Heartworm Infection Among Domestic Dogs in Canada with a Focus on Ontario: Temporal Trend, Spatial Distribution and Risk Factors

by

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ABSTRACT

HEARTWORM INFECTION AMONG DOMESTIC DOGS IN CANADA WITH A FOCUS ON ONTARIO: TEMPORAL TREND, SPATIAL DISTRIBUTION AND RISK FACTORS

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The heartworm infection status of domestic dogs in Canada, specifically Ontario is described in this thesis. The analysis was based on annual survey data collected from veterinary clinics across Canada during 1977 to 2010, as well as serological laboratory test results collected from 2007 to 2016. A temporal trend in heartworm infection was assessed using survey and laboratory data. Furthermore, the survey data were used to assess the efficacy of preventive medication through the Attributable Fraction Exposed. The spatial extent of heartworm was visually assessed through choropleth maps. The impact of reported risk factors on heartworm infection prevalence was assessed. Descriptive analysis revealed temporal changes in prevalence and changes in spatial distribution. Further analysis of risk factors illustrated that climate and human population size have impacted canine heartworm infection prevalence in southern Ontario. This thesis contributes to the understanding of heartworm infection in domestic dogs in both Ontario and Canada.

Dedication

I wish to dedicate this thesis to my father who passed away during the writing of this thesis. My father was a teacher who impressed upon me the value of education. He taught me the importance of hard work, determination and perseverance, and to be fair and kind to others. He was always supportive in any of my endeavours and had confidence in me, even if I did not. My dad was a wonderful father and a source of inspiration. Although he is not here, I think he would be proud as I accomplish this goal and obtain my MSc.

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Statement of Work

Erin Ruth Anne McGill, under the guidance of Dr. Olaf Berke, designed all the study objectives included in this thesis. She also performed the data cleaning, management and analysis of all descriptive and analytic data, interpreted results and was principal author of all chapters.

Dr. Olaf Berke provided assistance on study objectives included in the thesis, data analysis and interpretation, as well as critical feedback and edits on all chapters.

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Introduction and Literature Review

1.1 About Heartworm

Heartworm infection is caused by a vector-borne parasite known as *Dirofilaria immitis* (Atkins, 2005). Heartworm primarily infects members of the canine species, both wild and domestic (Jara *et al.,* 2016). In Canada, veterinarians are most concerned with infections among companion dogs. Heartworm infection can become severe if left untreated with life-threatening health conditions and even death (Bowman *et al.,* 2016; Silaghi *et al.,* 2017).

There are over sixty species of mosquitoes that are capable of transmitting *D. immitis;* however, the most important vectors are mosquitoes from the genera *Aedes, Anopheles, Culex, Culiseta,* and *Coquillettidia* (Atkins, 2005; Trájer *et al.,* 2016). Warmer climates allow for the presence of a number of mosquito species (Sassnau *et al*., 2014; Trájer *et al*., 2016). Heartworm is primarily found in countries with more temperate climates (Bowman *et al.,* 2016; Nguyen *et al.,* 2016). In North America, infection with *D. immitis* is perhaps the most important helminthic infection of dogs (Bowman *et al*., 2009).

Since 2001, heartworm infection among dogs has been detected at higher levels in Mediterranean countries, which could be due to climate change or migration of both humans and their canine companions (Ciucă *et al*., 2016). Heartworm distribution in the United States is also present over a wider area than previously acknowledged from 2001 to 2007; including north, central and southern regions of California, formerly considered free of heartworm (Bowman *et al.,* 2009).

1.2 Description of the Parasite

Dirofilaria immitis is a member of the Filarioidea family (Grieve *et al*., 1983). There is quite a size difference between adult male and female worms, with male worms 2-30 cm long and 0.7-0.9 mm wide with a tapered, spiral-coiled posterior end, and female worms being 25-31 cm in length and 1.0-1.3 mm in width with an obtuse posterior end (Grieve *et al*., 1983). Mature adult worms produce unsheathed motile vermiform embryos known as microfilariae (Grieve *et al*., 1983). Different diagnostic methods detect microfilariae at different lengths; 259.9 µm by filter technique, 308.7 µm by modified Knott test, and 323,8 µm by Mines technique (Watson, 1973). Microfilariae can travel through the dog's capillary beds and circulate in the vascular system in large concentrations 10^3-10^5 microfilariae per milliliter of blood (Grieve *et al*., 1983). The concentration of microfilariae in the blood varies based on season, with a higher concentration during the summer (mosquito season) (Grieve *et al*., 1983).

1.3 Life Cycle and Transmission

The most common hosts for *D. immitis* are domestic dogs and wild canids (Graham *et al*., 2012). However, there are other species capable of being infected with *D. immitis*, such as cats and ferrets; however, felines are not the ideal host for *D. immitis* because most larvae do not develop to the adult stage (Boss & Shearer, 2011). Heartworm is also a zoonotic infection capable of being transmitted to humans by mosquitoes, although it is rare (Diaz & Risher, 2015).

The *D. immitis* lifecycle has five larval stages (L1 to L5), of which stages L1 to L3 occur while in the mosquito (Hoch & Strickland, 2008). Mosquitoes become infected with *D. immitis* when taking a blood meal from a microfilaremic host and the microfilariae (pre-L1 stage) develop from L1 to L3 in the mosquito's malpighian tubules before migrating to the body cavity and ending up in the head and mouthparts of the mosquito (Graham *et al.*, 2012). During their time in the mosquito, the larvae molt twice (L1 to L2 to L3), in as few as 8 days at temperatures of 30 ℃ to as long as a month at temperatures of 18 ℃ (Knight & Lok, 1998).

The threshold temperature for development is 14℃; without sufficient heat during the lifetime of an infected mosquito, maturation to the L3 infective stage does not occur (Slocombe *et al.,* 1989). The heat required to reach the infective stage is 130 heartworm development units (HDUs); degrees over 14°C are summed until 130 HDUs are reached. The HDU model was pioneered in Canada based on weather station data collected from 1957 to 1986 for Ontario (Slocombe *et al.,* 1989). In particular, Windsor Ontario experienced warmer temperatures first, meaning the area was likely to reach the required 130 HDUs first, and was used to determine the transmission season (Slocombe *et al.,* 1989). *Aedes stimulans* was determined as the early season vector with a date of May 6th being the earliest blood feeding; HDUs were summed from this date onwards until 130 were reached (Slocombe *et al.,* 1989). End of transmission was based on the late season vector, *Ae. vexans,* and the last day 130 HDUs could be obtained within the previous 30 days (Slocombe *et al.,* 1989). Calculation of the start of transmission season did not use a 30-day period to accumulate 130 HDUs as *Ae. stimulans* has a longer life span and can be found into the summer months (Slocombe *et al.,* 1989). The HDU model has been used to examine and predict heartworm infection among dogs in many countries, including Argentina, Germany, and the United States, confirming the usefulness of this model (Vezzani & Carbajo, 2006; Brown *et al.,* 2012; Sassnau *et al.,* 2014).

Infective third stage, or L3, larvae are transmitted to a new host when the mosquito feeds and deposits the L3 in a drop of hemolymph (mosquito blood) (Vezzani & Carbajo, 2006). The puncture wound in the skin created by the mosquito's mouthparts allows for entry of L3 larvae into the host animal (Boss & Shearer, 2011). After three days, most of the larvae can be found in the subcutaneous tissues near the site of entry into the dog (Boss & Shearer, 2011). By day 21, the larvae in the dog will have migrated to the abdominal muscle tissues, and at day 41 the larvae can be found in either the abdominal or thoracic muscle tissues (Bowman &Atkins, 2009; Boss & Shearer, 2011). L3 and stage four larvae (L4) will travel between the muscle fibers during migration, but immature adult larvae will penetrate the veins to transport themselves towards the heart and lungs (Kume & Itagaki, 1955). It is during the early migration that the L3 larvae start molting to L4; this can start as early as day 3 of infection, and ends around day 9-12 (Bowman & Atkins, 2009). L4 larvae molt to L5 (the immature adult stage) 50-70 days after infection, and as early as day 70 the worms can reach the blood vessels in the lungs; all worms reach the lungs by day 120 (Boss & Shearer, 2011).

Parasites that first appear in the pulmonary arteries are 2-3cm long; the worms increase rapidly in size and at four months post-infection can be 10cm long (Bowman & Atkins, 2009). The female worms can reach sexual maturity as early as four months post infection; microfilariae can appear in the blood as early as six months and latest by nine months (Bowman & Atkins, 2009). This entire life cycle takes place between 184 to 210 days under optimal conditions; cooler temperatures and lower relative humidity result in a longer life cycle (Atkins, 2005).

The dog's blood pressure is the force that drives the juvenile heartworms (L4) into the small pulmonary arteries (Graham *et al*., 2012). The worms progressively occupy larger arteries as the L4 increase in size and molt into the final immature adult stage (Boss & Shearer, 2011). As the worm burden in the dog increases, the worms can be found in the right ventricle of the heart (Boss & Shearer, 2011). Dogs with "heavy" worm burdens (>40 worms) are at risk of having the caval syndrome, where many of the worms relocate into the right ventricle, right atrium, and the caudal vena cava, which interferes with the valvular function of the heart and can impact blood flow (Graham *et al*., 2012).

The primary damage caused by *D. immitis* in dogs occurs in the pulmonary arteries and lungs; the degree of damage depends on worm burden, duration of infection and the reaction of the host to the parasite (Hoch & Strickland, 2008). Adult worms release toxic substances and

cause physical trauma; worms living in the caudal pulmonary vascular tree cause inflammation, villous proliferation, pulmonary hypertension and can disrupt vascular integrity (Atkins, 2005). The activation of leukocytes and platelets by adult worms may eventually lead to smooth muscle proliferation and collagen accumulation, which results in fibrosis or pulmonary thromboembolism (Bowman & Atkins, 2009). A dog's affected vessels may become thickened, dilated, and non-functional; these changes are only partially reversible (Atkins, 2005). Compromised cardiac output in dogs, in part, is caused by the vasoactive substances released by heartworms which lead to hypoxia and vasoconstriction (Quinn & Williams, 2011). Severe cases with high worm burdens develop acute or chronic pulmonary hypertension and possibly congestive heart failure (Quinn & Williams, 2011).

Exercise in dogs tends to worsen the signs of thromboembolic heartworm disease and the development of pulmonary vascular disease (Atkins, 2005). Pulmonary hypertension is typically more severe in lightly infected dogs that exercise mildly, compared with dogs with a higher worm burden that do not exercise (Bowman & Atkins, 2009). As indication, the diseased pulmonary arteries are thickened, dilated and functionally incompetent, and resist increased demand, such as during exercise. Thus, infected dogs have a diminished capacity for exercise (Bowman & Atkins, 2009).

Dogs infected with heartworms can also have pulmonary parenchyma that is damaged due to the worms; eosinophilic pneumonitis is the commonly reported parenchymal lesion caused by the immune's response to the destruction of microfilariae in the pulmonary vasculature (Quinn & Williams, 2011). Less commonly reported is pulmonary eosinophilic granulomatosis, occurring when microfilariae are trapped inside the lungs with neutrophils and eosinophils that result in the formation of a granuloma (Hoch & Strickland, 2008).

Caval syndrome is the most severe manifestation of heartworm infection in dogs, where a portion of the worm burden is in the right ventricular inflow tract (Strickland, 1998). It is a multi-systemic disorder impacting both heart and liver and is life-threatening and a very serious complication of heartworm infection (Strickland, 1998). Treatment can be complicated as there are many preoperative considerations; the prognosis can be poor, with mortality rates of up to 42% (Strickland, 1998).

1.4 Clinical Signs

Clinical signs of heartworm in dogs depend on the severity and duration of infection, and in chronic cases reflect the effects of *D. immitis* on the pulmonary arteries and lungs (Atkins, 2005). Generally, no clinical signs are observed when low parasite burdens are present; infection is only recognized upon routine screening (Bowman & Atkins, 2009).

The observed signs of heartworm disease in dogs include weight loss, reduced exercise tolerance, poor body condition, lethargy, coughing, dyspnea, syncope and abdominal distention/ascites (Atkins, 2005). Physical examination of a dog with severe infection can show evidence of weight loss, right-sided heart murmur due to right atrioventricular valve insufficiency and a cardiac gallop (Atkins, 2005). It is uncommon that heartworm infection leads to cardiac arrhythmias (Hoch & Strickland, 2008). In patients with right-sided heart failure, jugular venous distension and pulsation are typical, along with hepatosplenomegaly and ascites (Atkins, 2005; Bowman & Atkins, 2009). Pulmonary signs of heartworm infection include coughing and pulmonary crackles, muffled lung sounds, dyspnea and cyanosis (Atkins, 2005).

Clinical signs of caval syndrome include acute anorexia, dyspnea, weakness, pale mucous membranes, anemia, jugular vein distention and pulsation, renal dysfunction and disseminated intravascular coagulation (Strickland, 1998).

1.5 Diagnostic Testing

      Heartworm tests for dogs were initially directed towards the detection of antibodies against *D. immitis*. However, these tests were often cross-reactive with other nematodes and thus had a poor specificity (Klotins *et al*., 2000). More specific techniques for detecting heartworm infection in dogs, like antigen detection, were discovered in the late 1980's, and these replaced the initial antibody tests in dogs (Klotins *et al*., 2000).

      Antigen testing is the most sensitive diagnostic method available to detect heartworm infection in dogs (Henry *et al.,* 2018). Heartworm antigen tests detect a uterine protein that is produced by *D. immitis* adult females (Jones *et al.,* 2014). Microfilaria testing may be done in combination with antigen testing to determine if that life-stage is present in dogs that test positive for the antigen (Graham *et al*., 2012). The sensitivity and specificity for microfilaria testing vary depending on the technique used; for the modified Knott's technique sensitivity was between 44.3% to 81.8% and specificity was 100% (Klotins *et al.,* 2000). Microfilariae are not always found circulating in the blood and so it is not recommended to perform microfilariae testing alone (Graham *et al*., 2012). The modified Knott's test remains the favoured method for examining body dimensions and morphology of *D. immitis* microfilariae and is one of the most sensitive diagnostic methods for microfilariae (Graham *et al*., 2012).

      Heartworm antigen tests use enzyme-linked immunosorbent assays (ELISA) and immunochromatographic test systems to detect the circulating antigen (Grieve *et al.,* 1983). These tests are nearly 100% specific but sensitivity can be impacted in cases with low worm burdens (Jones *et al*., 2014). An adult female worm burden or one to two worms decreased the antigen test sensitivity to 46% to 77% but did not affect test specificity (Courtney & Zeng, 2001). The current standard of antigen heartworm tests is to detect infections of at least one mature female worm (not circulating microfilariae) (Graham *et al.,* 2012). Heartworm infections with only male worms or less than five months old are usually not detected (Klotins *et al.,* 2000). The most commonly used heartworm test in Canada is the SNAP 4Dx Plus Test with a stated 99.0% sensitivity and 99.3% specificity (IDEXX, 2016; Herrin *et al.,* 2017). The heartworm component of the SNAP 4Dx Plus Test is an antigen detection system that uses ELISA methods to identify heartworm antigen in dogs (Herrin *et al.,* 2017). It is also used to detect antibody to other parasitic infections including *Borrelia burgdorferi* (Herrin *et al.,* 2017). Henry *et al.* (2018) assessed five different antigen diagnostic tests to determine their sensitivity and specificity in

detecting heartworm for a population of 250 dogs; the study found the SNAP 4Dx Plus Test had a 97.5% and 94.0% sensitivity and specificity, respectively.

