers emphasized the need to work across countries to "harmonize" their different policies on treatment (Millet et al. 2008).

Many different species of *Anopheles* mosquitoes across the world are capable of transmitting the malaria parasite (Kiszewski et al. 2004). However, most regions have one or a few key species that are the competent vectors for malaria in that area. Outside of North America, several studies have assessed different aspects related to the respective competent malaria vectors of each region. In one assessment of the regional competent malaria vector in Italy, a wider than expected range was found (Di Luca et al. 2009). Another European study examined how human-initiated changes in land use patterns have affected the

regional abundance of the local competent vector for malaria (Ponçon et al. 2007). Two additional studies assessed the potential for "airport malaria"—the movement of an infectious malaria vector via air travel—using data on air traffic patterns and volume, and information from a local case report, respectively (Tatem et al. 2006; Pomares-Estran et al. 2009).

Malaria has previously been endemic in North America, with transmission in most of the United States and parts of southern Canada (Zucker 1996). The parasite was introduced in the 16th-17th century with the arrival of European colonists and African slaves (Zucker 1996). The disease was eradicated through several different measures including vector control through changes in vector habitat, new antimalarial medications (such as quinine) for improved treatment, and a decrease in the contact between humans and the mosquitoes, due in part to changes in housing conditions (Zucker 1996). Malaria surveillance in the United States began in 1933 and by the 1950's the Centers for Disease Control and Prevention (CDC) determined that malaria transmission had been eradicated (Zucker 1996).

Malaria transmission in southern Canada was of historical importance due to the large population centers located in the southern areas of the country. Even today, the majority of the Canadian population is located in southern areas that have conditions for malaria transmission. Key populations that were affected by malaria transmission in Canada include migrant workers on the Rideau Canal in Ontario from 1826-1832 and police workers in the western prairie provinces (MacLean and Ward 1999). Malaria was eradicated in Canada through similar measures to those used in the United States: reduction in vector habitat, improved housing to limit the contact between the mosquitoes and humans and improved treatment of malaria patients (MacLean and Ward 1999).

The competent malaria vector species in the eastern half of the United States and parts of southern Canada is the *Anopheles quadrimaculatus* mosquito. Several studies from before the eradication of malaria in the United States investigated the degree to which *An. quadrimaculatus* could become infected with the *Plasmodium vivax* parasite (Kartman 1953; Jeffery et al. 1954; Eyles et al. 1948). Although temperatures in parts of the United States and Canada drop below the minimum suitable temperature for the survival of the vector in winter months, previous endemic transmission was potentially sustained through indoor transmission and hibernating mosquitoes, as it was in northern Europe (Huldén et al. 2005).

Both the Canadian and American governmental public health agencies track the number of imported malaria cases reported within their countries. The CDC publishes the Morbidity and Mortality Weekly Report, with a yearly summary of notifiable diseases that includes information on imported malaria cases by sex, race, age and geographic area (CDC 2009). The Canadian government publishes a summary of notifiable diseases by province (PHAC 2007). In addition, the Committee to Advise on Tropical Medicine and Travel (CATMAT) issues guidelines from a public health perspective on how to improve the prevention and treatment of malaria among Canadian travellers (PHAC 2009).

Other documents relating to imported malaria in North America generally take the form of journal articles. In both the United States and Canada, articles have been published that remind physicians that imported malaria is a current concern and that they should be aware of it when diagnosing patients (Boggild et al. 2009; Freedman 1992). In addition, there are specific suggestions related to pediatric malaria, with ideas about how to manage cases to improve time to treatment (Goldfarb et al. 2009). There is a need for increasingly forward reminders to physicians regarding imported malaria. Studies in both Quebec and

Ontario have identified concerns related to the timely and accurate diagnosis and treatment of malaria in their respective provinces (Ndao et al. 2005; Kain et al. 1998).

Other research aims to improve the understanding of the characteristics of imported malaria cases, to better target prevention and treatment efforts. One study examining imported malaria in Quebec found that most cases involved business travel and long-term (greater than 1 month) trips, whereas another article identified a common source of imported malaria cases as people who travel to visit friends and relatives (VFR) in countries with endemic malaria transmission (Provost et al. 2006; McCarthy 2001). Finally, a review article assessing the peak of imported cases of malaria in Canada in the late 1990's determined that surveillance is inadequate and needs improvement since changes in international travel and immigration could lead to an increase in imported cases (MacLean et al. 2004).

The current range of *Anopheles quadrimaculatus*, the main competent malaria vector in North America, is not well documented. The last assessment shows it covering most of the eastern half of the United States and parts of southern Canada (Ontario and Quebec) (Darsie and Ward 1981) and a recent large-scale study using mosquito collection data validates that range (Levine et al. 2004). Most of the recent research that has been conducted to better understand the biology and ecology of *An. quadrimaculatus* has focused on it as a nuisance mosquito moreso than as a potential disease vector. However, there seems to be an increasing awareness of its role in autochthonous malaria transmission, and investigations into local populations following local malaria transmission have added to the body of information available (Robert et al. 2005; Strickman et al. 2000). Since *An. quadrimaculatus* does not take avian bloodmeals it is not thought to be a vector for emerging infectious diseases with avian reservoirs

such as West Nile virus (Molaei et al. 2009).

An. quadrimaculatus is often classified as a "riceland" mosquito, and seems to prefer less densely populated areas that can provide it with its preferred habitat, such as large tree cavities for resting (Burkett-Cadena et al. 2008). The mosquito also requires a clean aquatic site for laying of eggs (O'Malley 1992). The behaviour of *An. quadrimaculatus* is highly dependent on the time of day (O'Malley 1992). It rests during the day and is most active at dusk and dawn when it is feeding (O'Malley 1992). Females hibernate over the winter and die after laying their eggs in the spring (O'Malley 1992).

2.2.2. Review of global models of malaria

Most current malaria models are global, describing how changes in climate could affect global transmission patterns (Kiszewski et al. 2004; Rogers and Randolph 2000; Martens et al. 1995a). A model by Rogers and Randolph examines potential changes in global malaria transmission based on statistic envelope modelling of existing global incidence ranges and predicts a negligible net change in global malaria distribution under projected climatic change (Rogers and Randolph 2000). However, this model does not take into account possible changes in non-climatic factors. In addition, the Rogers model does not address autochthonous incidence and limits malaria to regions with a similar climate to those already experiencing endemicity. The global model created by Kiszewski focuses on the role of the vector in limiting or intensifying malaria transmission (Kiszewski et al. 2004). A "stability index" was created to gauge the relative impact of the vector on malaria transmission. However, due to the large scale of the model, the results are perhaps not reliable on a smaller scale for individual regions.

Martens' 1995 global malaria model employs process-based modelling to predict

the regions with the potential to support transmission. This latter model was specifically targeted at identifying the causal contribution of climatic variables on malaria transmission, and all non-climatic factors were thus held constant (Martens et al. 1995a). This type of modelling may not be very useful for helping to understand how actual transmission could change in the future, because holding so many factors constant doesn't integrate all of the real complexity inherent in the system. Some of these global models do suggest that North America may be at-risk for future malaria transmission given the presence of competent vector species and projections of warmer temperatures, which may increase both the density and range of the vector populations, and increase the potential replication and survival of the pathogen (Martens et al. 1995a).

These global models, while useful for evaluation of potential worldwide patterns and trends in malaria incidence, have been constrained by the difficulty of integrating social determinants of risk at the global scale, and do not inform risk assessment at the local level or in non-endemic areas. This limitation has even been shown in the research done on global models, as researchers acknowledge that local conditions are the necessary lens through which to view global predictions (Martens et al. 1995a). Since most global models have looked at the expansion of endemic areas, there is a need for models that specifically look at re-emergent malaria and the ways in which new transmission zones might become established. Due to the importance of non-climatic determinants of transmission in non-endemic areas, models excluding non-climatic variables will be particularly unreliable. Risk projections in these regions are likely to be sensitive to local variations in non-climatic transmission determinants. Much of the current debate regarding climate change impacts on malaria is due to methodological issues and challenges. Regional studies incorporating new methodologies that look at social and travel factors in a non-endemic context have the potential to add valuable information to the discussion on climate and

malaria.

In recent years there has been an increasing interest in research on re-emergent malaria in a European context. Models of re-emergent malaria have been produced in France, Germany, Italy, and the United Kingdom (Ponçon et al. 2008; Linard et al. 2009; Schröder and Schmidt 2008; Romi et al. 2001; Kuhn et al. 2003). While these models provide important justification for the global importance of re-emerging malaria transmission, they have several limitations. Imported malaria patterns differ greatly, and the social factors that affect these patterns are not included in the European models. The idea of using spatial analogues for malaria models breaks down when social factors are not adequately addressed. This problematic absence is addressed in the wider emergent disease literature: "one limitation of current malaria models is that they rarely include key drivers besides climatic factors" (Ebi 2008; page 8). For re-emergent malaria models, in which transmission is dependent on the introduction of the parasite through imported malaria cases, it is important to include the key drivers of international travel and immigration patterns.

Most of the studies (both global and regional) outlined above have relied on a single method for their analysis of factors that could potentially impact malaria transmission, either endemic or autochthonous. There is a need for research that incorporates more diverse types of data and a greater variety of mixed methods to better understand the wider context of how malaria transmission in a particular region is impacted by factors that affect transmission globally, such as international travel and global climate change. Global models and studies that are conducted at a large scale are not always appropriate when applied to a regional small-scale context. Region-specific studies are necessary to evaluate which variables are the most significant to transmission in a particular area. Assessment of autochthonous malaria risk in Canada has not previously been

conducted (Berrang-Ford et al. 2009).

This thesis characterizes and evaluates the potential for autochthonous malaria transmission in the study area of Ontario, including factors related to international travel and immigration, imported malaria, and the competent vector population. The project incorporates quantitative temporal modelling, descriptive mapping, statistical analysis, qualitative interviews and historical reviews. To better understand the current geographical and temporal range of the vector and imported malaria cases, descriptive mapping is conducted. The human movement patterns that drive the imported malaria case patterns are assessed to frame the results of the descriptive mapping of the cases.

Chapter 3: A case-control analysis of imported malaria cases in Ontario, Canada

3.1. Abstract:

This thesis chapter characterizes imported malaria cases in Ontario, with an emphasis on examining the associations with neighbourhood-level immigration statistics and personal travel behaviour. Imported malaria data were provided by the Ontario Agency for Health Protection and Promotion (OAHPP) Malaria Reference Laboratory (OAHPP, Etobicoke) and included results of malaria tests conducted between 2008 and 2009 that identified subjects as either positive (cases) or negative (controls). Univariate tests and unconditional logistic regression were performed to assess the relationship between the predictor variables and case/control status. Malaria cases were found predominantly in the Greater Toronto Area (GTA), with more cases in suburban areas outside the city center. A statistically significant space-time cluster was found in an area northwest of the GTA, near Brampton. The case-control logistic regression analysis showed that malaria cases were positively correlated with living in a neighbourhood with a high proportion of residents who are immigrants from malaria endemic areas. Malaria cases were negatively correlated with population density and median household income. Statistically significant associations were found between case/control status and parasite species (*P. falciparum* or *P. vivax*) and personal travel history to areas with endemic malaria (Africa or Asia). Cases were more likely to report travel to areas with endemic malaria, and there was concordance between the parasite species and the region of travel. The associations between parasite species and geography held when neighbourhood immigration was examined as well: cases of *P. vivax* corresponded with immigration from endemic areas of Asia, and cases of *P. falciparum* were found in areas with immigration from endemic areas of Africa.

3.2. Introduction:

The global movement of people takes many forms. Two key types of human movement are travel and immigration. Travel patterns show where people move, and immigration patterns show where they settle. These processes are not necessarily independent of one another. Travel patterns can be influenced by immigration, as travellers may return to their country of origin to visit family or friends. Although the processes of international travel and immigration take place on a global scale, the outcomes can be seen through the local incidence of imported diseases.