Positive antigen test results in dogs lacking clinical signs should be confirmed before proceeding with adulticide therapy (Jones *et al*., 2014). Confirmation should initially be carried out using a second blood sample and a different antigen diagnostic test kit, then using tests for microfilariae (Jones *et al.,* 2014). Microfilaremia validates the serologic results, as well as identifies the patient as a functional reservoir of infection (Jones *et al*., 2014). It also alerts clinicians to the possibility of a severe reaction following the use of some heartworm preventives (Jones *et al.,* 2014).

Annual heartworm testing is recommended for dogs in endemic regions to ensure that prophylaxis is attained, and maintained, and because infections are often subclinical (Jones *et al*., 2014). Heartworm has a relatively long prepatent period, and as such, the earliest that heartworm antigen and microfilariae can be detected is about five to six months post infection (Graham *et al*., 2012). In countries that experience diverse seasons, such as Canada, the practice is to test dogs in the spring to check for exposure from the previous summer (Bowman & Atkins, 2009).

1.6 Heartworm Prevention

Historically, prophylaxis for heartworm infection in dogs is highly effective, nearly 100% effective when good compliance is used (Rohrbach & Patton, 2013). Preventive medication failure can result from lack of compliance with administration, or resistance of L3-L4 stage *D. immitis* to macrocyclic lactones (Rohrbach & Patton, 2013).

There are varying opinions regarding recommendations for prophylactic treatment. The American Heartworm Society recommends year-round prevention, and in regions with strong seasonality differences, the recommendation is heartworm prevention during the predicted transmission season (Bowman & Atkins, 2009). As indicated earlier, in Ontario, Canada the transmission period has been stated to run from June 1st to October 9th each year, based on weather data collected from 1957-1986 from Windsor, Ontario (Slocombe *et al.,* 1989).

The options that exist for chemoprophylaxis in dogs in North America are drugs that are administered either monthly as an oral tablet or as a topical liquid, or parenterally every six months (Jones *et al*., 2014). Puppies may be started on preventive medication as early as eight weeks old; however, it is recommended to test them six months later for possible exposure prior to being placed on a preventive (Jones *et al*., 2014). If a dog is seven months of age or older, a heartworm antigen and microfilaria test should be performed before placing them on prophylaxis (Jones *et al*., 2014).

Heartworm preventive drugs for dogs belong to the macrocyclic lactone (ML) drug class, and are ivermectin, milbemycin oxime, moxidectin and selamectin (Bowman & Mannella, 2011). These are considered very safe drugs that possess activity against L3 and L4 larvae and in some cases young adult heartworms (Boss & Shearer, 2011). A lapse in compliance of prevention of more than four weeks during the transmission season will increase a dog's chance of infection (Boss & Shearer, 2011).

Ivermectin is an oral ML preventative, administered at a 30-day dosing interval (Graham *et al*., 2012). The drug has a reach-back period of one month, where in situations of compliance failure, it prevents or reduces the development of L4 and L5 stages (McCall, 2005). It has also been noted that ivermectin can kill immature adult heartworms; although multiple doses are required for worms older than two months (McCall, 2005).

Milbemycin is also an oral ML class preventive, which is administered at a 30-day dosing interval (McCall, 2005). Milbemycin kills L3 and L4 heartworm larvae within the first six

weeks of infection, and like ivermectin, has a reach-back effect of one month (Hoch & Strickland, 2008). It is also approved to prevent hookworm, roundworm and certain whipworm infections (Hoch & Strickland, 2008).

Moxidectin is primarily used as a monthly topically applied liquid that is also approved to treat infections with multiple other internal, and external parasites including fleas and sarcoptic mange (Hoch & Strickland, 2008). It is also available as a slow-release product for dogs as a subcutaneous injection with a duration of six months (McCall, 2005).

Selamectin is a ML that is applied topically once a month (Atkins, 2005). Given at the preventive dose it is also capable of killing fleas, flea eggs, sarcoptic mange mites, ticks (*Rhipicephalus sanguineus* and *Dermacentor variabilis*) and ear mites (Hoch & Strickland, 2008). Selamectin gradually reduces the level of circulating microfilariae when administered as a preventive on a monthly basis, and also has adulticide efficacy, though not as effectively as ivermectin (Atkins, 2005; Hoch & Strickland, 2008).

When originally approved, ML heartworm preventives had a reported 100% efficacy. However, since 1998, there have been reports of lack of efficacy of chemoprophylactics for heartworm (Hampshire, 2005; Atkins *et al.,* 2014; Bourguinat *et al.,* 2017). According to the Center for Veterinary Medicine of the Food and Drug Administration (FDA), lack of efficacy (LOE) is testing heartworm positive regardless of appropriate dosage or administration consistency (Graham *et al*., 2012). Thus, the majority of LOE claims have subsequently been found to be due to compliance issues (i.e. not administering sufficient preventive or failing to administer the preventive when it was due) (Jones *et al*., 2014). It should also be recognized that dogs that vomit or who do not swallow a full dosage can still be at risk of infection (Boss & Shearer, 2011).

Moraes-da-Silva *et al.* (2016) studied the efficacy of selamectin in high-risk areas in Brazil (~31% heartworm infection prevalence) and selamectin treatment was found effective, when administered properly, against *D. immitis* for all 24 dogs during the 36-month period. In another high-risk area in Brazil, efficacy of 73.3% was reported, which compared to the 100% efficacy observed among dogs with correctly administered preventives in the same area, suggesting that compliance with use of a preventive is important for achieving optimal efficacy (Moraes-da-Silva *et al.,* 2016).

In a 2009 survey of veterinarians from Louisiana, 74% of those who responded claimed that they had seen cases of resistance to MLs when used as a preventive for heartworm in their clients' dogs (Wolstenholme *et al.,* 2015). Furthermore, 49% had dealt with cases in dogs where the microfilaremia persisted despite the use of an adulticide and ML treatment (Wolstenholme *et al*., 2015). Pulaski *et al.* (2014) illustrated through an *in vivo* study that it was possible for a second generation of *D. immitis* to develop within a group of ML-treated dogs which confirmed heritability of resistance despite a monthly dose of ivermectin given for three months. These studies illustrate that the historical efficacy of 100% may not be the case for certain *D. immitis* strains.

1.7 Risk Factors for Heartworm Infection

Canine population density can impact heartworm infection risk, as a high reservoir density may allow the parasite to be easily transmitted as mosquitoes do not have to travel far to take a blood meal. Urban coyotes and feral dogs may have a higher prevalence of heartworm due to increased proximity to a large reservoir of possible hosts; in Illinois, urban coyotes had ten-times the heartworm infection prevalence as rural coyotes (Aher *et al.,* 2016). In addition to density, movement of microfilaremic reservoirs pose a risk for the spread of infections; as dogs travel (for holidays or relocation to a new area) there is the risk of spread into new areas (Morchón *et al.*, 2012).

Host factors play a role in the risk of infection with *D. immitis*, as male dogs typically exhibit a higher prevalence than females (Vezzani *et al.*, 2011). However, there was not found to be a significant difference in infection prevalence between pure-bred dogs and mixed breeds, although pure-breeds with shorter fur had a higher prevalence (2.8% higher; $p<0.001$) than those with longer fur (Vezzani *et al.*, 2011). The longer fur could provide a mosquito repelling effect, as it is harder for them to reach the skin to take a blood meal. Larger dogs were also at greater risk of infection than small dogs (Vezzani *et al.*, 2011).

Environmental predictors for heartworm infection (i.e. risk factors and preventive factors as well as their indicators) include the presence or absence of mosquito species capable of transmission, warm and humid weather, precipitation, human density, prophylaxis compliance, distance to mosquito habitats, land use patterns and irrigation systems (Aher *et al.*, 2016).

Mosquitoes breed in water, and different types of water bodies are preferable to different mosquitoes for breeding (Trájer *et al.*, 2016). Urbanization and the distance to mosquito habitats are risk factors for transmission of heartworm (Trájer *et al.*, 2016). Major urban regions can support a diverse range of mosquito species and many of the largest and fastest growing cities are built close to water estuaries (mosquito habitats) (Crocker *et al.*, 2017). For example, the Sydney Olympic Park area has a large population of 50,000 persons, plus around 25,000 visitors per day and even if they do not use the recreation areas close to the swamps, they are within range of mosquito species, placing their pets at transmission risk (Crocker *et al.,* 2017). In Hungary, 90% of canine heartworm cases occurred within 524m of mosquito breeding sites (Trájer *et al.,* 2016).

Large cities may harbour "heat islands", creating more biologically favourable breeding conditions for mosquitoes than surrounding rural areas (Herrin *et al*., 2017). The "heat islands" are caused by buildings retaining heat from the sun during the day and radiating it at night - leading to microclimates that are suitable for the development of larvae in the mosquito and supporting vector populations during cooler months (Morchón *et al*., 2012). In cooler countries, such as Denmark, monthly or daily average temperatures can underestimate the potential for transmission of vector-borne infections as vectors do not experience a "mean temperature" but, instead, experience temperature fluctuations throughout the day (Haider *et al*., 2017). Thus, when modelling microclimatic temperatures, pathogens develop at a faster rate and there is a longer transmission season of vector-borne infections in such areas (Haider *et al*., 2017). This could lead to an extension of the heartworm transmission season in urban areas. Furthermore, construction such as housing developments or increased excavation activity, alter drainage of undeveloped land, providing pools of water at building sites that can serve as mosquito breeding grounds (Morchón *et al.,* 2012).

Agriculture specific to different countries has been linked with heartworm infection risk (Alho *et al.,* 2014). Rice fields serve as breeding locations for mosquitoes and lead to higher larval survival in rice fields in Portugal (Alho *et al*., 2014). Greece has wetlands and rice fields in the northern and western regions of the country; a map of the wetlands was similar in its geospatial distribution to a map of locations of *D. immitis* infections among dogs (Diakou *et al.,* 2016). Irrigated lands for farming provide a habitat for mosquitoes, contributing to the higher prevalence of heartworm in areas close to farmlands (Morchón *et al*., 2012).

Human socio-economic status (SES) is also associated with prevalence levels of *D. immitis* in domestic dogs (Wang *et al*., 2014). For example, United States households with higher median incomes were associated with reduced heartworm prevalence risk (Wang *et al.,* 2014). Pet owners with higher incomes can afford preventive medication for their dogs, so their dogs are protected.

Natural disasters, such as hurricanes and flooding, can impact heartworm infection risk (Levy *et al*., 2011). There may be an increased risk of exposure to heartworm infection, as standing water allows for mosquito breeding (Levy *et al*., 2011). After Hurricane Katrina in 2005, an estimated 50,000 cats and dogs were left behind by their owners and many shelters took in large populations of pets (Levy *et al.,* 2011). The heartworm infection status of the pets were

not known at the time of admission, and this could have led to transmission among the shelter population; heartworm prevalence in the Gulf Coast region is 45% to 50% (Levy *et al.,* 2011). Re-locating dogs from endemic natural disaster areas can contribute to the spread of heartworm infection.

1.8 Climate Change and Heartworm

Transmission of infection with *D. immitis* by mosquitoes is dependent upon a suitable climate that can support breeding of mosquitoes and development of parasite larvae in the vector (Genchi *et al*., 2011).

Both the mosquito lifecycle and the development of *D. immitis* within the mosquito are impacted by temperature and humidity (Simón *et al*., 2014). Climate change in certain regions may be supportive to mosquito habitats if the change leads to wet humid areas that promote breeding. However, some mosquito species can thrive in dry temperate countries with limited rainfall; thus, climate change that creates a dry, warm climate may still support the mosquito lifecycle (Simón *et al.,* 2014). Mosquitoes may use irrigated crop fields to breed if they are inhabiting a dry region (Simón *et al.,* 2014).

Climate change is causing a shift in average temperatures in Canada and different European countries, including Germany, which could lead to more suitable environments for the development of *D. immitis* (Ford *et al.,* 2011; Sassnau *et al.,* 2014). Increased temperatures allow for an increased maturation rate of larvae in the mosquito population, which would result in a shorter period before L3 are capable of transmission (Sassnau *et al.,* 2014). Temperatures of 30ºC allow *D. immitis* microfilariae to mature to L3 in only 8 to 9 days in the mosquito species *Aedes vexans, Ae. triseriatus* and *Anopheles quadrimaculatus* (Sassnau *et al.,* 2014).

An effect of climate change that is readily seen is increased populations of mosquitoes that occur after prolonged periods of rainfall (Ledesma & Harrington, 2011). Climate change has also allowed for the introduction and spread of vector competent invasive species, like *Aedes albopictus*, which has been implicated in the changing patterns of distribution of heartworm (Ledesma & Harrington, 2011). *Aedes albopictus* was native to southeast Asia and the western Pacific but has spread to Europe, Africa and North America in the past few decades (Morchón *et al*., 2012). Transport of used tires and gardening products has allowed for the emigration of this mosquito from endemic regions to new regions (Morchón *et al.,* 2012). The mosquito species *Ae. albopictus* is an invasive species because of its ability to adapt to various climates, as it can over-winter in an egg-state (Morchón *et al*., 2012).

1.9 Spatial Analysis

The motivation behind using spatial analysis for heartworm is that occurrence of infection varies from region to region as an effect of varying dog population density, prophylaxis use, mosquito vector abundance and climatic conditions (Brown *et al.,* 2012).

Spatial analysis is important to epidemiology as it provides insight into the geographic location and spatial patterns of a disease or infection. With climate change and the ability of people and animals to move around the world so quickly, understanding of spatial distribution and potential trends has become more and more important (Dowell *et al.,* 2016). Therefore Dowell *et al.* (2016) proposed to apply precision public health, whereby identification of small areas of highest risk or hot spots of disease under investigation are identified to allocate resources spatially and not waste efforts in unaffected regions. It is important to identify high-risk regions for heartworm infection and target these areas with prevention and control measures.

Spatial epidemiology is the branch of epidemiology that is concerned with the spatial patterns in disease occurrence including trend, cluster and clustering. A trend can be the directionality of disease occurrence (e.g. north/south) or disease occurrence increasing or decreasing in a given area. A cluster is a sub-population with a higher (or lower) intensity of disease occurrences. Clustering is the pattern of spatial dependence and a characteristic of the disease.

Trends are identified through regression modelling but can also be visible from a disease map. The most common form of mapping for regional data is choropleth mapping, where each region is coloured based on the values for that region (Berke, 2004). The problem with these maps is that the prevalence estimates are not comparable across a map due to differences in sample sizes, which can impact the precision of regional prevalence estimates (Berke, 2004). One solution is to generate a map based on empirical Bayesian smoothed estimates (Berke, 2004). Choropleth maps imply that prevalence is consistent across a region and changes at the border, which is unrealistic. Another problem is that of visual bias, where large areas dominate the map. These later two problems can be mitigated by isopleth mapping, e.g. via geostatistical kriging of smoothed regional prevalence estimates (Berke, 2004).

The pattern of clustering can be detected using Moran's I or variogram, of which the latter also provides a measure for the distance up to which spatial dependence lasts within the

sample data (Berke, 1999; Waller & Gotway, 2004). Both Moran's I and variograms can be applied to empirical Bayesian smoothed data (Waller & Gotway, 2004).

Cluster detection will result in the identification of high-risk areas or hot spots (within the study area). The flexible spatial scan test can be used to scan for regions that have higher or lower expected prevalence (Tango & Takahashi, 2012). The circular scan test uses a circular shaped scanning window, which has a limitation that not all disease clusters are circular in shape (Kulldorf & Nagarwalla, 1995). The scan test is based on a "likelihood ratio test" and thus the optimal statistical method when the assumption of a circular cluster holds (Tango & Takahashi, 2012). The flexible scan test in space does not rely on a circular cluster assumption in order to more accurately capture any disease cluster; however, the flexible scan test is based on the assumption of Poisson distributed data (Tango & Takahashi, 2012). The circular scan test can be applied to quantitative data such as residuals from a regression model to check for a potential residual cluster (Kulldorf & Nagarwalla, 1995). It should be noted that clusters are detected in relation to the surrounding prevalence. A heartworm infection prevalence cluster identified in Canada would not present the same as a cluster detected in the southern United States, where generally a higher prevalence of heartworm in dogs is observed (Brown *et al.,* 2012).

Spatial Poisson regression modelling can be used to examine spatial disease patterns, that is ecological relations between a disease agent, its host and the environment. Such models can adjust for clustering and overdispersion effects (Dean *et al.,* 2004). If there is no clustering present it is sufficient to fit an ordinary regression model, i.e. without spatially correlated random effects.

1.10 Objectives of this Thesis

Climate change has been implicated in the emergence of heartworm in Europe, as well as in the United States (Genchi *et al.,* 2011). As mentioned, climate change can lead to a longer transmission period and extend vector habitats (Greer *et al.,* 2008; Ford *et al.,* 2011; McPherson *et al.,* 2017). It is therefore possible that Canada has also seen changes in the distribution of prevalence of heartworm infection.

Previous research on heartworm infection in domestic dogs was based on annual surveys to veterinary clinics in each Canadian province including questions on when dogs were tested, how many were tested, the number of diagnosed cases, chronic cases, preventive medication usage, and other animals (i.e. cats) with heartworm that year (Slocombe, 2011). An investigation into heartworm prevalence has not been conducted in Canada since the annual surveys stopped in 2010, and evaluation of the putative emergence of heartworm, thought to be associated with climate change has not been carried out.

Ontario has previously been cited as an endemic area for heartworm infection in Canada (Klotins *et al.,* 2000); however, the prevalence may have changed since the last survey in 2010. It is possible that climate change may allow some areas in Ontario to be more supportive of the mosquito lifecycle which could contribute to the development of infection clusters.

The efficacy of heartworm preventive medication is of concern as loss of drug efficacy has been reported in the United States (Hampshire, 2005). Use of prophylaxis was reported in the aforementioned Canadian surveys, including the number of dogs who became infected but had been reported on preventive medication (Slocombe, 2011). Both prophylaxis and diagnostic statuses for the dogs were available for the years 1996 to 2010, which could be used to estimate the efficacy of historical prophylaxis in Canada, and if there was a change in efficacy.

Historic heartworm surveys did not investigate the effect of known risk factors from the literature on the Canadian dog population; risk factors assessed are often for countries of a temperate climate, such as the southern US, or Mediterranean countries (Genchi *et al.,* 2011; Brown *et al.,* 2012). It is not known whether these risk factors are applicable to the dog population in Canada. The province of Ontario conducts ~75% of heartworm testing in Canada; therefore, risk factors such as human population density and climate could be examined in a province that experiences strong seasonality and has a large sample size, such as Ontario, to see how their influence differs from warmer regions. Understanding how cited risk factors may be

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associated with heartworm infection in Canada could provide information on areas with higher risk.

The objectives of this thesis were therefore to (1) to determine if there is a temporal trend in heartworm infection in Canada, nationally and provincially, and estimate the efficacy of prophylaxis over time (2) visualize the spatial extent of heartworm infection in Canada with a focus on Ontario, and (3) assess the effect of known risk factors on the heartworm infection prevalence in southern Ontario.

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Chapter 2

Heartworm among domestic dogs in Canada, 1977-2016: prevalence, time trend and efficacy of prophylaxis

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2.1 Abstract

Dirofilaria immitis is a mosquito-borne parasite that primarily infects domestic and wild canids. The objectives of this study were to (1) determine if there has been a temporal change in heartworm infection prevalence among domestic dogs in Canada from 1977 to 2016, (2) explore the spatial extension of heartworm across Canada using choropleth maps, and (3) assess the efficacy of preventive drugs using the estimated Attributable Fraction Exposed. Heartworm surveys that collected data from 1977- 2010 and serological laboratory data from 2007-2016 were analyzed. The data depicted a decrease in heartworm prevalence, both nationally and provincially, from 1977 to the early 2000's. However, an increase in prevalence was identified for tested dog populations in Manitoba and Quebec from 2007 to 2016. Over the period evaluated, the burden of identified heartworm infection has shifted among provinces; Ontario was home to 80% of cases in 2008, but in 2015 only 60% of cases were diagnosed in Ontario. Chemoprophylaxis was associated with an estimated 94.7% (95% CI: 94.55, 94.92) reduction of heartworm infections in domestic dogs in Canada from 1977 to 2010.

2.2 Introduction

Dirofilaria immitis, also known as heartworm, is a mosquito-borne parasite that primarily infects domestic and wild canids (Atkins, 2005). Heartworm infection can be acute or chronic, and the disease can be fatal (Graham *et al.,* 2012). Heartworm can also be transmitted to humans by mosquitoes, but human infections are considered rare (Diaz & Risher, 2015).

Mosquitoes are the only known intermediate host of *D. immitis.* The rate of parasite development from microfilariae to the infective third-stage larvae is influenced by temperature. For the parasite to be transmitted from a mosquito to a dog there is a threshold temperature of 14°C that is required to support larval maturation to the infective stage and a linear relationship exists between the rate of development and temperature from 18 to 34°C (Slocombe *et al.,* 1989). The amount of heat required for microfilariae to reach that stage is 130 heartworm development units (HDUs) and is calculated using the daily sum of degrees Celsius above the threshold (Slocombe *et al.*, 1989; Sassnau *et al.,* 2014). In Canada, the transmission period for heartworm has historically been determined by summing daily HDUs from May 6th (date of earliest blood feeding for *Aedes stimulans* – an early season vector) until 130 HDUs are reached; this is regarded as the start of transmission season for that year (Slocombe *et al.*, 1989). The end of the transmission period was determined by looking at the last day 130 HDUs were accumulated within a 30-day period based on the late season vector *Aedes vexans* (Slocombe *et al.*, 1989). The start of the transmission season was not limited to 30 days because the early vector has a long life-span and can be found in August (Slocombe *et al.,* 1989). An assumption with these calculations was that mosquitoes do not overwinter (Slocombe *et al.,* 1989). Based on the most southern region of Ontario, for the years 1957 to 1986, the transmission period for Canada was estimated to be from June 1st to October 9th (Slocombe *et al.*, 1989). As a result, monthly heartworm preventive medication is suggested from June 1st to November 1st although the treatment on June 1st is likely not needed as preventives are approved with one month's reach back activity (Graham *et al.*, 2012).

Heartworm infection was first considered to be endemic to Canada in the 1970's and since then annual heartworm testing has been conducted at many veterinary practices (Klotins *et al.,* 2000). Historically, the risk in Canada was considered highest in southern Ontario, with two other foci in southern Manitoba and southern Quebec (Klotins *et al.,* 2000). For many years mail-back surveys were sent to veterinary clinics across Canada requesting information on the

heartworm infection status of dogs, cats and "other" animals. These annual surveys were conducted from 1977 to 2010 and collected data from 1977-89, 1991, 1996-98, 2000-02 and 2010. The surveys reported an aggregated prevalence of 3.7% for all dogs infected with heartworm and assessed by a veterinarian with a prevalence range from 2.4% in 1977 to 0.15% in 2010. However, heartworm prevalence among dogs not on preventive medication was 0.7% in 2010 (Klotins *et al.*, 2000; PubMed, 2017).

In Canada, heartworm is considered a rare infection compared to some other countries. For example, randomly selected companion dogs in Grenada displayed a prevalence of 25.3% (Lohman *et al.,* 2015).

As indicated, southern Ontario has historically been one of the foci of heartworm infection in Canada; however, the risk of infection across Ontario has been debated as heartworm maturation within mosquitoes requires sustained warm temperatures that are uncommon in more northern regions of the country (Fortin & Slocombe, 1981). The last nationwide mail-back survey in Canada was conducted in 2010. Anecdotally, heartworm risk may have changed in Canada since 2010 due to a changing climate, which is expected to increase prevalence and extend endemic areas (Bowman *et al.,* 2016). For example, climate change has been associated with the emergence of the parasite in Italy and parts of the United States (Genchi *et al.*, 2011; Diaz & Risher, 2015). If climate change is implied in the emergence of heartworm infections in these countries, the same could hold true for Canada. For example, Ontario has seen an increase in the number and extent of heatwaves (summers 2010-2012), i.e. ideal weather conditions for the development of heartworm in mosquitoes (Bishop-Williams *et al.,* 2016).

The goal of this study was therefore to estimate the prevalence of heartworm infection, and to study its geographic and temporal distribution in Canada from 1977 to 2016. The three objectives were to (1) determine if there has been a temporal trend in heartworm infection prevalence among domestic dogs in Canada, (2) explore the spatial extension of heartworm infection across Canada using choropleth maps, and (3) assess the efficacy of preventive drugs using the estimated Attributable Fraction Exposed (AFe).

2.3 Materials and methods

Data on the prevalence of heartworm infection in Canadian companion dogs were obtained from two sources. The first data set was retrieved from the Ontario Veterinary College (OVC) archives. It consisted of survey data collected for the years 1996, 1997, 1998, 2000, 2001, 2002 and 2010 (OVC, 2016). Additional, similar data from the years 1977 to 1989 and from 1991 were extracted from surveys conducted earlier (PubMed, 2017). The surveys described the number of cases per province (or grouped provinces by location, e.g. Atlantic provinces), the number of dogs tested, number of dogs on preventive medication diagnosed with heartworm, and the number of dogs on preventive medication. Surveys were sent to provincial, federal, industrial, institutional veterinarians (e.g. veterinary colleges) as well as mixed and small animal practitioners (OVC, 2016; PubMed, 2017). The surveys collected annual data, except for the 2010 survey which collected responses from 2010 and early 2011 (OVC, 2016). The definition of heartworm was determined by veterinarians participating in the surveys; the majority (~85%) used blood tests to determine heartworm infection status (OVC, 2016; PubMed, 2017). Data for Quebec were not available for the years 1996-98 and 2000-02. For privacy reasons, only the location of the veterinary clinics was provided. Information on the patient or the owner, including confirmatory diagnostics and clinical outcome for the patient were not available. The surveys included information on the travel history of only case dogs.

The second dataset analyzed was provided by IDEXX Laboratories Canada. The data were collected from 2007 to 2016 and were a combination of *D. immitis* antigen test results performed in clinics and results from submissions to the IDEXX Laboratory. The years 2007 and 2016 were partial years; 2007 had submissions from March to December and 2016 had submissions from January to July. There were three heartworm blood antigen tests used: the SNAP 3Dx, SNAP 4Dx and SNAP 4Dx Plus tests. The SNAP 4Dx test was released in 2007 and used concurrently with the 3Dx test for the years 2007 to 2011. The SNAP 4Dx Plus test was released in 2012 and that year all three tests were used. From 2013 onward only the 4Dx Plus Test was used. Dog signalment, test date and postal code of the veterinary clinic were provided. Information on the patient or the owner, including travel history, confirmatory diagnostics, preventative usage history and clinical outcome for the patient were not available.

The survey data were used to estimate the canine heartworm prevalence for Canada and separately by province, when/where possible. The denominator for Canada was all dogs tested

that year, which was available for all years included. Provincial estimates of prevalence depended on whether the published survey results that year included a breakdown of the reported positive dogs and number of dogs tested for that province. The laboratory data were used to estimate the prevalence of heartworm infection in Canada, and by individual province. To investigate the presence of a temporal trend in heartworm prevalence, the Cochran-Armitage trend test for proportions was applied (Lachin, 2011). The early surveys for 1977-1980 only reported data at a national level and were used to extend the national trend test from 1977 to 2010. In contrast, provincial data were only available from 1981-2010; provincial trend tests were examined for those years.

The reported sensitivity and specificity of the 4Dx Plus Test are 99.0% and 99.3%, respectively (IDEXX, 2016). The true prevalence for the study population was estimated using the Rogan and Gladen estimator based on the apparent laboratory prevalence and the sensitivity and specificity of the antigen test (Rogam & Gladen, 1978; Noordhuizen *et al.,* 2001). The predictive value for dogs testing positive was also estimated (Noordhuizen *et al.,* 2001). The prevalence referred to in this paper is the estimated apparent prevalence of heartworm infection and will hereafter be referred to as prevalence. It should be recognized that the apparent prevalence may differ from the true prevalence due to false negatives or false positives.

To visualize national temporal trends (as indicated by the trend test), sample size weighted trend lines based on smoothing splines were added to respective scatterplots for the survey data and the laboratory data separately. Provincial trend lines for Manitoba, Ontario and Quebec were added to the scatterplots.

To visually display the provincial prevalence estimates for both survey and laboratory data, a boundary map file was constructed that outlined the country and provincial borders. The boundary file for Canada and the province boundaries were retrieved from Statistics Canada for the 2011 census (Statistics Canada, 2018). The boundary file projection was converted from GCS North America 1983 to Universal Transverse Mercator 15N for better visualization of the provinces' landmass. The denominator was not consistent across provinces; thus, Empirical Bayesian smoothing was used to internally standardize the differences in sample size across provinces (Berke 2001; Berke, 2004; Beyer *et al.*, 2012). The annual prevalence was smoothed for each province and the smoothed prevalence estimates were aggregated over time using the mean to create a choropleth map for each dataset.

Geographic locations of veterinary clinics with laboratory submissions were extracted as centroids using Canadian postal code area information (DMTI Spatial Inc, 2007-14). The clinic locations were overlaid as points on top of the choropleth map of the smoothed prevalence. Point sizes were weighted by respective sample sizes (i.e. the number of reported tests).

The estimated AFe or Attributable Risk was estimated from the survey data to examine the efficacy of prophylaxis in preventing heartworm infection (Dohoo *et al.*, 2003). Exposure for heartworm infection constitutes mosquitoes and their ability to transmit the infective third-stage larvae to a dog. Heartworm preventive medication would create a 'lack of exposure' by killing the third-stage larvae transmitted to the dog by the mosquito (Graham *et al.,* 2012). The proportion of infections prevented by preventive medication was examined through the estimated AFe, where the exposed population were dogs not taking preventive medication and the unexposed population were dogs taking preventive medication. Thus, the equation used to calculate the AFe would be $AFe=[p(D+|E+)-p(D+|E-)]/p(D+|E+)$ (Doohoo *et al.,* 2003, equation 6.7). The assumption for this study is that all parasites are drug susceptible. The national annual efficacy was estimated by averaging the AFe for each province and year.

All data analyses were conducted in R and RStudio using a significance level of $\alpha=0.05$ (RStudio, 2016; R Core Team, 2017). The choropleth maps were created in ArcGIS (Esri, 2011).

2.4 Results

The heartworm prevalence for dogs in Canada in 1977 was estimated to be 2.4% (95% CI: 2.2, 2.6) and for 2010 (the last survey year) estimated to be 0.15% (95% CI: 0.14, 0.17). There was no information on the number of dogs on preventive medication in 1977 but 16% of the veterinarians who had participated in the 1977 survey said they recommended a preventive program for their clients (Slocombe, 1978). In contrast, the 2010 survey reported that 83% of dogs were on preventive medication (Slocombe, 2011). The prevalence based on laboratory data in 2015 was estimated to be 0.17% (95% CI: 0.16, 0.20), and for the partial year of 2016 (months January to July) estimated at 0.12% (95% CI: 0.10, 0.14). Using the laboratory data, the aggregated prevalence for all dogs tested from 2007 to 2016 was highest in Manitoba at 0.35% (95% CI: 0.31, 0.40). The aggregated prevalence for all dogs tested from 2007 to 2016 for Ontario was estimated to be 0.12% (95% CI: 0.11, 0.13), and the aggregated prevalence for all dogs tested from 2007 to 2016 for Quebec was estimated to be 0.27% (95% CI: 0.24, 0.31).