International travel and immigration flows have a significant impact on Ontario, Canada. Toronto, the largest city in the province and country, experiences a high volume of international travel, with 3.5 million passengers arriving at Toronto's Pearson International Airport in 2007 (Khan et al. 2009a). Toronto's air passenger volume is among the highest in the U.S. and Canada, outranked only by New York City (13.3 million), Los Angeles (6.5 million), and Miami (5.3 million passengers) (Khan et al. 2009a). Additionally, passenger volume to Toronto has grown 7% since 2001 (Khan et al. 2009a). After the U.S., Europe and Australia, the largest number of passengers to Toronto arrive from Hong Kong, Mexico, India and China (Khan et al. 2009a).

The high volume of international travel in Toronto is just one sign of its role as a hub for international immigration. Toronto is a major immigration destination, both globally and within Canada (Benton-Short et al. 2005). Although the 2006 census found that one in five Canadians was foreign-born, in the census metropolitan area (CMA) of Toronto the proportion is more than twice the national rate: 45.7% of the population is foreign-born (Chui et al. 2007). Patterns of the locations where immigrants choose to settle within the Greater Toronto

Area (GTA) show the strongest growth in cities outside of the City of Toronto (Chui et al. 2007).

Travel has always played a role in the spread of infectious disease, but the current prevalence of rapid air travel has increased the speed and density of connections between people and places (Khan et al. 2009a). One key aspect of international travel that has a large effect on infectious disease transmission is the movement of people between areas with different levels of risk (Gushulak and MacPherson 2004). While travel in general can be a driver of infectious disease spread, certain types of travel and travellers are more likely to play a role in emerging infectious diseases (Gushulak and MacPherson 2006).

Travellers visiting friends and relatives (VFRs) have special disease risks for a range of diseases (Bacaner et al. 2004; Angell and Behrens 2005; Angell and Cetron 2005; Fenner et al. 2007; Leder et al. 2006). There is a significant difference in the health outcomes of VFRs as opposed to tourists, or people who travel for vacation and recreational purposes (Hagmann et al. 2009). One of the key characteristics of VFRs that might be affecting their disease risk is their differing preventative care choices and their lower usage of pre-travel medical services (Baggett et al. 2009). Gender is another factor that seems to play a larger role in the incidence of travel associated diseases, with men and women having disproportionate risks for certain diseases (Schlagenhauf et al. 2010). Women are more likely to have diarrheal diseases and upper respiratory tract infections, whereas men are more likely to have vector-borne diseases and sexually transmitted infections (Schlagenhauf et al. 2010). It is not just the location of travel, but also the individual behaviours of the traveller that can affect disease risk.

Imported malaria in the context of international travel and immigration

Malaria is endemic in regions across the globe, and the specific role of human movement in malaria transmission is an important aspect of malaria emergence in some areas (Martens and Hall 2000). Humans can transport the malaria parasite from endemic areas to non-endemic areas, either in their own bodies or through the accidental movement of infectious mosquitoes (Martens and Hall 2000). Length and type of travel can be associated with risk of malaria transmission (Provost et al. 2006), and there appears to be a relationship between the behaviour of travellers and imported malaria incidence (Chen and Keystone 2005; Pavli and Maltezou 2010). Imported malaria is often associated with a lack of chemoprophylaxis or a lack of adherence to a recommended regimen (Chen and Keystone 2005). Additionally, not seeking pre-travel advice puts travelers at risk (Pavli and Maltezou 2010).

How travellers behave globally can have an affect on their risk of disease locally, when they return home. A frequent source of imported malaria cases are VFRs who are visiting friends and relatives in countries with endemic malaria transmission (McCarthy 2001; Pavli and Maltezou 2010). VFRs have been found to be an important source of imported malaria in the United Kingdom, the United States, and the Netherlands (Smith et al. 2008; Mathai et al. 2009; Schilthuis et al. 2007). One of the reasons for the increased risk of imported malaria among VFRs may be differing perceptions of the risk of malaria, leading to different behaviours in malaria endemic areas (Pavli and Maltezou 2010; Pistone et al. 2007; Schilthuis et al. 2007). It may also be due to how travel is patterned based on immigration, with more VFRs than tourists travelling to areas that have endemic malaria.

The global prevalence of malaria intersects with patterns of international travel

and immigration to lead to the local importation of malaria cases in Ontario, Canada. Significantly, Canada receives a reported 400 (approximately) cases of imported malaria a year, far more per capita than the United States (PHAC 2004; Kain et al. 1998). The reasons for the higher per capita rate of cases imported into Canada are not well understood, but may be due to differing proportions and source countries of immigrants.

Understanding the local patterns of imported malaria in Ontario has several key ramifications. First, the surveillance of imported malaria is significant in that imported malaria cases are a driver of autochthonous malaria transmission, since the parasite needs to be imported for a local transmission cycle to begin. Secondly, imported malaria cases can have severe or fatal outcomes (Humar et al. 1997). Lastly, a better understanding of imported malaria patterns in Ontario might point to how global travel and immigration could be impacting the local patterns of other infectious diseases.

This thesis chapter is a case-control study of imported malaria cases in Ontario, with an emphasis on examining the associations with neighbourhood-level immigration statistics and personal travel behaviour.

3.3. Methods:

Imported malaria data were provided by the Ontario Agency for Health Protection and Promotion (OAHPP) Malaria Reference Laboratory (OAHPP, Etobicoke). These data included results of malaria tests conducted between 2008 and 2009 that identified subjects as either positive (cases) or negative (controls). The Laboratory receives blood specimens from other labs throughout the province as well as from hospitals and clinics. The data include any test conducted at the Laboratory for malaria diagnosis, confirmation, or malaria

parasite speciation. The OAHPP Malaria Reference Laboratory handles approximately 75% of the malaria testing for Ontario (D. Pillai, personal communication, 2009). An ethics certificate was obtained from the McGill University Research Ethics Board and the data were stored in a confidential and secure manner.

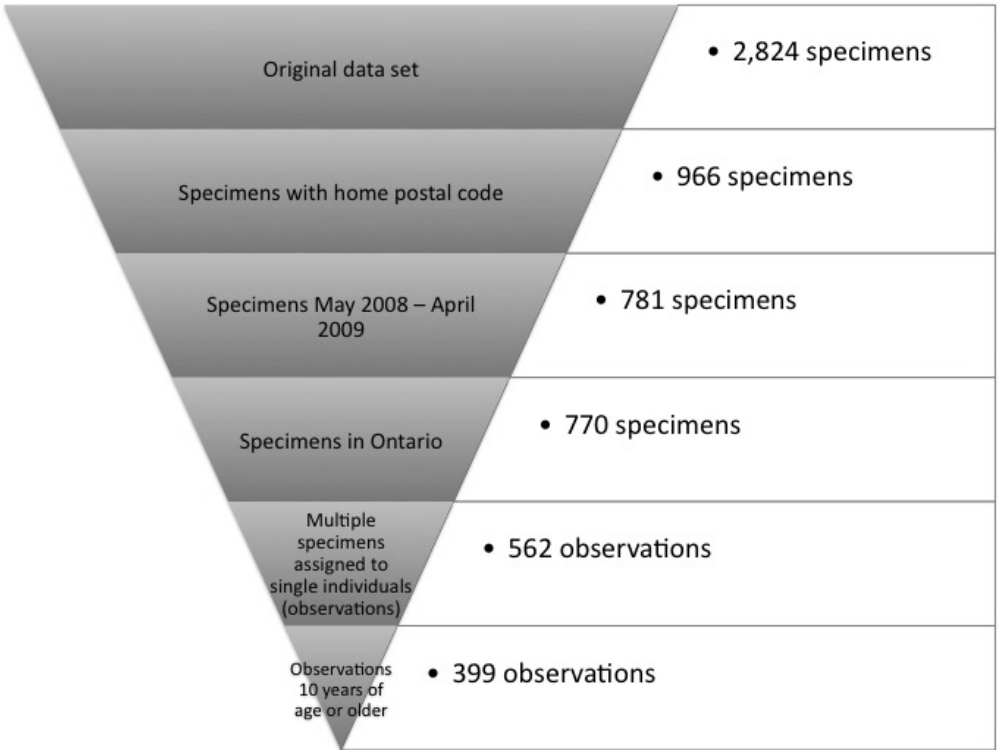

Figure 3.1: Malaria data exclusion diagram.

The OAHPP dataset contained 2,824 specimens (Figure 3.1). A specimen represented a blood sample that was tested for malaria. Data were converted from specimens to observations (individuals) in order to avoid duplication in the dataset due to repeat testing. Observations for individuals with no home postal code information and those with home addresses located outside of Ontario were excluded. All incident cases in children younger than 10 years of age were removed due to overdispersion (negative skewing) of the age distribution in the

control dataset. Different diagnosis patterns between children and adults are most likely responsible for the large number of negative tests in children. The symptoms of malaria in children are similar to those of other diseases (Stauffer and Fischer 2003). Due to this similarity, it is often recommended that children with appropriate travel history and symptoms be tested for an array of potential infections (Kuhn and McCarthy 2006). Entries were selected for the 12-month period from May 2008 to April 2009. The final dataset included 399 malaria test observations. These observations were categorized as cases (positive malaria tests, n=106) and controls (negative malaria tests, n=293). Travel history was reported for 60 of these individuals.

Statistics Canada census data from 2006 aggregated to the census tract level were obtained from the Data Centre of Computing in the Humanities and Social Sciences (CHASS) at the University of Toronto (CHASS, Toronto). Data describing population, immigration status and median household income were compiled for all Ontario census tracts. Census tracts were used as a proxy for neighbourhoods (Ross et al. 2004), and population density was calculated for each area. Endemic immigration proportion for each neighbourhood was calculated by dividing the number of respondents reporting immigration from an area with endemic malaria transmission (Southeast Asia, South Asia, East Africa, Central Africa, West Africa) by the number of census respondents. Geographic boundaries of both census tracts and dissemination areas were used for mapping, area calculation and spatial reference (CHASS Data Centre, Toronto).

All cartography was conducted using the software ArcGIS Version 9.3 (ESRI, Redlands). Several key variables were used in the descriptive mapping of malaria cases, namely case/control status, parasite species, age, and gender of patient. For case-control data, observations were placed at the centroid of the postal code recorded as the home address. Full six-digit postal codes were used for all

observations retained in the data set, and those without appropriate postal codes were removed (see Figure 3.1). CDC week codes (a numerical designation for each week of the year, 1-52) were assigned to each observation in the dataset to facilitate temporal graphing. Timeline graphs were created to evaluate the seasonal and temporal distribution of malaria observations. Incidents were stratified by parasite species. Due to a high concentration of cases in the Greater Toronto Area (GTA), a subset of the malaria case dataset was created with only observations occurring in the GTA. For purposes of analysis, Canadian Regional Municipalities were used to delimit the dataset and the GTA subset thus includes Toronto, Halton, Peel, York and Durham municipalities. Cluster analysis was performed on the GTA subset in order to assess the significance of any potential spatial clustering. A space-time scan statistic (Kulldorff et al. 1998) was performed on case/control status and parasite species using the software SaTScan Version 8.1.1 (M. Kulldorff, Boston). Analyses assumed a Bernoulli case-control distribution and a maximum cluster size of 50%.

Unconditional logistic regression analysis was conducted. The key variables included in the model were population density, median household income, endemic immigration proportion, and gender. The outcome variable was case/control status. All statistical analyses were performed using Stata 11 (StataCorp, College Station, Texas). Univariate tests were conducted to assess the relationship between the individual predictor variables and case/control status. One observation was removed because there were no census data available for the postal code centroid. All variables were checked for co-linearity and normality, and the model was checked for significant interaction, outliers, confounding, predictive ability and accuracy, leverage and goodness-of-fit. It was hypothesized that there might be interactions between population density, median household income and endemic immigration proportion. The best fit

model was chosen based on the inclusion of the key variables related to imported malaria cases given data availability, as well as the results of the tests for goodness-of-fit and other post-estimation procedures. The best fit model was interpreted using quartile values of each of the predictor variables, comparing the 1st and 4th quartiles.

Travel history was categorized using the regional groupings provided in the Canada census database. Univariate tests and graphing were performed on travel, neighbourhood immigration characteristics, case/control status and parasite species. For travel and immigration analysis, cases with a parasite species other than *P. falciparum* and *P. vivax* (n=10) were excluded due to the small number of observations.