The surveys consistently had a larger sample size (Table 2.1 and 2.2) than the laboratory register; however, the laboratory annual sample size steadily increased from 8082 samples in 2008 to 181,205 samples in 2015. Notably, most (75-90%) data were collected from dogs residing in the province of Ontario (Table 2.1 and 2.2).

There were differences in information provided between survey and laboratory data. Veterinarians from the territories did not mail back surveys and only one veterinarian from the Yukon submitted blood samples to the diagnostic laboratory. The surveys reported national heartworm information starting in 1977, but it was not until 1981 that the surveys displayed information at a provincial level.

Figure 1 is a proportional symbol or "bubble" plot showing the annual prevalence of heartworm across Canada, where the bubble size represents the sample size. Additional smoothing lines visualize time trends for selected provinces: Ontario, Manitoba and Quebec. These three provinces were selected because they had the largest sample sizes and had previously been suggested as provinces with an elevated risk of heartworm infection in companion dogs (Klotins *et al.,* 2000). The Cochran-Armitage trend test suggested a temporal trend at both the national level and for some provinces based on the survey data (p<0.05) (Table 2.1). Based on the laboratory data, the Cochran-Armitage trend test suggested a temporal trend from 2007 to 2016 for Manitoba and Quebec (p<0.05); with respect to the trend line for these

provinces, prevalence increased (Figure 1 - right). The trend test for Ontario suggested a temporal trend from 2007 to 2016 (p<0.05); the trend line showed a decrease in prevalence (Figure 2.1 – right).

The true prevalence for the laboratory data year 2015 was estimated to be 0.165% based on the calculation using apparent prevalence and Se and Sp of the antigen test, which was very close to the apparent prevalence of 0.17% (Noordhuizen *et al.,* 2001). The comparison of true to apparent prevalences were used with the aggregate prevalence for the years 2007 to 2016 for all dogs tested. The apparent prevalence for Manitoba was underestimated at 0.35% compared to the true prevalence of 0.37%. Ontario had a similar apparent and true prevalence with 0.12% and 0.11%, respectively. True and apparent prevalence were the same for Quebec at 0.27%. The positive predictive value was estimated at 96.1% for all dogs tested for the laboratory diagnostic year of 2015, indicating that only 3.9% of the positive tests that year were false positives.

The choropleth map for survey data indicated that Alberta and Quebec had the highest aggregate smoothed prevalence for 1981 to 2010 for all dogs (Figure 2.2). The choropleth map based on the laboratory data illustrated that Manitoba and Quebec had the highest aggregate smoothed prevalence from 2007 to 2016 for all dogs (Figure 2.3). Figure 2.3 also shows the spatial distribution of submitted samples from across Canada; the southern parts of provinces, especially southern Ontario, were the origin of most samples. A spatial comparison of the two maps show an increased prevalence in Manitoba and a decreased prevalence in Ontario (Figures 2.2 and 2.3).

Descriptive statistics for the apparent efficacy of preventive drugs are presented in Table 2.3. The AFe is interpreted here as the total amount of heartworm infections prevented by chemoprophylaxis aggregated for all years and was estimated to be 93.08% (95% CI: 92.85, 93.31). The AFe fluctuated between years, but the estimate for the year 1996 (AFe=62%) is far below that of the other years, which range between AFe= 93% and AFe=98%. The maximum AFe was attained in 2010 (Table 2.3).

2.5 Discussion

The prevalence of heartworm in Canada varied over time. However, results from surveys between 1977 and 2010 displayed a consistent decrease among all provinces. In contrast, the prevalence estimated from laboratory diagnostics conducted between 2007 and 2016 increased for Manitoba and Quebec (Figure 2.1 - right). It should be noted that the sample size has increased over time for both data sources, which may limit the potential for selection bias of veterinary clinics who consistently report cases. The increase in sample size over the years could also mean that more veterinary clinics are suggesting routine heartworm testing to their clients, or that there are more owners concerned about heartworm leading to an increase in testing. The antigen test is also used for detection of antibody to *Borrelia burgdorferi* and other tick-borne infections. Thus, the increase in testing may also be due to increased awareness/testing for tick-borne infections.

There were major differences between the survey and laboratory datasets. The annual surveys were based on veterinarian-reported cases of heartworm where the veterinarian acted as a "gold standard". However, there can be differences of opinion or method among veterinarians and without a diagnostic test it is not possible to estimate the true prevalence. The prevalence reported from the survey data from 1977 to 2010 could have been seriously over or under estimated. The laboratory data used an antigen test and although the test was updated from 2007 to 2016, the technique remained consistent. Despite the difference in methodology, the comparison of results was similar nationally and for Ontario. However, the possible variance in reporting measures used in the survey data makes a fair comparison to the laboratory data across space and time difficult.

The focus of the analysis was on Ontario due to the large sample size for that province, with additional specific study of Quebec and Manitoba, the provinces with second and third highest number of tests. A significant decrease in temporal trends for survey prevalence from 1981 to the early 2000's was noted in both Manitoba and Quebec. However, the prevalence among laboratory tested dogs from 2007 to 2016 depicted a significant increasing temporal trend (Figure 2.1 - right). Due to the large sample size from Ontario, the prevalence pattern seen for Canada closely follows the observed Ontario prevalence; despite the significant temporal decrease for Ontario laboratory prevalence reports there was not a trend at the national level (Figure 2.1 – right).

The western provinces do not appear to have experienced changes in heartworm prevalence, except for Manitoba. The western United States that border with the prairies in Canada have low heartworm prevalence compared to eastern states and this border relationship may contribute to the spatial distribution of heartworm among Canadian provinces (American Heartworm Society, 2018). There was no information regarding travel history for laboratory data, however the 2010 survey for western provinces stated majority of the cases never left the province (Slocombe, 2011). It is possible that recent changes in climate may have affected the mosquito populations of Manitoba impacting the heartworm prevalence among dogs.

The positive trend in prevalence seen in Manitoba and Quebec could be due to a changing climate in these provinces providing more suitable conditions for heartworm development in mosquitoes. The province of Ontario, for example, has experienced longer heatwaves during the summer (Bishop-Williams *et al.,* 2016). If other provinces, such as Manitoba or Quebec are experiencing similar heatwaves it could be produce a more supportive environment for the mosquito lifecycle; additionally, it could produce the heat required for quicker maturation to the infective third-stage larvae. Both of which could impact the transmission of heartworm infection among dogs.

The laboratory study limitations include the small sample sizes for the Atlantic provinces, Saskatchewan and the Yukon. A small sample size can impact the prevalence estimates as it is based on only a few dogs. The data were aggregated over time for choropleth mapping, as heartworm is a rare infection and some years had limited sample sizes. The aggregation of data to ensure adequate sample sizes was a limitation because it examines heartworm prevalence on a larger temporal scale than what occurs in a year; the choropleth maps represent the study period prevalence and not an individual year.

The limitations of the macroscopic view of heartworm infection in Canada from this study should be addressed. A detailed map of Ontario that depicts where most cases were diagnosed would provide better insight as to where hot spots of heartworm infection exist. Determining the location of hot spots might provide veterinarians with a communication tool for advising their clients regarding the necessity of preventive treatments. Additionally, regional climate data should be examined for spatial and temporal increases in *Dirofilaria*-development-units from 2005 to 2016 (the number of days over 14°C) (Sassnau *et al.,* 2014). There could be

changes in the spatial distribution or seasonality of development units that impact the transmission of heartworm.

Dogs can be expensive as there are many costs associated with owning a dog; the basics of food and medical care can be costly. A socio-economic status that permits paying for tests and regular veterinary visits may contribute to selection bias. Diagnosis is possible only among dogs who frequent a veterinarian and are tested, thus the prevalence may be under-reported as not all domestic dogs frequent the veterinarian or undergo testing. The subset of dogs not tested could also not have prophylaxis, thus they are at greater risk of heartworm infection, but their infections are also not being captured. In a United States survey of more than 50,000 households from 2011, 18.7% of dog owners reported not taking their dog to the veterinarian (American Veterinary Medical Association, 2013).

Compliance is a problem with prophylaxis, as some owners may not follow the instructions for dosing. However, information on prophylaxis compliance among dog owners is incomplete: it is only available for a few dogs and based on the assumption that owners correctly recall their compliance (Graham *et al.,* 2012). The issue of compliance can lead to a misclassification bias, where the protected group may include dogs that are not effectively protected. Furthermore, for oral products, gastrointestinal complications can lead to lower drug uptake by the patient and leave a dog vulnerable to infection (Graham *et al*., 2012). In Canada, the efficacy of prophylaxis among survey dogs from 1996 to 2010 has remained above 62% (Table 2.3).

This study shows that although previous reports of heartworm infection in Canada have indicated a decrease in cases from 1977 to 2010, in certain provinces such as Manitoba and Quebec, heartworm prevalence has been on the rise from 2007 to 2016 (Figure 2.1). Ontario, which has previously been a heartworm focus in Canada, displayed a decreasing temporal trend in prevalence from 2007 to 2016. Climate change may play a role in the epidemiological differences seen in heartworm infection across the country, although further investigation is required to separate this effect from the effect of preventive medication among dogs. The importance of chemoprophylaxis in preventing heartworm infection was illustrated, with an average of 93% of infections being prevented among dogs. This highlights the importance of regular veterinary visits and obtaining a preventive option for a dog that maximizes compliance.

Table 2.1. Descriptive summary table of heartworm survey data collected from 1977 to 2010.

Province	Year Range[1]	Sample Size Range[2]	Mean Sample Size[3]	Mean Case Size[4]	Temporal Trend[5]	Prevalence[6]
British Colombia	1981-2010	124-20,570	3,981	7	Not significant	0.26 (0.23, 0.30)
Alberta	1981-2010	21-9,959	2,973	3	Significant decrease	0.11 (0.09, 0.15)
Saskatchewan	1981-2010	76-822	333	1	Significant decrease	0.25 (0.15, 0.41)
Manitoba	1981-2010	1,074-23,192	8,360	29	Significant decrease	0.34 (0.31, 0.37)
Ontario	1981-2010	28,732-289,289	184,340	576	Significant decrease	0.31 (0.30, 0.32)
Quebec	1981-2010	252-48,301	16,955	50	Significant decrease	0.30 (0.28, 0.33)
New Brunswick	1981-2010	30-1,729	722	1	Not significant	0.10 (0.06, 0.17)
Nova Scotia	1981-2010	101-4,685	1,421	2	Significant decrease	0.10 (0.07, 0.15)
Prince Edward Island	1981-2010	26-136	65	0	Not significant	0.36 (0.14, 0.93)
Newfoundland	1981-2010	3-154	51	1	Significant decrease	1.1 (0.59, 1.0)
Canada	**1977-2010**	**16,563-419,381**	**197,203**	**622**	**Significant decrease**	**0.32 (0.31, 0.32)**

Source: National Library of Medicine National Institutes of Health [PubMed] Available from:
https://www.ncbi.nlm.nih.gov/pubmed/?term=heartworm+and+slocombe :Last accessed January 29, 2018.
[1] Year range that provinces provided data. Survey was not sent out every year, and some years there were provinces who did not return the survey.
[2] Range of minimum to maximum sample size per province for the years data were supplied
[3] Mean sample size calculated as the cumulative n = sample size, divided by year range data were obtained from the province
[4] Mean case size calculated as the cumulative k = cases, divided by the year range data were obtained from the province
[5] Result of Cochran-Armitage trend test, either significant or not significant at the $\alpha=0.05$ level
[6] Raw aggregated prevalence estimates (%) that were smoothed to create the choropleth maps, 95% CI in brackets

Table 2.2. Descriptive summary table of diagnostic heartworm test (SNAP 3Dx, 4Dx and 4Dx Plus Test) results collected in a diagnostic laboratory database from 2007 to 2016.

Province	Year Range[1]	Range of Sample Size[2]	Mean Sample Size[3]	Mean Case Size[4]	Temporal Trend[5]	Prevalence (%)[6]
British Colombia	2007-2016	11-1,135	543	1	Not significant	0.13 (0.06, 0.27)
Alberta	2007-2016	7-1,222	718	1	Not significant	0.19 (0.12, 0.33)
Saskatchewan	2008-2016	0-163	57	0	Not significant	0.22 (0.04 ,1.0)
Manitoba	2007-2016	1,050-11,560	8,057	28	Significant increase	0.35 (0.31 ,0.40)
Ontario	2007-2016	3,782-136,581	66,097	76	Significant decrease	0.12 (0.11, 0.12)
Quebec	2007-2016	7-17,028	10,378	28	Significant increase	0.27 (0.24, 0.30)
New Brunswick	2007-2016	6-591	242	0	Not significant	0.16 (0.06, 0.42)
Nova Scotia	2007-2016	29-1,456	713	1	Not significant	0.18 (0.10, 0.31)
Prince Edward Island	2013-2015	0-1	0	0	Not significant	0 (0, 1.0)
Newfoundland	2007-2016	0-53	30	0	Not significant	0.81 (0.22, 1.0)
Yukon	2011-2015	0-5	2	0	Not significant	0 (0, 1.0)
Canada	**2007-2016**	**4,893-166,904**	**86,822**	**136**	**Not significant**	**0.16 (0.15, 0.17)**

Source: IDEXX Laboratories Canada Corp.
[1] Year range that SNAP 3Dx, 4Dx and 4Dx Plus Tests were submitted for laboratory testing for that province
[2] Range of minimum to maximum sample size per province for the years data were supplied
[3] Mean sample size calculated as the cumulative n = sample size, divided by year range data were obtained from the province
[4] Mean case size calculated as the cumulative k = cases, divided by the year range data were obtained from the province
[5] Result of Cochran-Armitage trend test, either significant or not significant at the $\alpha=0.05$ level
[6] Raw aggregated prevalence estimates (%) that were smoothed to create the choropleth maps, 95% CI in brackets

Table 2.3. The estimated annual Attributable Fraction Exposed (AFe) with provinces aggregated to determine the percentage of cases prevented by chemoprophylaxis

Year	AFe	95% Confidence Interval
1996	62.38%	59.30, 65.18
1997	92.63%	92.06, 93.14
1998	93.51%	92.93, 94.03
2000	93.90%	93.24, 94.48
2001	97.59%	97.27, 97.88
2002	96.07%	95.61, 96.48'
2010	98.26%	98.08, 98.42
Total	**93.08%**	**92.85, 93.31**

Source: National Library of Medicine National Institutes of Health [PubMed] Available from:
https://www.ncbi.nlm.nih.gov/pubmed/?term=heartworm+and+slocombe
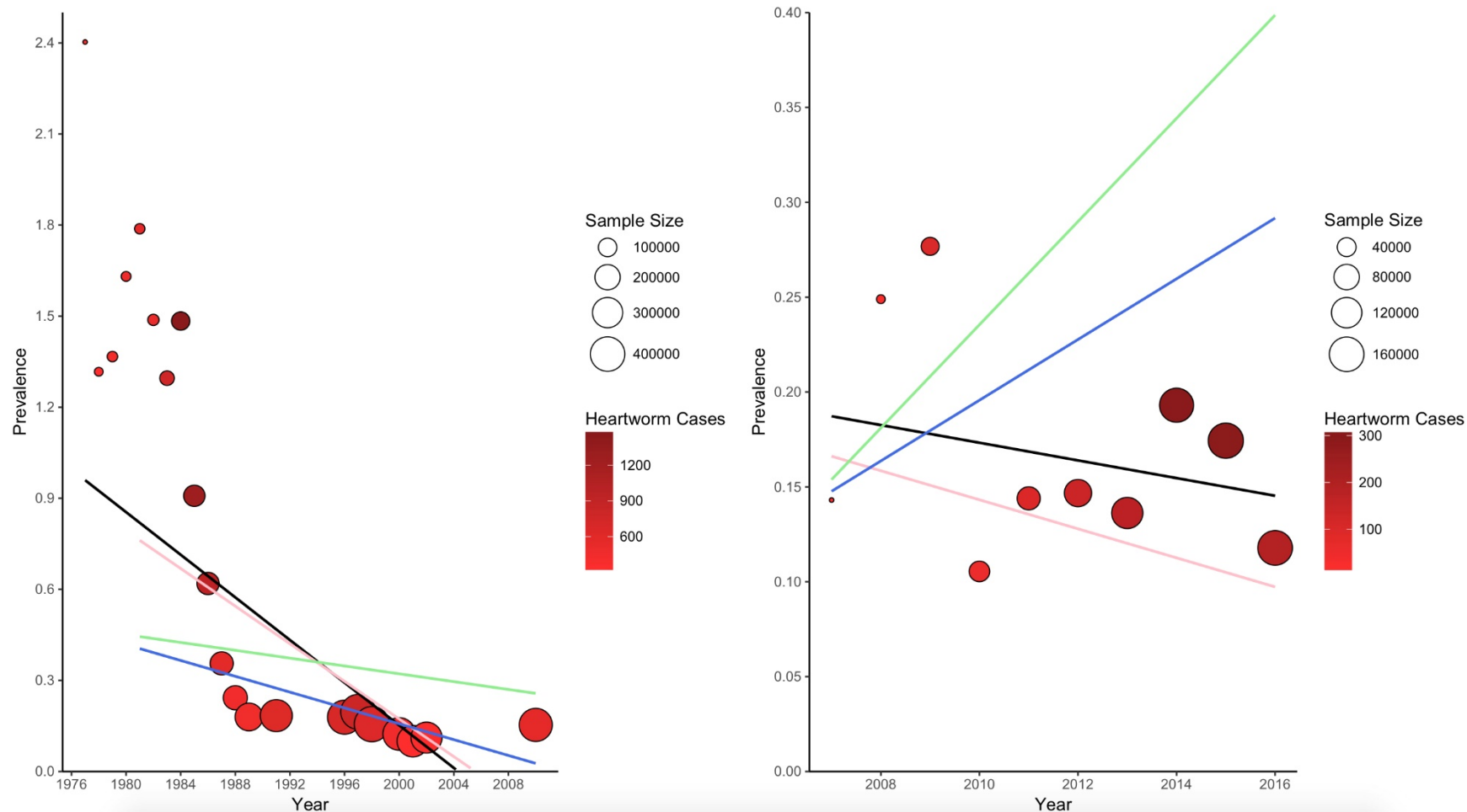
Figure 2.1. Heartworm prevalence scatterplots weighted by sample size with smoothing lines for Canada (black), Manitoba (green), Ontario (pink) and Quebec (blue). X-axis depicts years of data collection, and y-axis depicts prevalence of heartworm infection as a percentage. Left panel, survey data; right panel, laboratory data.

Figure 2.2. Choropleth map of aggregated smoothed heartworm prevalence using heartworm survey data for Canada for all dogs tested from 1981 to 2010.

Figure 2.3. Choropleth map of aggregated smoothed heartworm prevalence from 2007 to 2016 using laboratory data for Canada for all dogs tested. The distribution of veterinary clinics with submitted samples is overlaid; the size of the point is weighted by the number of submissions.

2.6 References

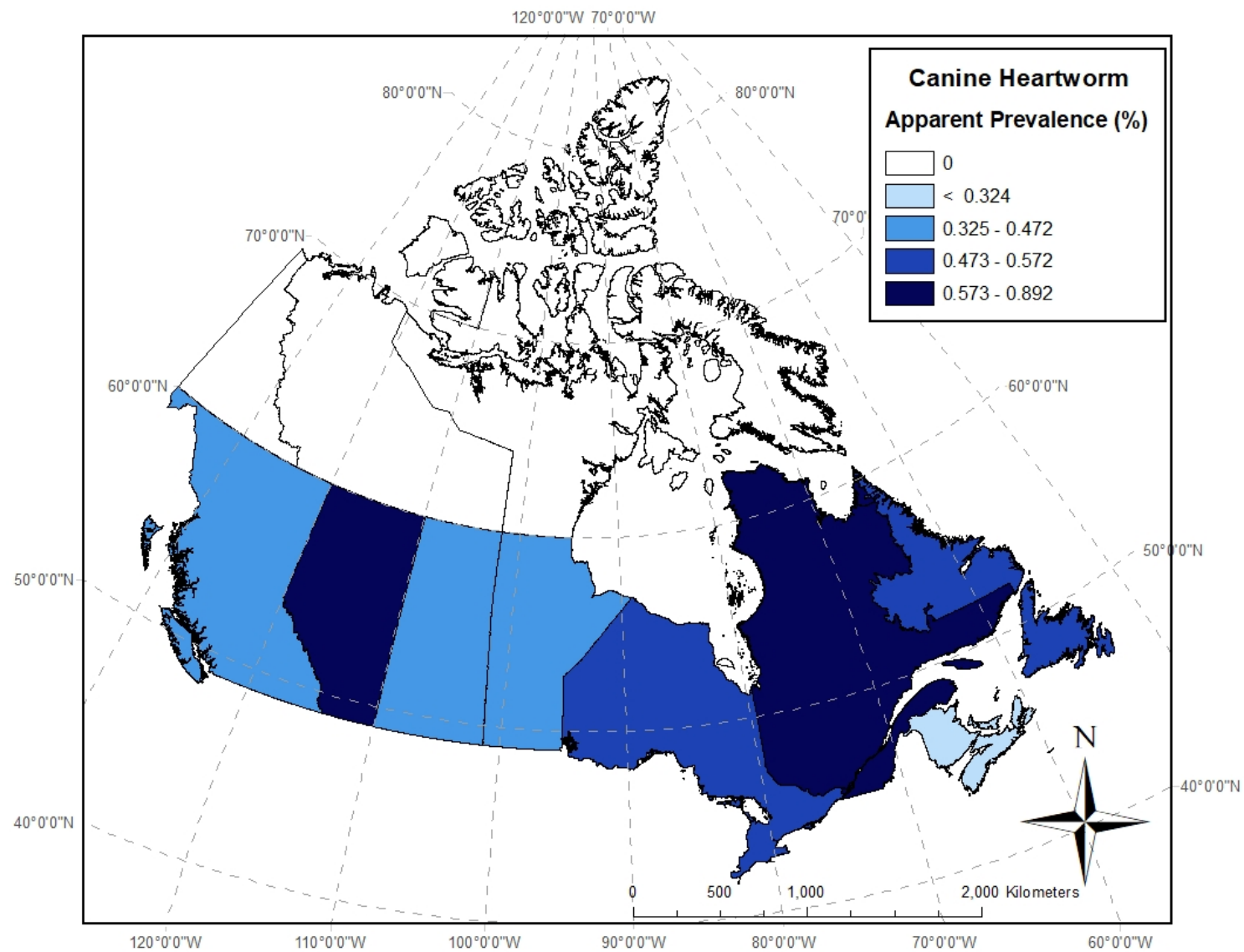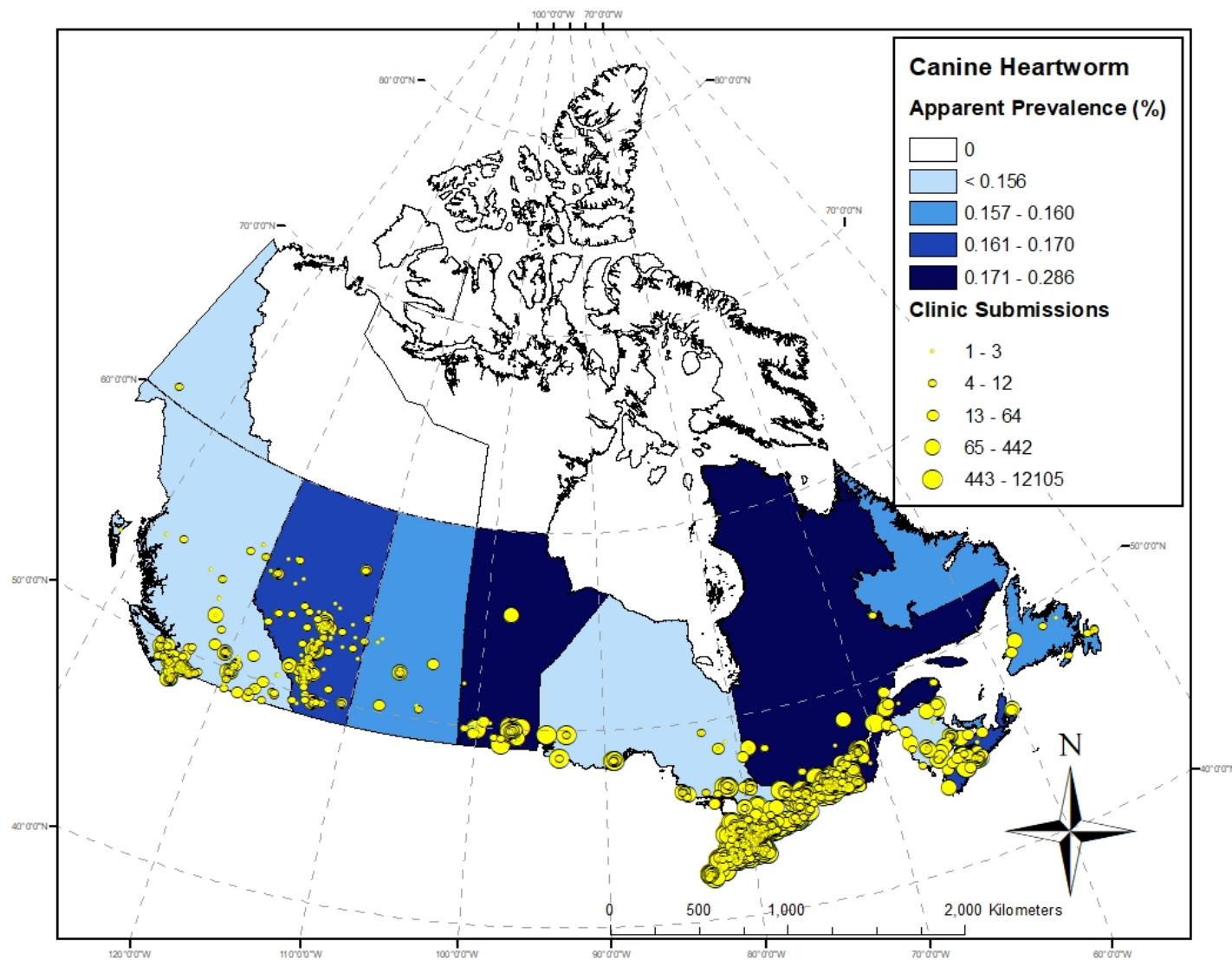American Heartworm Society [homepage on the Internet] c2018 Heartworm incidence maps. Available from: https://www.heartwormsociety.org/pet-owner-resources/incidence-maps Last accessed March 1, 2018.

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Chapter 3

Epidemiology of canine heartworm (*Dirofilaria immitis*) infection in domestic dogs in Ontario: geographic distribution, risk factors and effects of climate

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3.1 Abstract

Dirofilaria immitis is the causal agent of heartworm infection, a mosquito-borne parasite that primarily infects domestic and wild canids. Heartworm is endemic in parts of Canada, and Ontario has been identified as the province where the majority of heartworm infections occur. In Canada, the recommendation for preventive treatment is to treat dogs in risk areas seasonally (June to November) with anthelmintic drugs. However, the status of heartworm infection among domestic dogs in Canada requires re-evaluation due to climate change, as countries in Europe and the US have reported how climate change has impacted the distribution of heartworm infection. Test results for blood samples submitted by veterinary clinics for the years 2007-2016 were used to conduct a spatial risk analysis of heartworm among domestic dogs in Ontario. The geographic extent of the apparent heartworm prevalence was examined through smoothed choropleth maps for all 49 census division regions. Furthermore, the regions were assessed for local clusters in apparent prevalence using the flexible spatial scan statistic. Three clusters were found: western Ontario (Rainy River), southern Ontario (Haldimand-Norfolk, Oxford, Elgin, Chatham-Kent and Lambton) and eastern Ontario (Lanark and Renfrew). A spatial Poisson regression model for heartworm prevalence among pet dog populations in southern Ontario census divisions was fit to determine the association between human population size, heartworm development units (HDUs), climate moisture index (CMI), precipitation and east/north directions with heartworm infection. The model identified the spatial distribution of HDUs and CMI as important predictors, positively associated with heartworm infection. In contrast, human population size, increasing northern latitude and drier areas were negatively associated with heartworm infection. The direction east and precipitation were not significant.

3.2 Introduction

The parasite *Dirofilaria immitis*, commonly known as heartworm, primarily infects dogs but can also infect felids, wild canids, and very rarely humans (Vezzani and Carbajo, 2006). Domestic dogs and wild canids serve as the primary reservoirs for infection (Ciucă *et al.*, 2016). Mosquitoes are the only known intermediate host and vector of *D. immitis* (Slocombe *et al.*, 1989).

In Ontario, Canada there are at least 22 mosquito species reported as potential vectors of *D. immitis* (Ludlam *et al.,* 1970). On the basis of historical data, *Aedes vexans, Culex pipiens-restuans* and *Mansonia perturbans* are the most common and widespread in southern Ontario, capable of supporting *D. immitis* larvae development to the infective stage (Ernst & Slocombe, 1984; Slocombe *et al.,* 1989). *Aedes vexans* is the most common vector in the fall and survives into at least mid-September; its life span is approximately 30 days (Slocombe *et al.,* 1989). *Aedes stimulans* can be found as early as mid-May and can live into the summer months (Slocombe *et al.,* 1989). Ernest and Slocombe (1984) captured over 3000 mosquitoes of ten different species in Ontario to observe how many contained third-stage larvae; the mouthparts of six of the ten species contained infective larvae. A recent report estimated there are 67 mosquito species endemic to Ontario and *Ae. albopictus* specimens were found, although it is not considered to be endemic (Giorgano *et al.,* 2015). *Aedes albopictus* is considered an invasive species and the northern range of its habitat has been increasing in the United States (Armstrong *et al.,* 2017).

The threshold temperature to allow for parasite maturation to the infective stage within the mosquito is 14°C; there is a linear relationship between rate of development of the parasite and temperatures from 18 to 34°C (Fortin & Slocombe, 1981). The amount of heat required for larval development to the infective stage is 130 heartworm development units (HDU), which are calculated as the cumulative sum of daily average degrees centigrade above the 14°C threshold temperature (Slocombe *et al.,* 1989). In Canada, these warmer temperatures are generally first observed in southern Ontario compared to other areas in the province and country. Thus, the transmission period for Canada is considered June 1st to October 9th (Slocombe *et al.,* 1989) and is based on weather data from 1957-1986 collected from Windsor, Ontario; analysis of similar data from weather stations across Ontario determined that Windsor had the earliest and latest dates of transmission. However, the wide range of climate across Canada means that the true risk

period varies geographically. The start of the transmission risk period for Canada has historically been calculated by summing daily HDUs from May 6th (date of earliest blood feeding for *Ae. stimulans*) until 130 HDUs are achieved (Slocombe *et al.,* 1989). The end of the transmission period has historically been calculated as the last day in the year when 130 HDUs can be reached within 30 days (based on the life span of *Ae. vexans* a late season vector) (Slocombe *et al.,* 1989). Some mosquitoes are capable of overwintering; however, it is assumed that the effect of overwintering mosquitoes on the overall heartworm transmission pattern is negligible (Knight *et al.,* 1998).