Table 3.1: Variable definitions and sources

Variable Name	Definition	Data Source
Case/control status	Positive/negative malaria test	OAHPP Malaria Reference Lab
Gender	Male, female or not available	OAHPP Malaria Reference Lab
Age	Age in years at time of test	OAHPP Malaria Reference Lab
Parasite species	*Plasmodium* species	OAHPP Malaria Reference Lab
Population density	2006 Canadian census tract population divided by census tract size	CHASS Data Centre
Median income	2006 Canadian census value in Canadian dollars	CHASS Data Centre
Endemic immigration proportion	Proportion of residents who reported immigrating from Southeast Asia, South Asia, East Africa, Central Africa, or West Africa in the 2006 Canadian census	CHASS Data Centre
Endemic immigration from Asia	Proportion of residents who reported immigrating from Southeast Asia, or South Asia in the 2006 Canadian census	CHASS Data Centre
Endemic immigration from Africa	Proportion of residents who reported immigrating from East Africa, Central Africa, or West Africa in the 2006 Canadian census	CHASS Data Centre
Travel to endemic Africa	Country of reported travel within East Africa, Central Africa or West Africa, or general African travel	OAHPP Malaria Reference Lab
Travel to endemic Asia	Country of reported travel within South Asia or Southeast Asia	OAHPP Malaria Reference Lab

3.4. Results:

Table 3.2 summarizes the individual characteristics of the imported malaria data set, for all of Ontario and the GTA. There are 106 cases and 293 controls, approximately a 1:3 ratio. The general distributions of gender, age, and relative parasite proportions do not differ greatly between all of Ontario and the GTA subset. The spatial distribution of the cases and controls is consistent with Ontario's areas of highest population density, with most of the observations located in southern Ontario and the GTA. There appears to be a large concentration of controls in the downtown core of Toronto, with more cases in the suburban areas.

There was significant clustering of cases in suburban Toronto during the summer months (Figure 3.2). A significant space-time cluster was identified from May 1, 2008 - September 27, 2008, in an area northwest of Toronto, near Brampton ($p < 0.01$, Relative Risk = 3.96). This is near the location of the main international airport in the Greater Toronto Area. In this area during the summer months, tested individuals were almost 4 times more likely to be diagnosed with malaria infection than individuals outside of this area and period. No significant clustering of individual parasite species (*P. falciparum* or *P. vivax*) was identified.

Table 3.2: Summary statistics of imported malaria cases and controls

	Cases (%)	Controls (%)	Total (%)
Ontario	106 (27)	293 (73)	399 (100)
Gender:			
Male	72 (33)	146 (67)	218 (100)
Female	28 (16)	147 (84)	175 (100)
Not Available	6 (100)	0 (0)	6 (100)
Mean Age	39.3	38.1	38.4
Species:			
P. falciparum	64 (60)		
P. vivax	32 (30)		
P. ovale	5 (5)		
P. [unidentified species]	3 (3)		
P. [mixed species]	1 (1)		
Babesia	1 (1)		
GTA subset	93 (28)	238 (72)	331 (100)
Gender:			
Male	63 (36)	112 (64)	175 (100)
Female	24 (16)	126 (84)	150 (100)
Not available	6 (100)	0 (0)	6 (100)
Mean Age	39.7	37.2	37.9
Species:			
P. falciparum	55 (59)		
P. vivax	31 (34)		
P. ovale	4 (4)		
P. [unidentified species]	2 (2)		
P. [mixed species]	1 (1)		
Babesia	0 (0)		

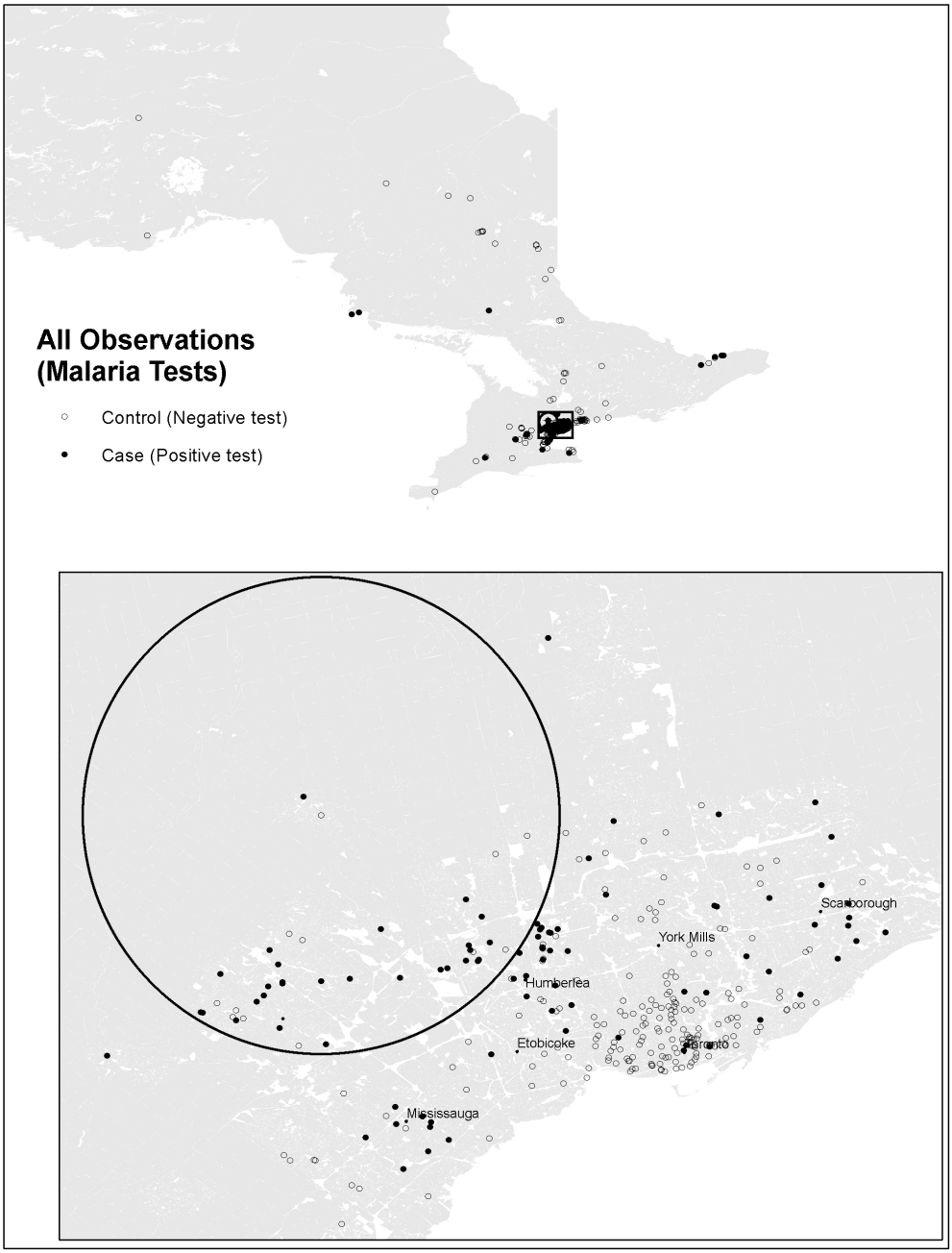

Figure 3.2: All observations, stratified by cases and controls, including the most significant space-time cluster.

Cases were significantly more likely to occur in the summer months (June - September) compared to the non-summer months for both the GTA and all of Ontario (chi-squared: $p = 0.02$ and $p = 0.01$, respectively). This is consistent with the space-time cluster analysis and historical travel patterns, which point to potential seasonal patterns in imported malaria cases (K. Kain, personal communication, 2009). Visual observation of monthly incidence, however, does not indicate any strong or clear trends, possibly affected by low observation numbers, as well as incidence and seasonality in endemic countries from which malaria is imported (see Figure 3.3).

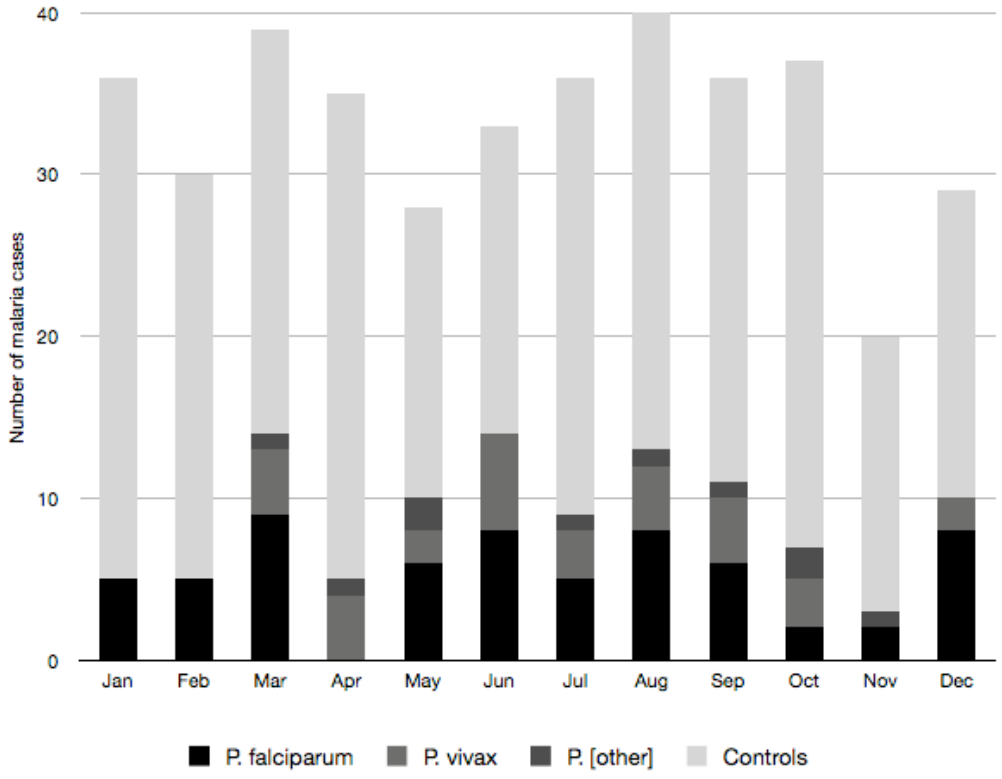

Figure 3.3: Cases (by species) and controls, monthly.

Population density is highly variable in the study area, with more densely populated areas near the downtown area of the GTA. Median household income, in contrast, has negligible clustering except for low-income suburbs east and west of the downtown core. Endemic immigration proportion is highest in the suburban areas to the east and west of Toronto (see Figure 3.4).

Over 95% of cases with recorded travel history indicate recent travel to Africa or Asia (Figure 3.5), with African travel most frequently reported. These results are consistent with known spatial ranges of malaria endemic areas (Griffith et al. 2007).

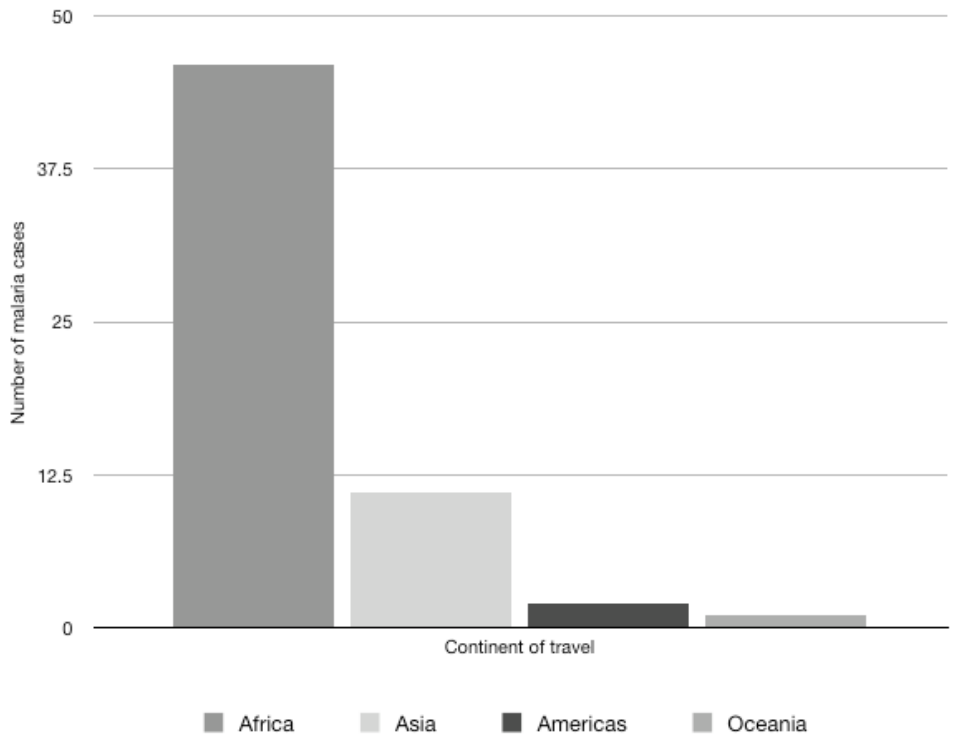

Figure 3.5: Number of malaria cases by continent of travel.

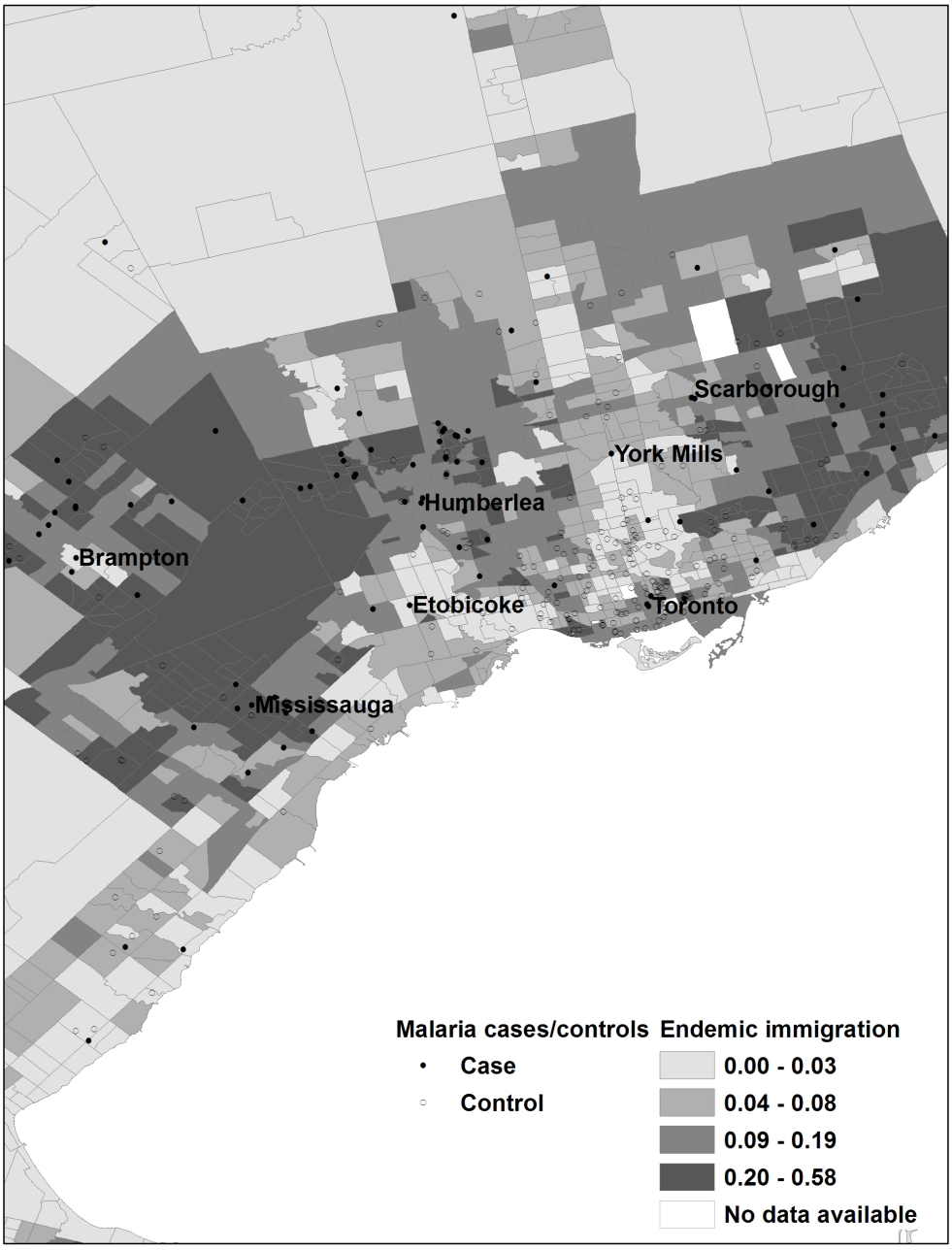

Figure 3.4: Proportion of residents in a neighbourhood reporting immigration from areas with endemic malaria, and malaria cases/controls, GTA.

Cases are significantly more likely than controls to be male, and live in lower-income neighbourhoods with a higher proportion of immigrants from malaria endemic regions (Table 3.3). The proportion of immigrants from endemic countries was, for example, almost twice as high in neighbourhoods with cases than in neighbourhoods where controls were identified. The average income of case neighbourhoods was approximately $28,000, compared to $31,000 in control neighbourhoods (T-test, p = 0.02). The subset of cases with travel history (n = 56) are more likely to report having travelled to Africa and to live in a neighbourhood with a higher proportion of immigrants from endemic countries than controls with travel history (Table 3.4).

Table 3.3: Univariate statistics

	Cases		**Controls**		**p-value**
Individual Variables	**n**	**percent**	**n**	**percent**	
Total	106	26%	292	73%	
Gender:					
Males	72	68%	145	50%	p < 0.01 (!)
Females	28	26%	147	50%	
Not reported	6	6%	0	0%	
	Mean	**95% CI**	**Mean**	**95% CI**	
Age	39.2	(35.9 - 42.5)	38	(35.9 - 40.2)	p = 0.58 (^)
Neighbourhood-level Variables					
Population Density (persons per square km)	4,750	(4,084 - 5,415)	6,406	(5,620 - 7,193)	p = 0.18 (*)
Median Income (CAD$)	27,878	(25,991 - 29,766)	30,877	(29,592 - 32,163)	p = 0.02 (^)
Percent immigrant from a malaria endemic area (%)	18.0	(15.6 - 20.5)	9.6	(8.4 - 10.8)	p < 0.01 (*)

Notes: [! Chi-squared], [^ T-test], [* Mann-Whitney]

Table 3.4: Travel and immigration and case/control & parasite species univariate tests

	Cases	Controls	p-value	*P. falciparum*	*P. vivax*	p-value
Neighbourhood-level variables	**Mean**	**Mean**		**Mean**	**Mean**	
Immigration from endemic Africa (%)	3.3	1.5	< 0.01 (*)	4.0	2.0	0.27 (*)
Immigration from endemic Asia (%)	15.3	8.0	< 0.01 (*)	12.4	21.1	< 0.01 (*)
Individual-level variables	**n**	**n**		**n**	**n**	
Travel to endemic Africa	45	32	< 0.01 (!)	44	1	< 0.01 (~)
Travel to endemic Asia	11	36	0.10 (!)	0	11	< 0.01 (~)

Notes: [* : Mann-Whitney][! : Chi-squared test][~ : Fisher's exact test]

The best-fit logistic regression model is shown in Table 3.5. The results are consistent with univariate analyses: cases are more likely to be male (OR 2.33, CI 1.36 - 3.99) and live in neighbourhoods with high endemic immigration (OR 1.08, CI 1.05 – 1.10). Population density was a weak predictor of outcome, but was retained in the model to account for potential confounding with endemic immigration. Endemic immigration showed the strongest effect on case status: for every one percent increase in endemic immigration, the odds of being a case increase by six percent. The odds of being a case are more than 10 times higher in neighbourhoods in the top quartile of endemic immigration than in the neighbourhoods in the lowest quartile. There were slight negative effects from median household income and population density. For every one unit increase in either, the odds of being a case decrease by one percent.

The pseudo R-squared of the model is 0.16, with a predictive accuracy of 68% correctly classified using a cutoff of 0.25 (67% sensitivity, 69% specificity). The model is a good fit (Hosmer-Lemeshow test, p=0.88), and leverage values did not indicate any significant outliers that required removal (see Figure 3.6). There were a number of high positive residuals, as shown in Figure 3.6. This is due to the low sensitivity of the model using a default cutoff (0.5). This low sensitivity reflects the limited number of variables available for analysis. The model was developed, however, for explanatory rather than predictive purposes; low sensitivity reflects the small number of covariates and use of neighbourhood-level variables.

Table 3.5: Model results

	Null model		**Fully-adjusted**		**1st vs. 4th Quartiles**	
	OR	95% CI	OR	95% CI	OR	95% CI
Percent endemic immigration	1.06	1.04 - 1.08	1.08	1.05 - 1.10	10.77	4.96 - 23.38
Median income	0.99	0.99 - 0.99	0.99	0.99 - 0.99	2.78*	1.25 - 6.22*
Population density	0.99	0.99 - 0.99	0.99	0.99 - 0.99	5.71*	2.42 - 13.47*
Gender (males)	2.61	1.59 - 4.27	2.33	1.36 - 3.99	N/A	N/A
(* Reverse coded to show 4th vs. 1st quartiles)						

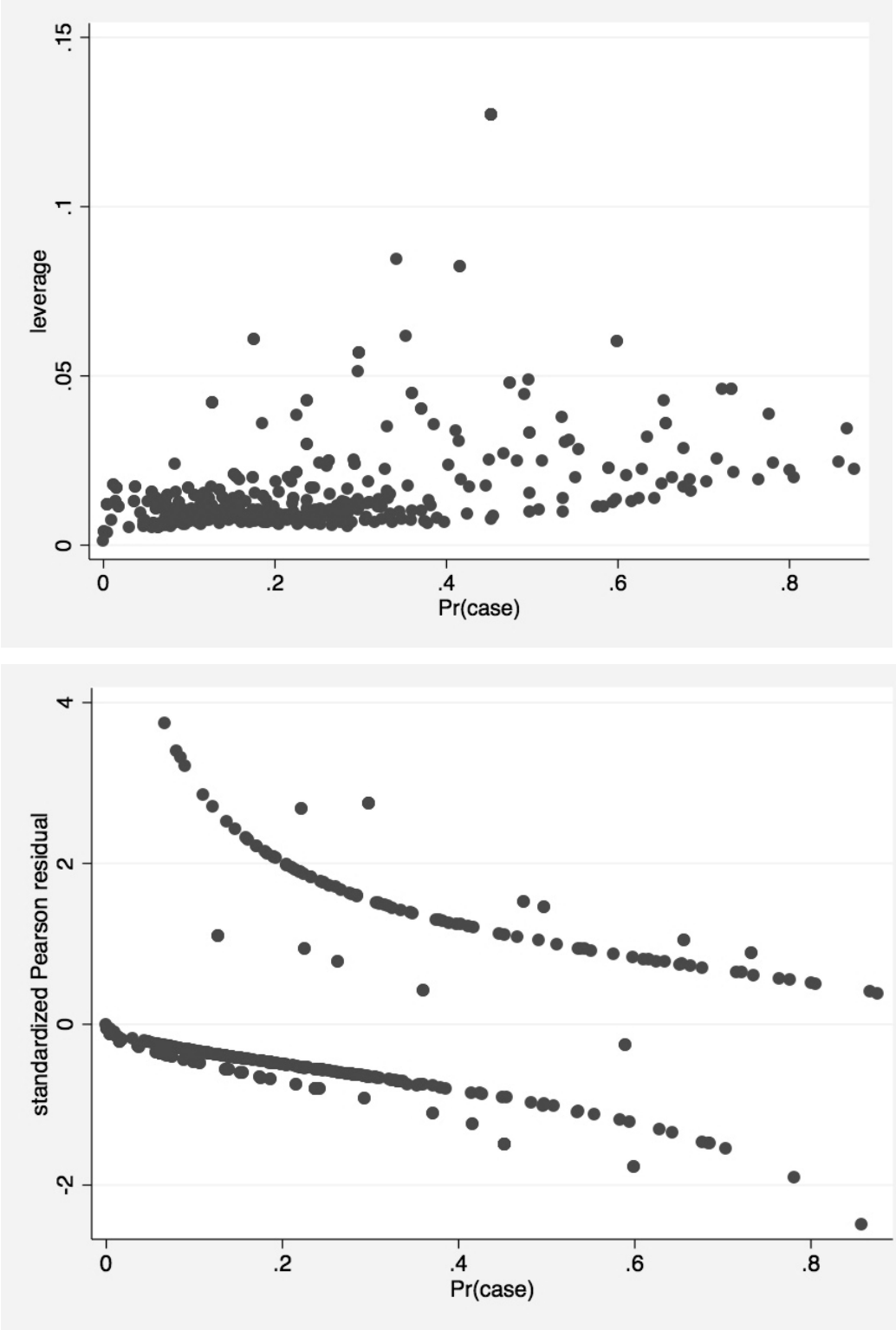

Figure 3.6: Composite of post-estimation graphs (leverage and residuals).