On the basis of the aforementioned transmission season, the recommendation for preventive medication in Canada is July 1st to November 1st, as seasonal preventive treatment is to begin within the first month of the start of the transmission season and continue until the first of the month following the end of the transmission period (Graham *et al.,* 2012). As a conservative approach, the recommended start date for prophylaxis is June 1st. Macrocyclic lactones are the drug family used for preventive medication and are marketed as oral (ivermectin and milbemycin oxime) and topical (moxidectin and selamectin) products (Graham *et al.,* 2012); all products have one month's reachback activity.

Climate change has been implicated as a possible factor for increases in heartworm prevalence that have been observed in the United States in dogs, due to faster development to the infective larval stage in mosquitoes (Ledesma and Harrington, 2011). An increase in temperatures can also lead to the emergence of the parasite in new areas, as mosquitoes are able to extend their habitat range (Sassnau *et al.,* 2014). Furthermore, climate change can lengthen the annual transmission season by extending the periods when temperatures are sufficient for parasite development (Morchón *et al.,* 2012). Climate-based models for heartworm infection that examine the effect of temperature on larval development within the mosquito assume the required 130 HDU's and mosquito life expectancy of 30 days, based on Fortin and Slocombe's study (1981; Genchi *et al.,* 2011).

Many factors influence transmission of heartworm including those that impact mosquito populations. Precipitation, vegetation and moisture can impact mosquito breeding, consequentially impacting *D. immitis* transmission (Alho *et al.,* 2014). The moisture index may be a more meaningful predictor of heartworm infection than precipitation amount, because the

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moisture index is calculated as the difference between annual precipitation and potential evaporation. As such, it estimates the available standing water in the environment for egg-laying mosquitoes (Brown *et al.,* 2012). In addition to moist areas, mosquitoes require heat; large cities may produce "heat islands" creating more favourable conditions for mosquito breeding compared to rural environments (Morchón *et al.,* 2012; Herrin *et al.,* 2017). However, on the basis of data from the United States, Wang *et al.* (2014) found that heartworm prevalence in dogs decreased with increasing human population density and elevation; a possible explanation being the difference in humidity and temperature at higher elevations.

The majority of testing of dogs for heartworm in Canada occurs in Ontario (Klotins *et al.,* 2000). The spatial distribution of heartworm infection among dogs in Ontario was most recently assessed from 2013-2014; however, the spatial pattern over a longer time period has not been determined (Herrin *et al.,* 2017). Although historic areas of risk are known, those regions may have experienced changes. Furthermore, the association between heartworm infection and risk factors such as HDUs, precipitation, moisture and urban density have not been examined in Ontario.

The objectives of this study were therefore, firstly, to determine the prevalence of heartworm in dogs at the Ontario census division level and visualize its geographic distribution by choropleth mapping. Secondly, to assess the presence of spatial clusters of heartworm infection in dogs in Ontario using the flexible scan test. And thirdly, to assess the association between environmental predictors including HDUs and human population size and heartworm infection prevalence in companion dogs using a spatial Poisson regression model.

3.3 Materials and methods

The dataset for this study consisted of *D. immitis* antigen tests performed at a commercial diagnostic laboratory (IDEXX laboratories) and in veterinary clinics from March 2007 to July 2016. Three different antigen tests were used during the study period: the SNAP 3Dx antigen test for the years 2007 to 2012, the SNAP 4Dx antigen test for 2011 to 2012, and the SNAP 4Dx Plus Test for 2012 to 2016. Data consisted of test result, dog age and postal code of the veterinary clinic where the sample was collected, or the test was performed.

The dataset was cleaned by removing duplicate sample submission numbers and non-canine species. Heartworm test results that were not coded as 0 (negative) or 1 (positive) in the electronic file were removed. Dog age was reported in months and the final dataset only included dogs that were at least 6 months of age (minimum age the antigen test would be able to detect heartworm) and a maximum age of 240 months (20 years) to capture possible age outliers and retain biological plausibility.

Natural Resource Canada supplied HDUs and climate normals for the centroid of all 49 Ontario census divisions. The climate normals included the mean moisture index and mean precipitation observed at different weather stations for the years 1980 to 2010 modelled on a grid to provide an estimate for that location. The mean climate moisture index (CMI) was provided in cm and mean precipitation in mm. Natural Resource Canada also applied the HDU model (Slocombe *et al.,* 1989) to the observed recorded daily temperature at weather stations, modelled on a grid to provide an estimate for the census division centroids to provided HDU data at a weekly level for the years 2005 to 2016. The weekly HDU data were then used to determine the transmission season and cumulative HDUs for that season. Start of transmission was defined as the accumulation of a minimum of 130 HDUs within 4 weeks or 28 days (using the weekly data provided), which is roughly the lifespan of a mosquito (Fortin & Slobombe, 1981; Sassnau *et al.,* 2014). End of transmission was the last week that 130 HDUs could be accumulated within 4 weeks. Annual cumulative HDUs were determined as the sum of HDUs for the transmission season of each year. These annual cumulative HDUs were then aggregated at the census division level over the years 2005 to 2016 (i.e. the years prior to and concurrent with heartworm testing).

Information regarding human population size and dwelling number was retrieved from the 2011 census conducted by Statistics Canada (Statistics Canada, 2011).

The prevalence of companion dog heartworm infections was examined at the Ontario census division level. The six-digit postal codes of the veterinary clinics (as supplied with the diagnostic test results) were matched to their Multiple Enhanced Postal Code (MEP) identification number (DMTI Spatial Inc., 2011). The MEPs were then matched to the corresponding census division number using a postal code to census division translation table (DMTI Spatial Inc., 2011). Both current and retired MEPs were used because the data spanned from 2007 to 2016. Duplicate postal codes were combined, and clear typographical errors based on the pattern followed by Canadian postal codes (e.g. "0" "O") were corrected. Reports without a postal code were excluded. Reports that were labeled Ontario but had a non-Ontario postal code were also excluded.

To visually display the heartworm prevalence estimates across Ontario, a boundary map file of the province's 49 census divisions was retrieved from Statistics Canada for the 2011 census year (Statistics Canada, 2016); the census division boundaries were extracted from the respective Canada census division boundary file. The boundary file projection was then converted from Mercator to Universal Transverse Mercator (UTM) Zone 17N for better visualization of the province's landmass.

The denominator of the census division prevalence estimates, i.e. the number of tests performed, was not consistent across the divisions. Thus, empirical Bayesian smoothing was used to internally standardize the differences in sample size (Berke, 2004; Beyer *et al.,* 2012). The annual prevalence was smoothed for each census division and the smoothed prevalence estimates were aggregated over time to generate a single choropleth map for the years 2006 to 2016. The choropleth map visualized the geographic variation in heartworm prevalence and any cluster of census divisions with a higher than expected prevalence. A flexible spatial scan test was applied to determine the location of probable clusters and estimate the relative risk of prevalence for the respective clusters compared to regions outside the cluster (Tango and Takahashi, 2005). The maximum number of census divisions used for the flexible scan test was set as 7 regions to ensure the populations assessed did not constitute more than 50% of the Ontario population. The significance of the test result was assessed using estimated p-values based on 999 Monte Carlo simulations. The clustering of infections, i.e. strength of spatial correlation of heartworm infection among domestic dogs, was tested through the Moran's I

correlation coefficient using the queen spatial structure where all touching polygons are considered neighbours (Assução & Reis, 1999; Waller & Gotway, 2004).

In additional work, a model was built to examine the risk of heartworm infection among domestic dogs in Ontario. Initially, a Generalized Linear Model (GLM) was used to assess putative risk factors. To correct for overdispersion, a Generalized Linear Mixed Model (GLMM) of the Poisson family with an exponential spatial correlation structure was fit using the Penalized Quasi-Likelihood (PQL) estimation method. The following factors that have been shown to have a positive association with heartworm infection risk in dogs were included in this model building process to assess their effects in the current study population: average dog age, human population size, human population density, agricultural regions, number of human dwellings, CMI, precipitation and HDUs. The potential for a spatial trend in east/west and north/south directions was assessed using polynomials in the centroid coordinates of the census division. Collinearity of the quantitative predictor variables was assessed by the variance inflation factors (VIF), where VIF > 10 was considered to indicate multicollinearity (Dohoo *et al.,* 2003).

The spatial GLMM model fit was assessed by examining the predictive properties, linearity and normality. The predictive properties of the model were assessed through a plot that examined the predicted and observed prevalence. Normalized residuals were evaluated with a scatterplot of the residuals against the quantitative predictors of the model and a line of best fit. A QQ-plot was used to check the normality of the normalized residuals. A circular scan test under the normal model was performed on the normalized residuals to detect any potential clusters not explained by the predictors in the regression model (Kulldorf *et al.,* 2009). The spatial scan was for both high and low residual values; significance of the test results was assessed using estimated p-values based on 999 Monte Carlo simulations.

ArcGIS was used for conversion of postal codes to the census division level (Esri, 2011). R and RStudio were used to generate the choropleth maps, and to perform the flexible scan test (smerc package) and for the spatial regression model analysis (RStudio Team, 2016; R Core Team, 2017). Normalized residual analysis was conducted in SaTScan (SaTScan, 2018). A significance level of α=0.05 was applied for all analyses, unless otherwise indicated.

3.4 Results

A total of 660,946 Ontario dog test results were included in this study of which 629,720 were from dogs that inhabited southern Ontario census divisions. There were 762 test positive dogs in Ontario between 2007 and 2016; 722 lived in southern Ontario. The total Ontario heartworm prevalence over 2007 to 2016 was 0.12% (95% CI: 0.10, 0.12), and ranged over time from 0.32% (95% CI: 0.20, 0.52) in 2008 to 0.13% (95% CI: 0.11, 0.15) in 2015. With respect to geographic variation, Haldimand-Norfolk census division had the highest prevalence over 2007 to 2016 with 0.64% (95% CI: 0.55, 0.75) followed by Chatham-Kent at 0.59% (95% CI: 0.49, 0.71) and Rainy River at 0.50% (95% CI: 0.33, 0.75). The ten census divisions of Prince Edward, Kawartha Lakes, Dufferin, Muskoka, Haliburton, Parry Sound, Manitoulin, Sudbury, Timiskaming, Cochrane and Algoma had no positive test results from 2007 to 2016. The sample size from 2007 to 2016 increased on by a factor of ~1.5 each year. The number of positive test results also increased from 16 in 2008 to 176 in 2015; the years 2007 and 2016 were partial years.

Figure 3.1 is a choropleth map of the smoothed heartworm prevalence for the 49 Ontario census divisions from 2007 to 2016; southern Ontario data are presented at higher resolution in Figure 3.2. There were three hot spots for heartworm infection identified by the flexible scan test: southern Ontario (Haldimand-Norfolk, Oxford, Elgin, Chatham-Kent and Lambton), western Ontario (Rainy River), and eastern Ontario (Lanark and Renfrew) (Figure 3.1 & 3.2). Each hot spot is outlined in a different colour for identification and was significant at the α=0.05 level. The cluster in southern Ontario had 328 positive test results, where the expected number was 77, and the prevalence risk ratio (PRR) of canine heartworm infection of the cluster was 6.7, i.e. the risk in the cluster was almost 7 times greater than the risk in the rest of Ontario. Rainy River had 22 positive test results from 2007 to 2016; the expected number was 5 test positives and the PRR=4.3. Eastern Ontario had 36 positive test results from 2007 to 2016; the expected number was 14. The PRR was lowest in this eastern hot spot at 2.6. The value of Moran's I correlation coefficient for Ontario was 0.17, p=0.008; indicating the presence of spatial clustering.

The final spatial Poisson regression model included several risk factors for heartworm infection among domestic dogs and is summarized in Table 3.1. Human population size had a

protective effect on heartworm infection prevalence; as the human population size within increased, the risk of heartworm infection in dogs decreased (p=0.0074; PRR=0.63 with a population of 500,000 and decreased to 0.40 with a population of 1,000,000 people per census division). There was a positive association between HDUs and heartworm prevalence (p=0.006); the PRR doubled for each increase of 100 HDUs within a transmission season. There was an interaction effect between CMI and increasing north latitude (p=0.01) on the heartworm prevalence. Neither variable was associated with the outcome on its own; however, their interaction was an important model component and indicated an association between heartworm infection and CMI. Heartworm prevalence infection risk increased with increasing CMI conditional on the interaction effect.

Precipitation was not significant but was kept in the model because it has been cited in the literature as a positive risk factor (Alho *et al.,* 2014; Wang *et al.,* 2014). It also had a large effect within the model, as the prevalence risk of heartworm doubled for each additional 5mm of rain, meaning that in areas that experience greater amounts of precipitation there may be an increased risk of heartworm.

Dog age was originally included in the spatial regression model for a basic epidemiological understanding of population dynamics; it was found to have a negative association with heartworm infection (i.e. older dogs had a reduced risk of infection). However, this was not significant, and the effect was very small; thus, it was excluded. Agricultural regions were also originally included in the model but were found to be not significant and without a large effect. Human population density was cited as a risk factor for dog populations in the United States (Brown *et al.,* 2012; Wang *et al.,* 2014); however, population density was not associated with heartworm infection in this study. Lastly, the total number of private human dwellings per census division was initially considered for inclusion, but when assessed for collinearity was highly influenced by population size and excluded.

The scatterplot of the predicted versus observed prevalence of heartworm infection in dogs estimated by census division (Figure 3.3) did not indicate a lack of fit for the regression model. Furthermore, the circular spatial scan test applied to the normalized deviance residuals did not indicate the presence of any residual cluster. Thus, the predictors in the regression model explained the observed clusters (Figure 3.3).

3.5 Discussion

Southern Ontario has historically been the focus of heartworm prophylaxis and concern for this infection in dogs in Canada (Klotins *et al.,* 2000). The results from this study, by looking at data over 2007 to 2016, show that there was also a cluster in northern Ontario, in Rainy River (Figure 3.1). Historically, northern Ontario did not have a cluster of heartworm (Slocombe, 2011; Herrin *et al.,* 2017). It is possible that veterinarians in Rainy River did not expect heartworm due to being further north and therefore did not recommend a preventive medication to dog owners. A changing climate provides an alternative explanation for the emergence, or newly discovered, cluster of heartworm infection in Rainy River; warmer temperatures may have allowed mosquitoes and *D. immitis* to move further north (Genchi *et al.,* 2011). A national serological survey in the United States found that heartworm was present over a wider area than generally acknowledged, extending beyond the endemic southern states (Bowman *et al.,* 2016). Furthermore, forecasting models using heartworm risk factors in the United States have predicted heartworm infection prevalence will increase in the northern regions of some states like California (Bowman *et al.,* 2016). Canada is expected to experience warmer temperatures, more rainfall and increased droughts due to climate change (Cox *et al.,* 2013). The province of Manitoba has seen an increased prevalence in heartworm infections from 2002 to 2010 (Slocombe, 2011), which could be associated with a changing climate. There could be new vector species with longer life spans than the average 30 days that have emerged, allowing for *D. immitis* to develop and be transmitted even at cooler summer temperatures (Ludlam *et al.,* 1970).