The distribution of parasite species infection in cases reflects the patterning of global malaria, with *P. falciparum* cases associated with African travel or immigration and *P. vivax* cases associated with Asian travel (Table 3.4). Globally, it has been demonstrated that there is a significant spatial patterning of malaria parasite species, with *P. falciparum* found predominantly in Africa and *P. vivax* found predominantly in Asia (Griffith 2007). Cases infected with *P. falciparum*, for example were significantly more likely to report recent travel to Africa than *P. vivax* cases (Fisher's exact test, $p<0.01$). Conversely, *P. vivax* cases were more likely to report travel to Asia (Fisher's exact test, $p<0.01$). *P. vivax* cases live in neighbourhoods with significantly higher proportions of endemic immigration from Asia (Mann-Whitney, $p<0.01$). In contrast, however, *P. falciparum* cases did not necessarily live in neighbourhoods with high African endemic immigration (Mann-Whitney, $p=0.27$). This may be due to the much lower level of African immigration seen overall.

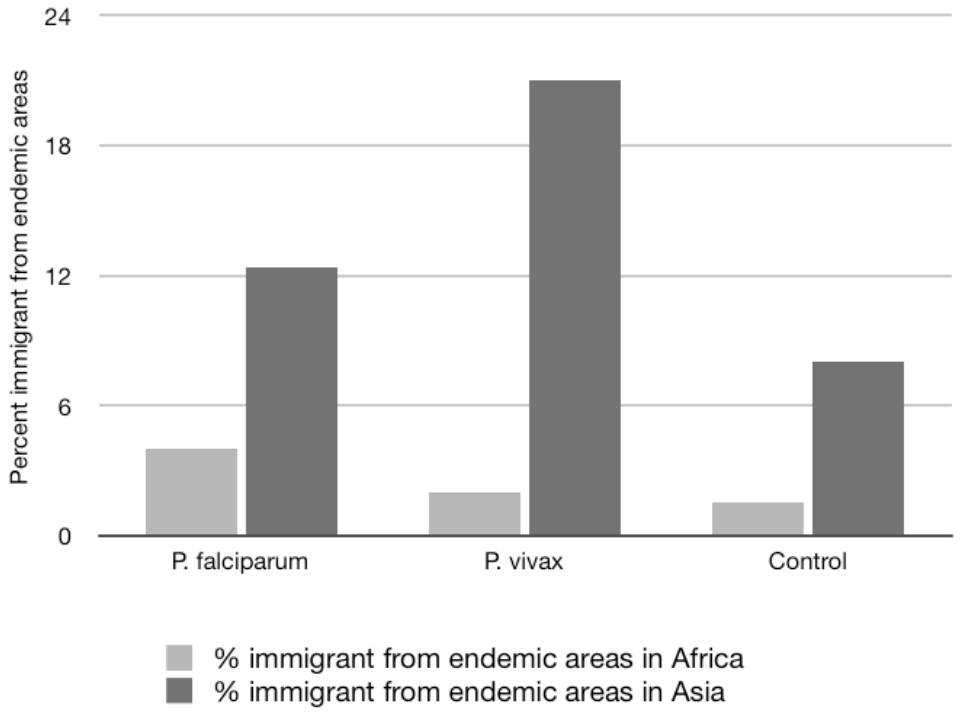

Figure 3.7: Parasite species and neighbourhood immigration statistics.

3.5. Discussion:

Malaria cases in Ontario were found predominantly in the Greater Toronto Area (GTA), with more cases in suburban areas outside the city centre. Malaria cases are more likely to live in suburban neighbourhoods with a high proportion of residents who are immigrants from endemic areas. Cases were more likely than controls to report travel to areas with endemic malaria, and there was concordance between the parasite species and the region of travel. The association between parasite species and geography held when neighbourhood immigration was examined as well—cases of *P. vivax* corresponded with immigration from endemic areas of Asia, and cases of *P. falciparum* were found in areas with immigration from endemic areas of Africa.

The geographical findings of the cluster analysis and the statistical findings of the relationship between case/control status and neighbourhood-level immigration statistics are supported by the results of the 2006 Canadian census. Brampton (the location of the significant space-time cluster of malaria cases), Markham and Ajax all showed significant increases in the proportion of foreign-born residents (Chui et al. 2007). As immigration to the neighbourhoods in the suburbs of the GTA grows, more imported malaria cases could result.

Cases were more likely to be male than female. This is consistent with the literature on gender and travel-associated diseases, which finds that males are more likely than females to have vector-borne diseases, including malaria (Schlagenhauf et al. 2010). This may be due to mosquitoes being more attracted to men than women (Schlagenhauf et al. 2010). Another potential cause could be differing travel behaviours between men and women (Schlagenhauf et al. 2010). However, it is not known whether potential differences in health care utilization patterns between men and women affected the results in this study.

Further research into how men and women seek pre- and post-travel medical care in Ontario would improve the understanding of this result.

When assessing imported malaria cases, underreporting of cases is a significant issue. There is likely to be a high rate of underreporting of malaria in Ontario, estimated to be from 10 - 40% (D. Pillai, personal communication, 2009; K. Kain, personal communication, 2009; Kain et al. 1998; Watkins et al. 2003). 226 malaria cases were documented by laboratories in Ontario in 1998 (Watkins et al. 2003). However, in that same year, only 160 cases in Ontario were reported to Health Canada (Watkins et al. 2003). No recent published or quantified estimates of actual incidence are available, and the true level of current underreporting of malaria in Ontario and Canada more generally remains unclear. The data used in the study are strengthened by the inclusion of negative and positive malaria tests. The use of controls allows for a better understanding of the factors that correlate with malaria cases, unbiased by underlying patterns of malaria testing.

The cluster analysis of the malaria cases has implications for potential preventative measures that could be taken before individuals travel abroad. Targeted prevention could focus on hospitals and clinics found in the cluster area, and try to improve their travel medicine screenings and increase the amount of information available on the importance of malaria prophylaxis.

Although the use of travel clinics is not associated with income in Canada (Duval et al. 2003), there might be other reasons that could cause differential pre-travel usage among Canadians. Ethnicity has been found to impact physician choice in Toronto (Wang et al. 2008), and it could be at play in travel clinic usage issues. The recent growth of immigrants in Brampton is an example of a population change that area physicians might not have responded to yet (Chui et al. 2007).

Creating targeted prevention materials in appropriate languages would be an important step in improving the disproportionate burden of imported malaria cases in neighbourhoods with a large proportion of immigrants from malaria endemic areas.

Chapter 4: An assessment of the potential for autochthonous malaria transmission in Ontario, Canada: The influence of scale

4.1 Abstract:

Southern Ontario is host to the three conditions required for theoretical malaria transmission: importation of *Plasmodium* parasites in travellers, competent *Anopheles quadrimaculatus* vectors, and temperatures that allow the parasite to replicate within the vector. This thesis chapter is a spatially-explicit assessment of the potential for autochthonous malaria transmission in Ontario, Canada. Data for the competent malaria vector species, *Anopheles quadrimaculatus*, were obtained from the Ontario Ministry of Health and Long-Term Care (Ontario MHLTC, Ontario). Data on imported malaria cases in Ontario were obtained from the Ontario Agency for Health Protection and Promotion (OAHPP) Malaria Reference Laboratory (OAHPP, Etobicoke). To identify the potential for interaction between the malaria cases and the vector population, the datasets were overlaid for combination spatial and temporal descriptives. The locations of traps where *An. quadrimaculatus* was found at some point during the summer of 2008 extends farther north than shown in previous maps (Darsie and Ward 1981). While vectors are predominantly observed in the outer suburban areas of the Greater Toronto Area, cases are reported closer to Toronto where vectors are generally absent. Areas of limited spatial coincidence include the area northwest of the GTA, near Brampton, and in the east near Scarborough. Based on the maximum biologically plausible flying range of the vector, only 9% of reported malaria cases fall within a geographic area where autochthonous transmission would be possible. There is very low likelihood of malaria transmission in Ontario, even under increased immigration and climate change, due to limited spatial and temporal coincidence of vectors and imported cases at the local scale.

4.2 Introduction:

Our knowledge of disease risk factors is often based on area-level data. Disease risk estimates sometimes focus on regions or administrative units. However, disease processes occur at a small scale on an individual level. The importance of scale in spatial epidemiology research highlights the significance of the chosen scale of analysis. This chapter assessing the potential for autochthonous malaria transmission in Ontario, Canada, explicitly includes the influence of scale in the methodology. The necessary factors for malaria transmission are examined at both the provincial and the more local level, utilizing small-scale data. The way in which scale can influence the outcome of disease risk assessment illustrates the importance of spatially-specific disease surveillance and analysis.

The issue of scale in the assessment of disease risk is exemplified by current models of malaria risk. Most current malaria models describe how changes in climate could affect global transmission patterns (Kiszewski et al. 2004; Rogers and Randolph 2000; Martens et al. 1995a). Some of these global models do suggest that North America may be at-risk for future malaria transmission given the presence of competent vector species and projections of warmer temperatures, which may increase both the density and the range of the vector populations, and increase the potential replication and survival of the pathogen (Martens et al. 1995a). However, these global models may not be useful for understanding potential changes in autochthonous transmission in non-endemic areas, which occurs on a local-level. This limitation has even been shown in the research done on global models, as researchers acknowledge that local conditions are the necessary lens through which to view global predictions (Martens et al. 1995a).

The particular case of autochthonous malaria transmission is generally absent

from global malaria models. Autochthonous transmission is characterized as sporadic, locally-acquired mosquito-transmitted cases of malaria. Autochthonous malaria cases in non-endemic areas around the world have been documented, including in the United States (Zucker 1996). Autochthonous cases occur when an individual with malaria travels from an endemic area to a non-endemic area, and a local mosquito bites that individual, contracting the malaria parasite and later passing it on to another individual. Sporadic autochthonous cases can be indicative of a potential for malaria emergence or re-emergence, but are not considered evidence for significant risk of such. Autochthonous cases are potentially more significant as an infectious disease event demonstrating the risks of imported pathogens into non-endemic zones.

Although only autochthonous cases of malaria are found in North America today, malaria was previously endemic in a large area of the United States and parts of southern Canada (Zucker 1996). The parasite was introduced in the 16th-17th century with the arrival of European colonists and African slaves (Zucker 1996). The disease was eradicated through several different measures including vector control through changes in vector habitat, new antimalarial medications (such as quinine) for improved treatment, and a decrease in the contact between humans and mosquitoes, due in part to changes in housing conditions (Zucker 1996). Malaria transmission in southern Canada was of historical importance due to the large population centers located in the southern areas of the country. Even today, the majority of the Canadian population is located in southern areas that have conditions for malaria transmission.

Southern Ontario is host to the three conditions required for malaria transmission: the parasite, the vector and temperatures that allow the parasite to replicate within the vector. The parasite arrives via imported malaria cases, approximately 400 of which are reported in Canada every year (PHAC 2004). The

competent malaria vector species in southern Ontario is the *Anopheles quadrimaculatus* mosquito. The current range of *An. quadrimaculatus* is not well documented, and most research has focused on it as a nuisance mosquito moreso than as a potential disease vector. Summer temperatures in southern Ontario often exceed the minimum necessary for the malaria parasite to survive within the mosquito, and potential transmission could occur in the summer months (Berrang-Ford et al. 2009).

But the knowledge of the presence of the three factors necessary for autochthonous malaria transmission in southern Ontario only allows for a large-scale assessment of disease risk. Is there true coincidence on the individual scale necessary for disease transmission? This thesis chapter aims to assess spatial and temporal overlap between the parasite and the vector to evaluate the potential for autochthonous malaria transmission in Ontario, Canada.

4.3. Methods:

Data for the competent malaria vector species, *Anopheles quadrimaculatus*, were obtained from the Ontario Ministry of Health and Long-Term Care (Ontario MHLTC, Ontario). These data were collected in 2008 in the context of West Nile virus surveillance using CDC miniature light traps and include several mosquito species. The data were collected by the individual health units within Ontario, using the same type of trap and sampling methods throughout (C. Russell, personal communication, 2010). Traps were set up in locations determined by the health unit, such as city parks, backyards and marshes. The traps were operational for one night each week and were used on a weekly basis or a bi-weekly basis in smaller communities (C. Russell, personal communication, 2010). Since *An. quadrimaculatus* was not the main species of interest for the vector surveillance activities, the type of trap was not chosen to be the most attractive

to *An. quadrimaculatus*. Due to this bias, there could be a large degree of under-surveillance of *An. quadrimaculatus* in the dataset. Data from the year 2008 were selected for comparison with available parasite data from the same time period.

The vector data set obtained from the Ontario Ministry of Health and Long-Term Care contained 1,139 female *An. quadrimaculatus* observations with geographic information for the year 2008. After duplicate observations from the same sampling site were combined, 390 unique presence locations remained in the final spatial dataset. In constructing the vector dataset, only presence data, not absence data, were used. This is because it is unclear if all traps in Ontario were looking for *An. quadrimaculatus*. Measures of mosquito density by location were available but not considered to be sufficiently reliable due to differential sampling effort.

Data on imported malaria cases in Ontario were obtained from the Ontario Agency for Health Protection and Promotion (OAHPP) Malaria Reference Laboratory (OAHPP, Etobicoke). These data included positive and negative malaria tests conducted at the laboratory, located in Etobicoke, Ontario. The Laboratory receives blood specimens from other labs throughout the province as well as from hospitals and clinics. The data include any test conducted at the Laboratory for malaria diagnosis, confirmation, or malaria parasite speciation. The OAHPP Malaria Reference Laboratory handles approximately 75% of the malaria testing for Ontario (D. Pillai, personal communication, 2009). The original dataset contained specimens representing blood samples that were tested for malaria. Data were converted from specimens to observations (individuals) in order to avoid multiplication in the dataset due to repeat testing. Observations with no home postal code information or located outside of Ontario were excluded. All incidents associated with children under 10 years were removed

due to over-dispersion (negative skewing) of the age distribution in the control dataset. Different diagnosis patterns between children and adults are most likely responsible for the large number of negative tests in children. Entries were selected for the 12-month period from May 2008 to April 2009. The final dataset included 399 malaria test observations. Of these malaria test observations, 106 are positive tests, and represent the distribution of imported malaria cases in Ontario.

All cartography was conducted using the software ArcGIS Version 9.3 (ESRI, Redlands). The current geographical distribution of the vector was mapped using ArcGIS and the point locations of each trap. For malaria case data, observations were placed at the centroid of the postal code recorded as the home address. Presence and the number of consecutive weeks with *An. quadrimaculatus* were mapped.

CDC week codes were assigned to each observation in the dataset to facilitate temporal graphing. Timeline graphs were created to evaluate the temporal distribution of the geographic presence proportion of *An. quadrimaculatus*. The geographic presence proportion is the number of locations where *An. quadrimaculatus* was found during one week divided by the total number of geographic locations where *An. quadrimaculatus* was ever found throughout the summer.

Spatial interpolation of the point locations of the mosquito traps was also performed using kernel density estimation. This method was used to create an estimate of the current geographic range of *An. quadrimaculatus*. Kernel density estimation creates a surface that represents the intensity of events in the surrounding area, and is considered useful for exploratory visualization (Allen and Wong 2006). However, it was not possible to utilize the kernel density

surface for analysis, as the small number of observations was insufficient in the context of the normal flying range of the vector. After kernel density estimation, data falling outside the provincial boundaries were removed.

To identify the potential for interaction between the malaria cases and the vector population, the datasets were overlaid for combination spatial and temporal descriptives. Maps were created that compare the locations of the malaria cases with that of areas where the vector was present for a range of consecutive weeks. The temporal graph compares the number of malaria cases with the number of traps with *An. quadrimaculatus* in the study area.

Distance buffers of the vector trap locations were created to determine the extent of geographical coincidence between the vector and the parasite. For this map, only traps with four or more consecutive weeks of *An. quadrimaculatus* presence were used, as the goal was to evaluate the current degree of spatial overlap between malaria cases and vectors. The buffers used distances from 0.5km up to 10km, to represent a wide range of different possible mosquito distributions. The normal flight range of the *An. quadrimaculatus* mosquito is 1 mile (1.6 km) or less, although it can be up to 3 miles (4.8 km) (O'Malley 1992). Based on this information, a graph was created to compare the number of malaria cases within each buffer range. The results of this graph were used to assess the degree of spatial overlap between the malaria parasite and vector in Ontario.

4.4. Results:

The locations of traps where *An. quadrimaculatus* was found at some point during the summer of 2008 extends farther north than shown in previous maps (Figure 4.1) (Darsie and Ward 1981).

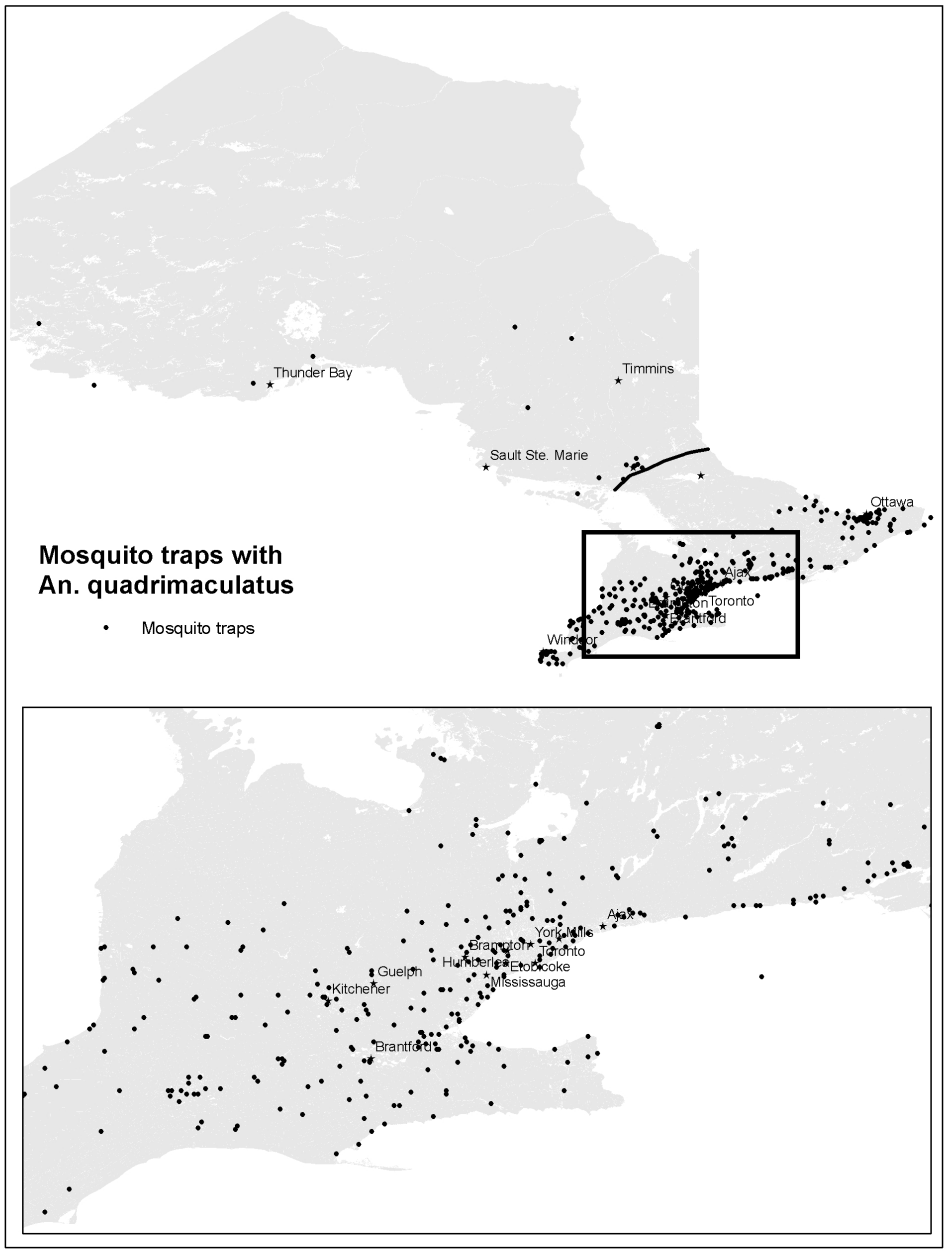

Figure 4.1: Mosquito traps with *An. quadrimaculatus*, Ontario. The black line represents the most recent known extent of the vector (Darsie and Ward 1981).

This indicates the approximate current distribution of *An. quadrimaculatus*, which may be much wider than previously known, either due to imprecise mapping or actively expanding distributions. In particular, *An. quadrimaculatus*

are found throughout the Greater Toronto Area. *An. quadrimaculatus* was observed in areas of northern Ontario, though these distributions were limited to a short period during the summer season. Areas with highest occurrence of the vector were found in southern Ontario, especially in the areas just outside of Toronto and near Ottawa (Figure 4.2). These results indicate spatial coincide of the vector with major population centres at the provincial scale.

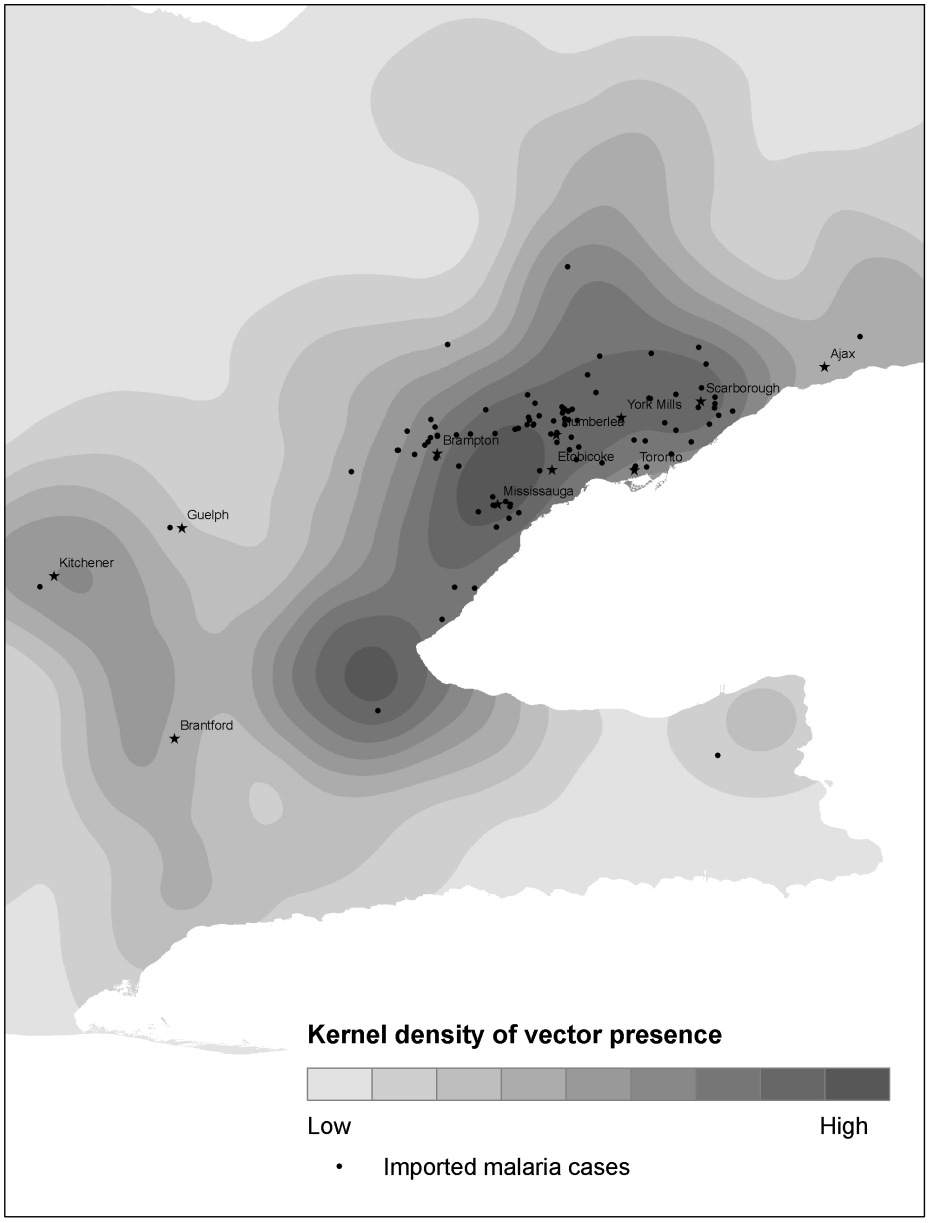

Figure 4.2: Kernel density of *An. quadrimaculatus* presence proportion, GTA.