Climate change has been implicated in the changing tick distribution, including *Ixodes scapularis* the vector of *Borrelia burgdorferi*; thus, Lyme disease has become a growing concern in Ontario (Greer *et al.,* 2008; McPherson *et al.,* 2017). As there is growing concern, more veterinarians are recommending testing for *B. burgdorferi* infection and the same diagnostic test kit is used for the detection of heartworm. It could be that increased testing for tick-borne pathogens is leading to the detection of heartworm infections, which could explain the newly detected cluster in northern Ontario. The increasing sample size each year may account for more infections being detected. Furthermore, as *I. scapularis* increases its northward expansion due to climate change, it would follow that other vector-borne pathogens, such as *D. immitis*, may also experience a change in distribution (McPherson *et al.,* 2017).

The transmission season of *D. immitis* is estimated using the HDU model, built upon historical work by researchers at the Ontario Veterinary College; the threshold required for maturation of the parasite within the mosquito is 14°C and the model assumes mosquitoes do not overwinter (Fortin and Slocombe, 1981; Slocombe *et al.,* 1989). This historic model was built using weather data from 1957-1986 and research on Ontario mosquito species from the 1970's and 80's; both of which may have experienced changes. It is important for further research to determine the mosquito species which are currently most common in Ontario, and elsewhere in Canada, and capable of supporting *D. immitis* development to the infective stage. The spatial model using the newly estimated HDUs supplied by Natural Resource Canada (based on the historic model) accurately predicted the heartworm prevalence observed from the data, which illustrated the validity of the HDU model. It could be beneficial to determine if there have been any changes to the beginning and end of the transmission season due to climate change. Climate change may contribute to the early season vector, *Ae. stimulans,* being found earlier than the historic May 6th date (Slocombe *et al.,* 1989). Understanding how climate change has impacted the transmission season in different regions in Ontario would be a helpful tool for veterinarians.

The prevalence referred to in this paper is the estimated apparent prevalence of heartworm infection among domestic dogs in Ontario. The sensitivity and specificity of the 4Dx Plus Test are 99.0% and 99.3%, respectively (IDEXX, 2016). False positives and false negatives from the heartworm antigen tests are a limitation and influence estimation of the true heartworm risk among dogs; false positives lead to an over-estimation of the prevalence, while false negatives lead to an under-representation of the true prevalence. Thus, the positive predictive value with an overall prevalence of 0.1% over 2007 to 2016, with a test 99.0% sensitivity and 99.3% specificity, is 95%, leaving 5% of test positives misclassified as positive. The negative predictive value estimated using the same values is 99.9%, meaning the antigen tests used are better at classifying negative test results accurately than positive results. Berke and Waller (2010) showed that spatial data analyses for the detection of geographic/spatial patterns in infection occurrence are not seriously affected by diagnostic misclassification if the sample size is large in each region. However, what large sample sizes are is not specified (Berke & Waller, 2010). In the current study, large numbers of dogs were tested, overall, but heartworm is a rare event in Ontario which might be the reason that veterinarians in some census divisions did not detect cases.

The spatial Poisson regression model accurately predicted the observed census division heartworm prevalence from the laboratory dataset. Thus, variables that were included based on literature from Europe and the United States (Brown *et al.,* 2012; Sassnau *et al.,* 2014), were applicable to the Ontario population of dogs. The model initially was built for all 49 Ontario census divisions. However, the geography of Ontario (with extreme large northern regions that supplied few samples for testing as opposed to smaller regions in the south that supplied the majority of samples) required splitting-up the study area. Indeed, the PQL estimation algorithm for a model for the northern and southern Ontario heartworm prevalence combined would not converge. Since most of the Ontario human population and their domestic dogs inhabit southern Ontario, the model was built for this population only.

Limitations of this study include lack of information regarding repeated testing and clinical outcome (i.e. whether a test positive dog was re-tested and whether a veterinarian decided a test-positive dog was genuinely infected with *D. immitis*). The dataset included a postal code, but that was for the veterinary clinic location, not the residence of the dog. Furthermore, dog owners walk their dogs and may also take them hiking or camping, which increases the exposure area from a single point. In addition, dog owners often have a favourite veterinarian and may travel to that veterinary clinic which could mean the point associated with that dog is not close to their place of residence. Lastly, travel history outside the province or the country, was not available for this study. To avoid spatial misclassification bias, the point data were aggregated to a census division level; any small discrepancies due to human error recording of postal codes should not have influenced the data interpretation because of the aggregation from point data to regional data.

As climate change continues and leads to warmer temperatures supportive of maturation and transmission of *D. immitis,* it is important to determine areas of risk and be aware of vector spread into new environments. Historically, most heartworm cases in dogs were discovered in south-western Ontario (Klotins *et al.,* 2000; Slocome *et al.,* 2010). However, the most southern census division of Essex, home to Windsor city (and the weather stations that supplied the data for the original HDU model for Ontario) was not included in the hot spot identified in this study. It is possible that there is a heightened awareness in that region which contributes to high prophylaxis and therefore the dog population is less susceptible to infection. There could also

have been changes in heartworm risk for that census division, and dogs are now not exposed to the same level of heartworm risk as they were historically.

In conclusion, this study has shown that, overall, heartworm infection is a rare event in Ontario, but that geographic variations in heartworm risk exist. The geographic variation is strongly associated with variation in HDUs and CMI. Traditionally, the focus of heartworm testing has been southern Ontario, but heartworm is widespread among dogs in the province. It is not known if the spread of heartworm infection is endemic or related to travel; however, historically around 75% of heartworm infections were locally acquired (Slocombe, 1990). There was an increase in the number of regions in Ontario where heartworm infections were found from 2007 to 2016. It is possible that the increased testing in Ontario from 3,782 test results in 2007 to 136,581 test results in 2016 is likely due to increased concern regarding tick-borne pathogens, in both humans and dogs (Bouchard *et al.,* 2015; PHO, 2018) and that this contributed to more heartworm infections being detected. Overall, the combination of increased testing over the study period and climate change may explain the heartworm infection clusters and the change in distribution seen in Ontario over 2007 to 2016.

Table 3.1. Summary of the output from a spatial GLMM-PQL model for heartworm infection prevalence among dogs in southern Ontario over 2007 to 2016, including the descriptive measures for the variables included in the model.

Variable	Coefficient Value	Variable Range[1]	P-value	PRR[2]
East	-0.0030	352.8-975.7 km	0.1967	0.15
North	0.0088	4669.4-5058.0 km	0.0866	30.55
Precipitation	0.0976	74.5-94.6 mm	0.0926	7.08
CMI[3]	31.97	-0.73-1.96 cm	0.0100	2.23×10^{37}
HDU[4]	0.0101	211.3-816.8 HDU	0.0006	453.07
Human population size	-9.26×10^{-7}	17,026-2,615,060	0.0074	0.09
I(north*CMI)	-0.0066	-3408.7-9913.7	0.0104	0.001

[1] Minimum and maximum value for that variable
[2] The prevalence risk ratio for the total variable (exp[coefficient*range of variable])
[3] Climate moisture index
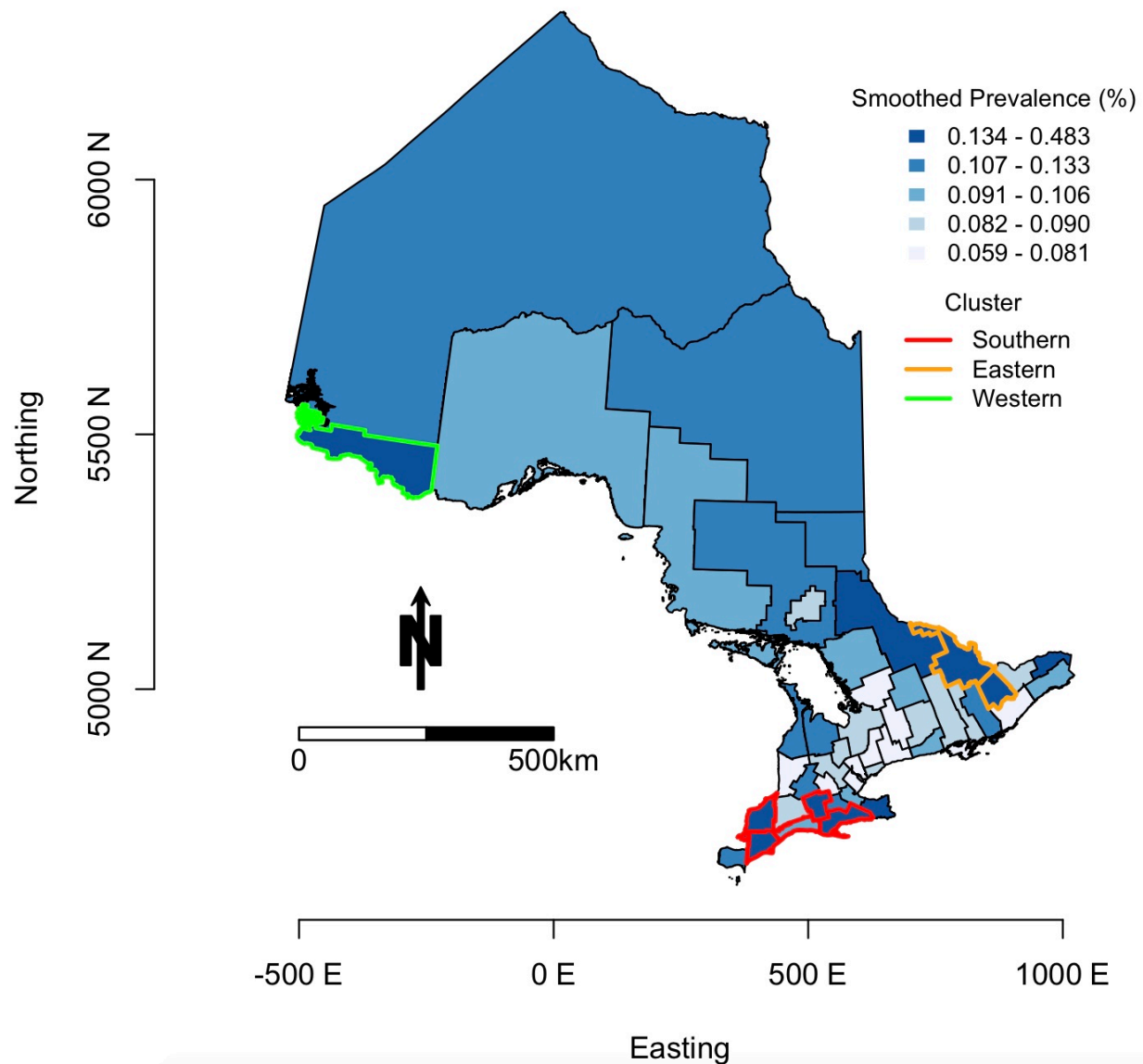[4] Heartworm development units

Figure 3.1. Choropleth map of all 49 Ontario census divisions with aggregated smoothed heartworm infection prevalence from 2007 to 2016 using laboratory data for all dogs tested. Primary cluster (southern Ontario) in red, secondary cluster (eastern Ontario) in orange and other secondary cluster (western Ontario) in green.
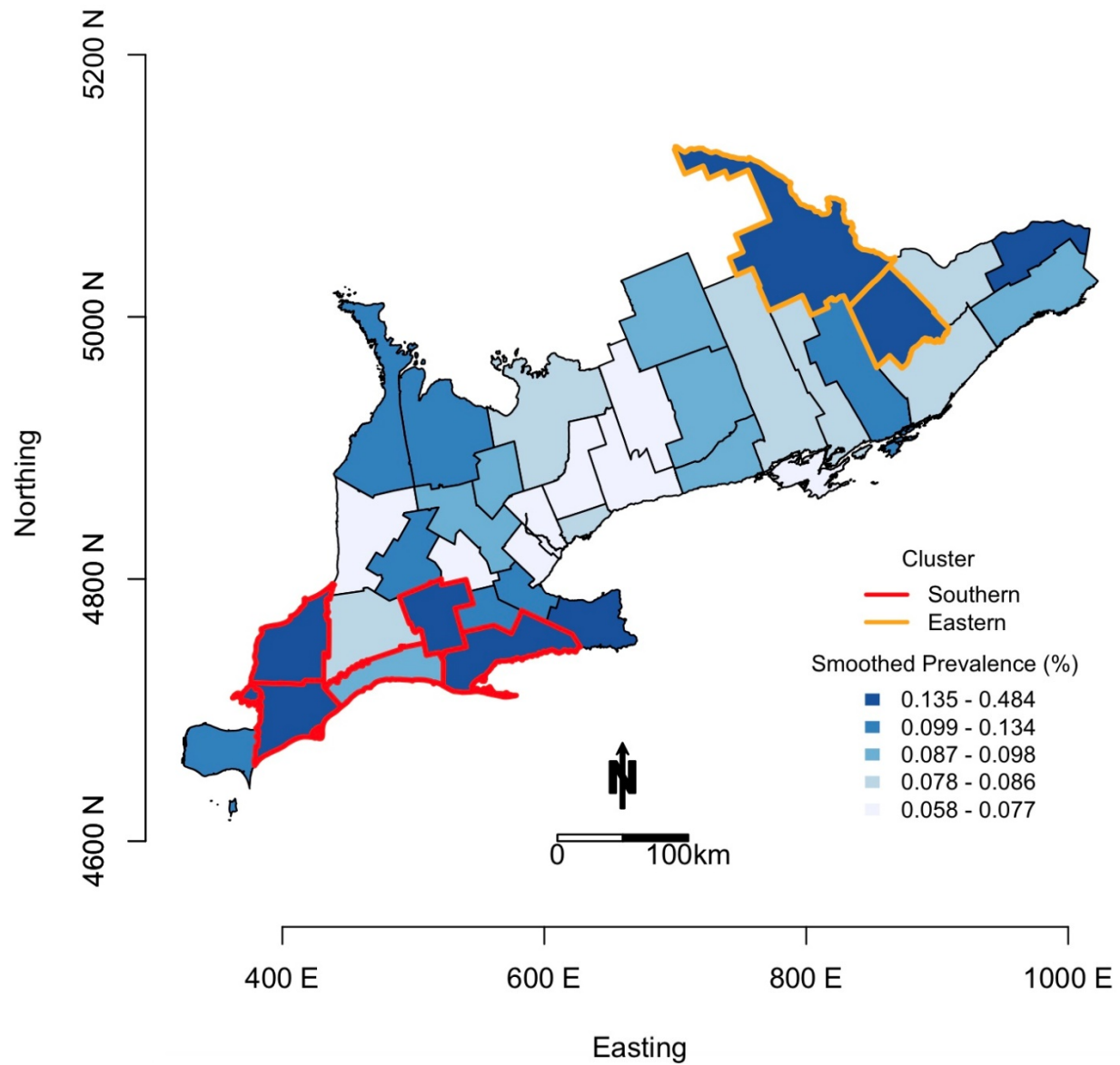
Figure 3.2. Choropleth map of the 37 census divisions in southern Ontario with aggregated smoothed heartworm infection prevalence from 2007 to 2016, using laboratory data for all dogs tested. Primary cluster (southern Ontario) in red, secondary cluster (eastern Ontario) in orange.
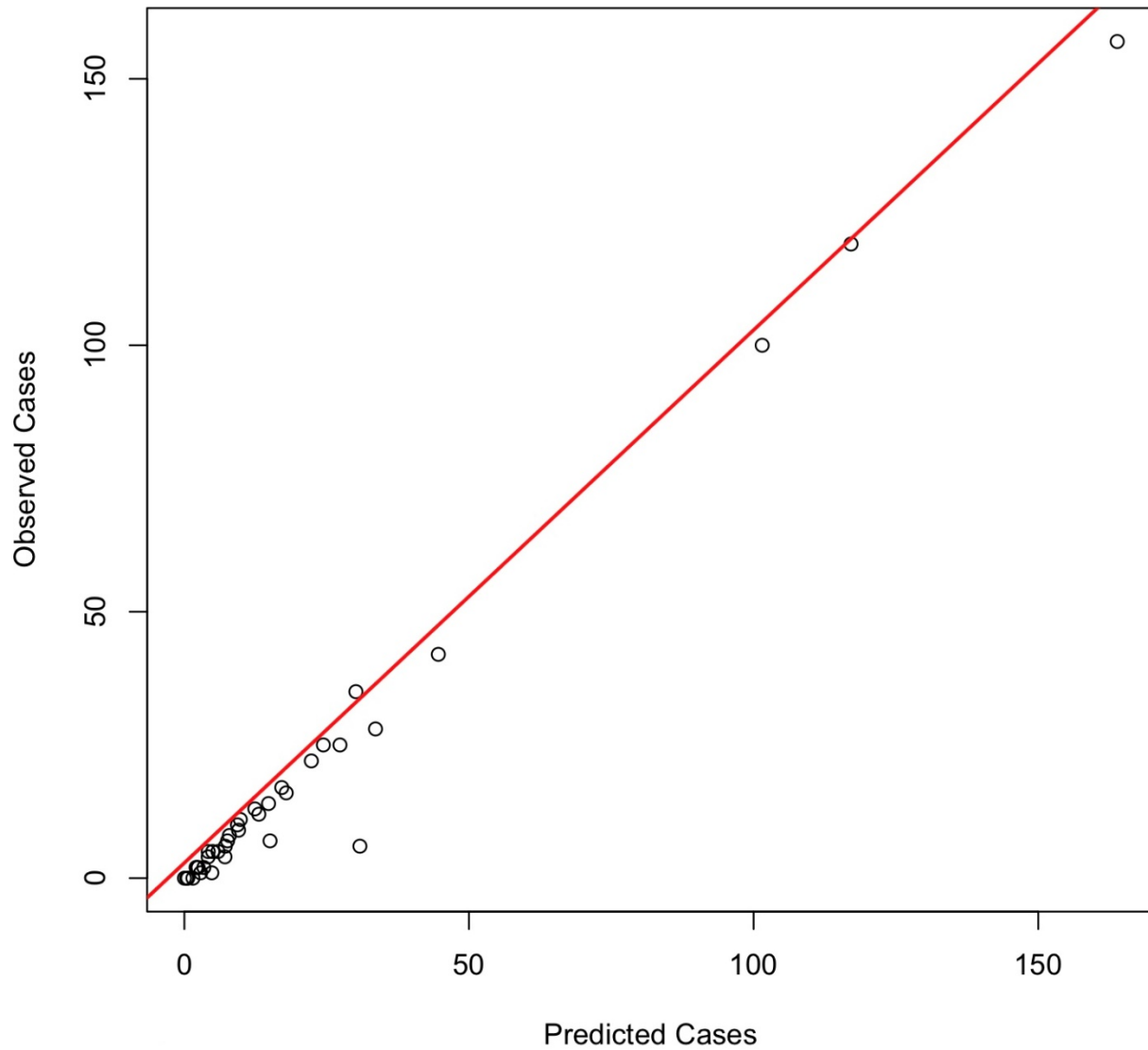
Figure 3.3. A plot of the observed heartworm infection cases in dogs from the laboratory data for the 37 southern Ontario census divisions against the predicted cases based on variables included in the spatial GLMM-PQL model.