Figure 4.3 shows the temporal intersection of the vector population and the imported malaria cases. The temporal graph of *An. quadrimaculatus* geographic presence proportion in traps extends from week 23 (June) to week 38 (September). The peak of the vector distribution appears to be between weeks 30 and 34 (July - August). The relative presence proportion of *An. quadrimaculatus* is graphed along the same time scale as the malaria cases. On the weekly timescale, no strong patterns in malaria case distribution emerge, but there are a large number of cases occurring during the summer months. It appears that malaria cases are found at times of the year when vectors are also present.

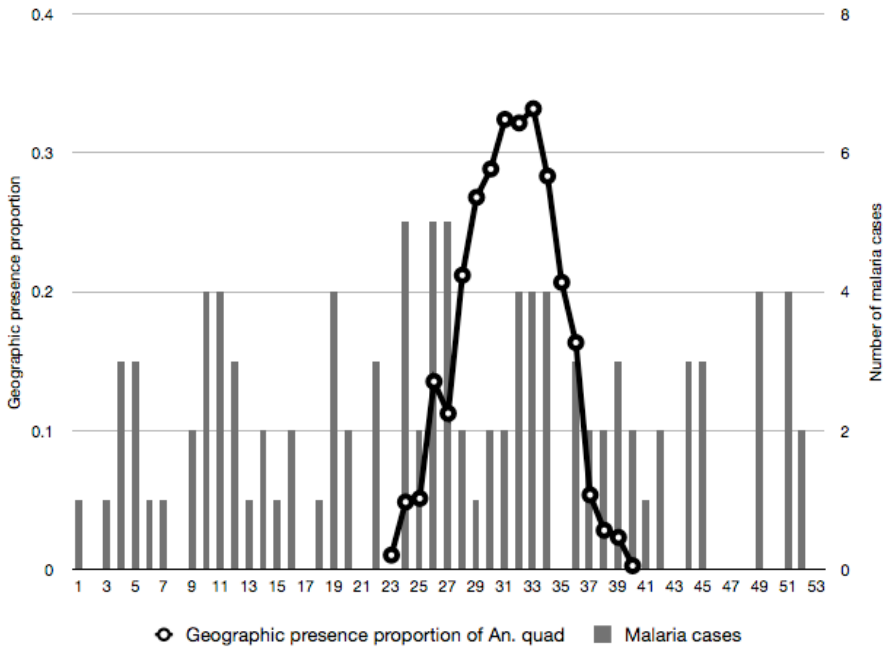

Figure 4.3: Malaria cases and geographic presence proportion of *An. quadrimaculatus*.

To assess the potential for geographic overlap between the malaria cases and the vector population, the specific time period of four consecutive weeks of *An. quadrimaculatus* presence was mapped (as well as three weeks, since these areas could potentially have four consecutive weeks if a change in temperature were to occur). Four weeks is approximately the period of time it takes the malaria parasite to replicate within the mosquito at 18-20 degree C (Berrang-Ford et al. 2009); replication will occur faster at temperatures beyond this range. Figure 4.4 shows that areas with four consecutive weeks of *An. quadrimaculatus* are present throughout southern Ontario, especially in the GTA, but not in Toronto central. This is consistent with the preference of *An. quadrimaculatus* for resting sites such as hollow trees, stumps, culverts and dams (O'Malley 1992), which are unlikely to be highly prevalent in urban centres.

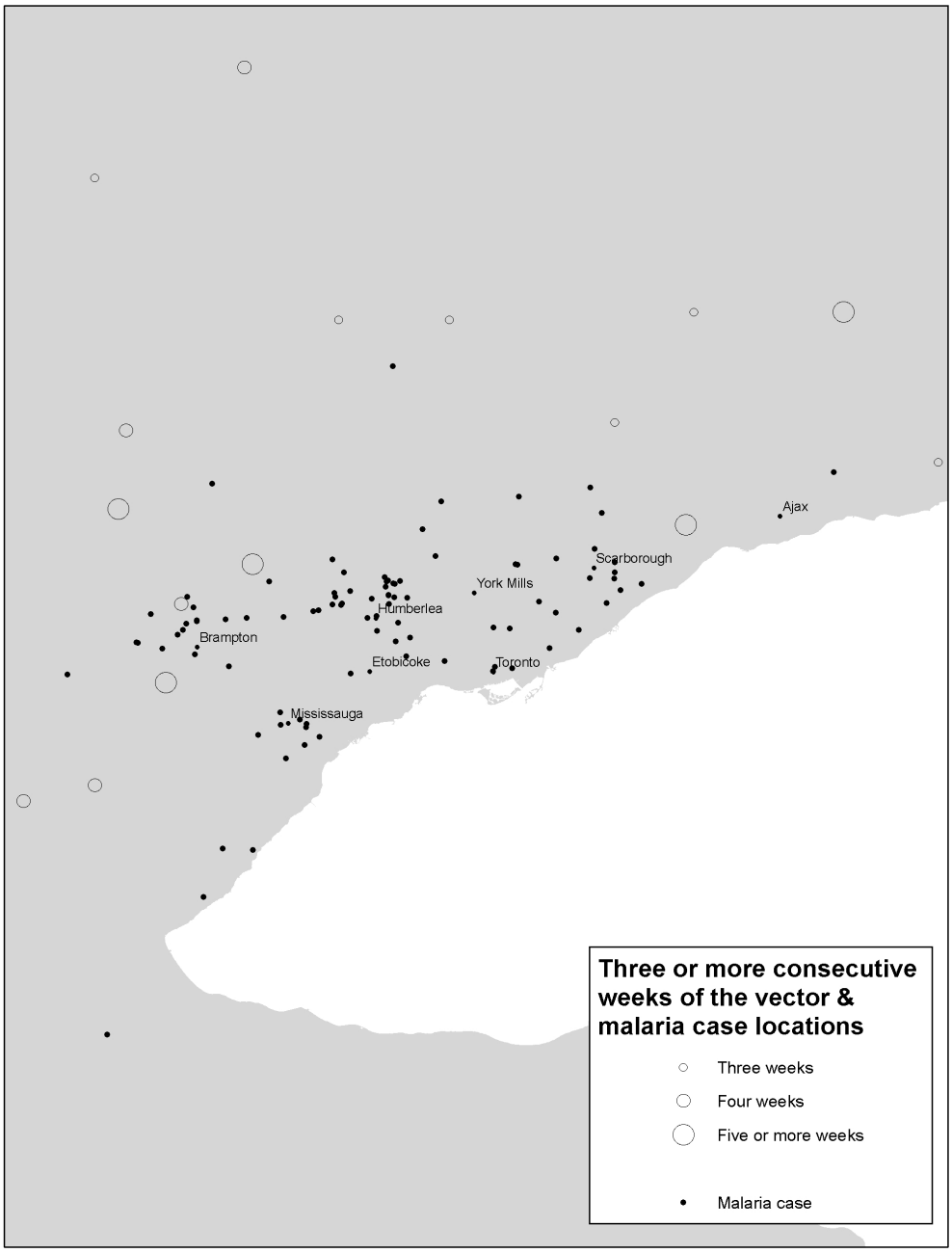

Figure 4.4: Malaria cases and consecutive weeks of *An. quadrimaculatus*. Note that vector observations with fewer than three weeks of consecutive presence are not shown.

There is negligible overlap of imported cases and extended vector distributions within the GTA (Figure 4.5). Even at the largest buffer distance (10 km), there is minimal concurrence of imported malaria cases with extended occurrence of

observed vectors. While vectors are predominantly observed in the outer suburban areas of the GTA, cases are reported within the residential core where vectors are generally absent. Areas of limited spatial coincidence include the area northwest of the GTA, near Brampton, and in the east near Scarborough.

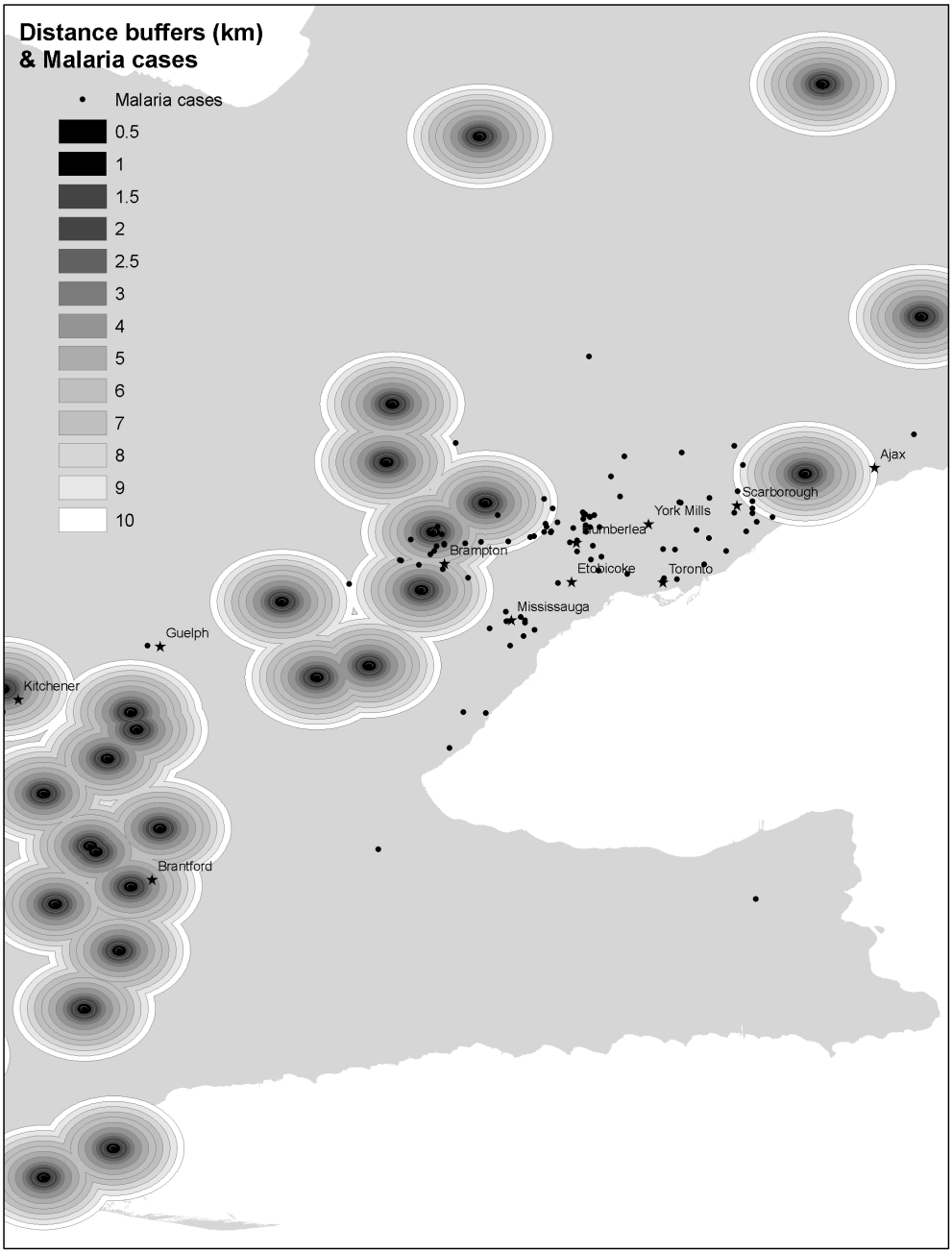

Figure 4.5: Distance buffers of four or more weeks of *An. quadrimaculatus* and malaria cases.

Using the number of malaria cases falling within the different buffer ranges, the proportion of malaria cases that occur in locations where malaria transmission is possible can be quantitatively assessed. Figure 4.6 shows the number of malaria cases falling within a certain distance of a trap with four or more weeks of *An. quadrimaculatus* presence. The data points are based on the buffer map, and a polynomial regression line was fit to the data (r-squared = 0.97). Based on the current distribution, there are approximately 2 cases (2%) within the typical fly range of *An. quadrimaculatus* (1.6 km) and approximately 10 cases (9%) within the maximum fly range (4.8 km) (O'Malley 1992). Based on the maximum biologically plausible flying range of the vector, only nine percent of reported malaria cases fall within a geographic area where autochthonous transmission would be possible.

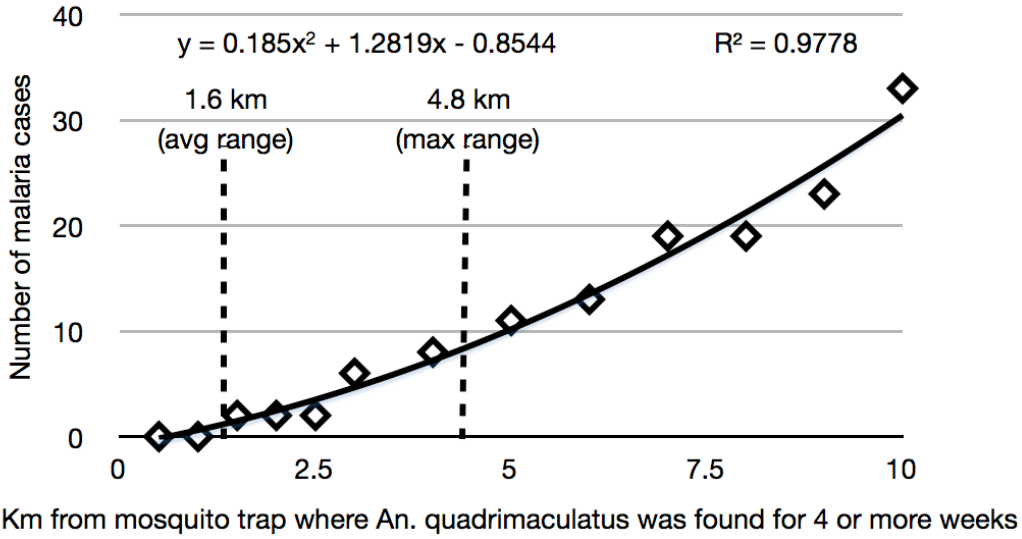

Figure 4.6: Malaria cases within a given distance of a trap with four or more weeks of *An. quadrimaculatus* presence.

4.5. Discussion:

Viewed at a regional scale – the province of Ontario − there is a significant occurrence of imported malaria cases within population centres, as well as the presence of a competent malaria vector, *An. quadrimaculatus*. However, finer scale mapping, focusing on the Greater Toronto Area (GTA), highlights the lack of significant overlap (both temporal and spatial) between the parasite and the vector. Imported malaria is generally reported in the urban areas of Toronto, while extended vector presence is predominantly reported in sub-urban areas of the GTA. These results suggest that despite the concurrence of transmission requirements in the province, there may be very limited opportunity and minimal probability of autochthonous transmission given non-concurrence within the GTA's urban environments. If there was to be autochthonous malaria transmission in Ontario, theoretically risk would be highest during the summer months when the vector is present for several consecutive weeks, and would potentially occur in the suburban areas outside of the GTA where there are imported malaria cases.

The results are subject to several potential sources of bias and error. The mosquito data were collected within the context of West Nile virus surveillance and thus the type of trap used was not chosen to be optimal for *An. quadrimaculatus* collection. *An. quadrimaculatus* was not the species of interest during collection, and sampling method may not have been ideally suited to maximum and precise observation of this species. Additionally, the malaria case data represents only 75% of the total number of malaria cases imported into Ontario, and it is unclear if there is differential bias in this sample. The cases identified through the OAHPP Malaria Reference Lab may not be representative of the cases imported into Ontario. Additionally, the use of home address data to assess case locations does not completely capture the true nature of the risk

landscape. Information related to local travel behaviours of subjects would strengthen the analysis, as exposure to vectors varies greatly depending on location (homes vs. parks or outdoor recreation areas). However, when it is not possible to obtain local travel behaviour data, home address locations are used in spatial epidemiological studies (Bastin et al. 2007).

Coincidence of risk factors is dependent on the habitat of the competent vector and whether it is likely to be compatible with the habitat environments where imported cases are most likely to be found. Additionally, temporal travel patterns may not necessarily coincide with limited periods of vector presence and duration. This chapter highlights for the first time a lack of small-scale geographical overlap between the vector and the parasite in Ontario. Mosquito surveillance confirms that *An. quadrimaculatus* is not an urban mosquito, and the majority of malaria cases are located in urban areas. These patterns likely differ significantly from historical risk in southern Canada when urbanization was more limited in extent, and the potential for spatial concurrence of imported cases and vectors may have been higher. Rapid urbanization, particularly in the GTA region, has contributed to decreased availability of vector habitat in the urban core. Imported cases are observed predominantly in urban areas, a reflection of immigrant settlement and travel patterns within the GTA.

Chapter 5: Conclusion

5.1. Key findings:

Imported malaria cases are the key driver of autochthonous malaria transmission. A better understanding of imported malaria cases is an essential component of the risk assessment of autochthonous transmission. Chapter 3 fulfilled the first objective, the characterization of the spatial and temporal profile of imported malaria cases in Ontario, Canada. Malaria cases (positive tests) were compared to controls (negative tests) to assess patterns independent of factors related to access to testing. There were many controls in the downtown core of Toronto, with more cases located in suburban areas of the Greater Toronto Area (GTA). Significant clustering was also found in a suburban area, northwest of Toronto, near Brampton. Cases were significantly more likely to occur in summer months. However, there were no significant temporal patterns at a monthly or weekly scale.

When examining the cases and controls in concert with neighbourhood-level predictor variables, significant associations were found. Cases were more likely to live in neighbourhoods with lower median household income and lower population density. Cases were also more likely to live in neighbourhoods with a higher proportion of residents who were immigrants from areas with endemic malaria transmission. The logistic regression model returned results that were consistent with the univariate findings. Population density and median household income were weak predictors, but the proportion of residents who were immigrants from endemic areas was a very strong predictor: for every one percent increase in immigration from an endemic area in a neighbourhood, the odds of being a case increase by six percent.

Significant relationships were also found between the specific parasite species for each case and individual travel history, as well as neighbourhood-level immigration statistics. The distribution of parasite species infection reflected the patterning of global malaria. *P. falciparum* cases were associated with African travel and residing in a neighbourhood with a high proportion of immigration from areas of Africa with endemic malaria transmission. Conversely, *P. vivax* cases were associated with Asian travel and residing in neighbourhoods with a high proportion of immigration from areas of Asia with endemic malaria transmission.

Building on the characterization of the imported malaria cases in Ontario, the potential for interaction with the vector population was evaluated. In Chapter 4, the imported malaria case data was compared to the vector data to assess the potential for autochthonous transmission. This chapter fulfilled the second objective, the evaluation of potential interactions between the vector population and imported malaria cases. First, the current distribution of the vector population was assessed. The current geographic range of *Anopheles quadrimaculatus* extends farther north than shown in previous maps. *An. quadrimaculatus* is found throughout the GTA, with the greatest concentration of vector observations during July and August.

When examining the presence of the vector for a duration of four weeks or longer, as is necessary for the replication of the parasite within the vector, the spatial distribution changed. Locations with four or more weeks of vector presence were only found in areas at the outer edge of the GTA. This contributes to the very limited overlap of imported malaria cases and extended vector presence within the GTA. Based on the maximum biologically plausible flying range of the vector, only nine percent of reported malaria cases fall within a geographic range where autochthonous transmission would be possible.

The aim of this thesis was to characterize and evaluate the potential for autochthonous malaria transmission in Ontario, Canada, including factors related to international travel and immigration, imported malaria and the competent vector population. Both objectives served this aim and I conclude that there is very low risk of autochthonous malaria transmission in Ontario, Canada. The low level of risk is due to the lack of spatial coincidence between the parasite and the vector. Although both the malaria parasite and vector are present in southern Ontario, this small-scale evaluation concludes that they do not currently share sufficient time and space at the individual level as would be required for transmission. However, if there were to be changes in the spatial distribution of the mosquito vector or the imported malaria cases, this conclusion would require re-evaluation. Changes in vector habitat or changes in immigrant settlement patterns could alter the risk landscape.

5.2. Emerging infectious diseases and social factors:

Infectious disease transmission is more than the simple biological transfer of a pathogen. Infectious disease risk is often patterned by social factors unrelated to biological susceptibility. The role of social factors in the potential for autochthonous malaria transmission in Ontario, Canada, is an argument in favour of greater inclusion of social factors in research related to emerging infectious diseases. The social factors that were shown to contribute to the risk of imported malaria included factors that are created at a personal level: income and immigration status. These personal attributes aggregate to create environmental variables of risk at the neighbourhood level. The analysis in Chapter 3 used this neighbourhood-level data to demonstrate how the urban landscape of social factors affects the patterning of emerging infectious diseases in cities. The profile of a city is relevant to its emerging disease risks. In the case

of Toronto, the settlement of immigrants in the suburbs and the reduction of green space have contributed to reduced risk of autochthonous malaria transmission.

5.3. Social factors and global malaria models:

The social factors that impacted imported malaria patterns in Ontario and the low level of autochthonous malaria risk in the GTA need to be included in global malaria models as well. Chapter 3 focused on the importance of social determinants of malaria risk, and the findings illustrate the role that social factors can play in patterning disease risk. Both neighbourhood-level variables related to immigration and personal variables related to travel have a significant effect on the likelihood of being a malaria case instead of a control, as well as the species of parasite among the cases. Global malaria models need to try to include social factors by integrating measures of urbanization, travel and immigration into their structure. This is especially important in malaria models that include non-endemic areas, as the likelihood of the emergence or re-emergence of malaria is significantly affected by the local patterns of the social factors that relate to malaria risk.

5.4. Global malaria models and scale:

Global malaria models also need to try to integrate small-scale patterns that can affect risk. This might not be possible in broader models, but the results of Chapter 4 show that analysis needs to be local to see true disease risk. The small-scale patterns of the imported malaria cases and the vector population had a significant effect on the autochthonous transmission risk assessment, and it was found that the risk is low. Global malaria models that don't allow for small-scale variation in risk factors will not generate results with enough nuance

to be applicable at a local level. Local level results are critical, as this is the level of actual disease transmission, and often the level at which prevention or treatment can occur.

5.5. Importance of scale in disease risk assessment:

This thesis demonstrates that the evaluation of disease risk is highly scale-dependant. Differences in disease risk emerge at different scales, and the chosen scale of an analysis or model can have a significant effect on the result. Although it is important to have an understanding of global patterns and measures of disease risk and incidence, it is also equally vital to conduct infectious disease surveillance and modelling on a small-scale that can incorporate the specific context and conditions of a locality. The risk of autochthonous malaria transmission in Ontario, Canada is highly scale-dependent. At the provincial scale, all of the necessary factors for autochthonous transmission are present. However, when examined at a local scale, there is a lack of significant geographical coincidence between the imported malaria cases and the mosquito vector population. As such, there is not significant risk of autochthonous malaria transmission in Ontario, Canada.

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