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Chapter 4

Conclusion

Canada has been affected by climate change, with some regions in southern Ontario experiencing longer summer heatwaves from 2010-2012 (Bishop-Williams *et al.,* 2016). Thus, heartworm infection prevalence and geographic distribution is expected to have changed in this region since it was last monitored in 2010 (Slocombe, 2011). A more recent estimation of heartworm prevalence among dogs is required in order to compare it to historic prevalence estimates. Two datasets, annual surveys and cumulative laboratory diagnostics, were used to examine heartworm infection in dogs in Canada from 1977 to 2016. The combination of datasets provided a long-term insight into the spatial-temporal distribution of heartworm and efficacy of prophylaxis in Canada with a focus on the southern Ontario dog population and the impact of putative risk factors and climate change on the heartworm infection prevalence.

Heartworm in dogs in Canada has changed across space and time from 1977 to 2016. Although there is an indication of heartworm prevalence changes as illustrated by the distribution in Ontario and increased prevalence in Manitoba and Quebec from 2007 to 2010, this effect is likely confounded by prophylactic treatment and veterinary testing habit changes due to concern over tick-borne pathogens. There may be other unknown risk factors contributing to the epidemiological change seen in heartworm infections among dogs in Canada. Addressing the changes seen in heartworm infections should be done at the animal and human level through discussions around prophylaxis and better understanding of the local exposure risk.

4.1 Summary of Findings

The two datasets were consistent in showing a decreasing trend for heartworm infection prevalence in Ontario and Canada over the entire study period from 1977 to 2016 (Chapter 2 – Figure 2.1). However, the laboratory data illustrated that there has been an increase in apparent heartworm prevalence in Quebec and Manitoba from 2007 to 2016, whereas the survey data showed a decrease in these provinces from 1977 to 2010 (Chapter 2 – Figure 2.1). There was a difference in diagnostic methods used between the two datasets which could explain why results were conflicting. Around 80% of veterinarians in the survey dataset used antigen testing as a diagnostic (Slocombe, 1978); however, the veterinarian made the final decision regarding heartworm status of the dogs. This may have led to a misclassification of cases and may explain

why the two datasets did not provide congruent results. Where the survey dataset may have had inconsistent reporting, the laboratory dataset was comprised of only antigen test results with a consistent heartworm diagnostic component.

Climate change may be associated with the increasing prevalence seen in Manitoba and Quebec that has provided more adequate conditions for heartworm maturation to the infective stage within the mosquito population (Sassnau *et al.,* 2014; Bouchard *et al.,* 2015). Other vectors have experienced increased distribution across Canada due to a changing climate providing warmer temperatures, such as the vector for *Borrelia burgdoferi* (Greer *et al.,* 2008; McPherson *et al.,* 2017). However, climate change as an explanation conflicts with Ontario prevalence results, which have seen a decrease in heartworm infection prevalence from 1977 to 2016 (Chapter 2 – Figure 2.1). It could be possible that climate change has created a more supportive mosquito and *D. immitis* environment in Manitoba and Quebec, while providing a less hospitable environment in Ontario. There may be regions in Ontario that through climate change are drier and do not provide a suitable mosquito habitat.

The increase seen in Manitoba and Quebec could also be attributed to selection bias from increased testing over 2007 to 2016, because of concern over tick-borne infections – the 4Dx test is a combination test for heartworm and tick-transmitted *B. burgdoferi* among others. Heartworm selection bias would occur when clinics who have diagnosed heartworm cases before request their clients to agree to testing, while clinics that have less experience with heartworm are less concerned and do not suggest testing to dog owners. However, with a diagnostic focus on Lyme disease, the potential for heartworm selection bias would be minimized. The focus on Lyme explains the increase in test population, although it does not explain the increase in heartworm infection prevalence.

Spatial investigation of the heartworm infection prevalence in Ontario from 2007 to 2016 uncovered three hot spots: southern Ontario (Chatham-Kent, Lambton, Elgin, Oxford and Haldimand-Norfolk), eastern Ontario (Renfrew and Lanark) and western Ontario (Rainy River). The respective infection map from 2007 to 2016 illustrated that heartworm infection can be found across Ontario and is not restricted to a single region (Chapter 3 – Figure 3.1). Historically, the foci of heartworm infection in Ontario was in the south-west, defined as being below the highways 402 and 403 that connect from Sarnia across to Hamilton (Klotins *et al.,*

2000); however, it appears to be spreading further north (Appendix III – Figure 3). The apparent prevalence of heartworm in Ontario decreased from 1977 to 2016, providing conflicting results as there are heartworm infection clusters across the province despite the decreased prevalence (Chapter 2 – Figure 2.1; Appendix III – Figure 3). It is also surprising that Essex census division, home to the city of Windsor, was not included in the southern Ontario cluster; it was weather data from Windsor that informed the historic HDU model which the Canadian transmission season is based on (Slocombe *et al.,* 1989).

The decrease in Ontario prevalence could be due to the increased sample size for both datasets during the study period; from 28,732 in 1981 (first year with provincial summaries) to 289,229 for the survey dataset and from 3,782 in 2007 to 136,581 for the laboratory dataset. The number of positive test results also increased for the laboratory dataset from 16 in 2008 to 176 in 2015 (2007 and 2016 were partial years). However, the positive test results decreased for the survey data from 528 to 431. If early reports of heartworm infection in the survey dataset were indeed impacted by selection bias, the bias would be moderated by a moving focus from heartworm to Lyme testing in areas where the risk of heartworm is lower than the historical provincial average. Thus, there are confounding factors at work that make it difficult to distinguish a true decrease in the risk of heartworm from a decreasing prevalence due to changes in sampling (i.e. diagnostic testing) and uptake of prophylactic measures.

Heartworm preventives were approved with 100% efficacy; however, preventive efficacy was tested on a small sample size of dogs, generally 8-14 dogs, which may not be generalizable to a larger dog population (Hampshire, 2005; Vidyashankar *et al.,* 2017). The Attributable Fraction Exposed was used as a measure to estimate the efficacy of heartworm prophylactics over 1996 to 2010; overall efficacy during that time was 93%, with lowest efficacy in 1996 at 62% and the highest efficacy of 98% in 2010 (Chapter 2 – Table 2.3). In 2010, the last year preventive history was available, efficacy was high in Canada at 98%; although the estimated efficacy may have changed since. Efficacy of preventive medication is dependent on compliance and the drug susceptibility of parasites; since there is currently no evidence that drug-resistant parasites are endemic within Canada, the true efficacy may be higher than this study estimated. A survey assessing compliance with products used to control fleas and ticks among 559 dog owners across the United States found that only 62% correctly recalled their veterinarians'

recommendations (Lavan *et al.,* 2017). Furthermore, based on medication sales, compliance was 50% (Lavan *et al.,* 2017).

Risk factors for heartworm infection including climate and human population size were modeled using a spatial Poisson regression model to assess their impact on heartworm infection prevalence among dogs in southern Ontario. The results indicated that heartworm development units (HDUs) and climate moisture index (CMI) were positively associated with prevalence of heartworm infection (p<0.05), whereas lower human population was negatively associated with heartworm infection prevalence (p<0.05). The predicted relative prevalence from the model, when compared to the observed prevalence, exhibited a good fit, meaning the risk factors included in the model accurately predicted the observed prevalence and explained the hot spots seen in Ontario. Hot spot regions may have environments that are more supportive for the maturation of the parasite; thus, contribute to a locally increased number of infections, i.e. higher than would be expected. The risk factors in those regions may be useful in explaining the higher number of infections.

4.2 Study Limitations

Limitations of the survey dataset included likely selection bias towards veterinary clinics which had experience with heartworm and thus a natural interest in heartworm. Diagnostic methods for heartworm were not consistent and not fully reported. Around 80% of veterinarians in 1977 used blood tests for microfilariae as the primary diagnostic and 19% of veterinarians in 1989 reported using immunodiagnostic tests, although several practices still used blood smears (Slocombe, 1978; Slocombe, 1990). Veterinarians determined the positive/negative status of dogs based on test results without a case definition (Slocombe, 1978). There was also a lack of information regarding confirmatory testing, which possibly lead to misclassification of cases.

Limitations of the laboratory dataset were an overrepresentation of Ontario dogs (~75%, of all records). Thus, some provinces were under-represented, for example Prince Edward Island had only two observations from 2007 to 2016 in total. There was also a lack of information provided on the dogs' travel history, prophylaxis status and confirmatory testing. And finally, the sensitivity and specificity of the tests used may have been different for the study population compared to the population from which these measures were estimated (Noordhuizen *et al.,* 2001).

There was a spatial scale limitation to the choropleth maps, as the scale was either at the provincial level or census division and did not depict small area variation. Choropleth maps imply constant risk across a region, while variation within a region is to be expected. The risk of heartworm infection is for the locations where testing occurred and not for the entire region. For example, the map indicated non-zero risk for vast uninhabited areas in northern Ontario; however, the prevalence represented would be for nearby the veterinary location of the test submissions not the entire region. The choropleth map of the geographic distribution of veterinary clinics that submitted samples in Figure 2.3 (Chapter 2), illustrates this point; clinics were mostly spread across southern borders of Canadian provinces and were not evenly distributed within each province. Similarly, the risk of an infection is not expected to "know border lines" and does not increase or decrease when crossing into a different region, which is implied by choropleth maps. As an alternative, Berke (2004), has proposed the use of isopleth maps to visualize the spatial variation without borders.

4.3 Future Research

As a recommendation from this study, further investigation should be conducted on the mosquito species currently inhabiting Canada both transiently and permanently. There may be species capable of supporting the heartworm lifecycle that prior to the changing climate were not present, including the invasive *Aedes albopictus*, which has expanded its northern range in the United States (Morchón *et al.,* 2012; Armstrong *et al.,* 2017). Surveying of mosquito species should be conducted in the identified risk areas, such as the clusters in Ontario and the provinces of Manitoba and Quebec.

The heartworm development unit model for predicting the transmission season was pioneered in Canada; the transmission season was determined based on the mosquito species inhabiting Canada in the 1970's, and climate data from 1957-1986 (Slocombe *et al.,* 1989). The predicted transmission season should be re-examined as Essex census division was not included in the Ontario clusters, yet the predicted transmission season was based on climate data from this region. There may now be mosquito species other than the early season vector, *Ae. stimulans,* that should be used to determine the start of the transmission season. *Aedes stimulans* was observed to take blood meals as early as May 6th; the HDUs were summed from this date onwards (Slocombe *et al.,* 1989). The HDU data from 2005 to 2016 had days with temperatures

above the 14°C threshold prior to May 6th, possibly suggesting transmission may occur prior to June 1st in certain regions (Slocombe *et al.,* 1989). Furthermore, there were days above the 14°C threshold past the predicted end of transmission date of October 9th (Slocombe *et al.,* 1989). This is of specific importance, because the predicted transmission season is linked to recommendations for prophylactic treatment across Canada.

The changing heartworm infection distribution in dogs in Canada requires investigation and examining Quebec and Manitoba at a finer scale would provide further information. The laboratory dataset for these two provinces was comprised only of a small number of samples (i.e. not all census divisions in these provinces supplied samples). It would be beneficial to combine heartworm antigen tests from other sources, e.g. Antech Diagnostics (Antech, 2018); this would allow for more detailed spatial analysis in these provinces, including the search for possible clusters or points of emergence with the flexible scan test. Risk factors for heartworm infection in Manitoba and Quebec could also be evaluated to assess their impact on infection and compared to Ontario to determine why Ontario has seen a decrease in heartworm prevalence from 1977 to 2016, while Manitoba and Quebec have seen an increase from 2007 to 2016.

4.4 References

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Appendix 1: Data Cleaning

The raw data received from IDEXX Laboratories Canada Corp were cleaned before any analysis was performed. Below is a step-by-step guide through the data cleaning process:

1. Datasets were supplied in different CSV files: Database_2007_2010, Database_2011, Database_2012, Database_2013, Databse_2014, Database_2015 and Database_2016

2. The datasets were merged into one table and exported to a CSV file using R/RStudio

3. The raw combined dataset in a CSV format were imported back into R/RStudio

4. Data were examined

5. Duplicate "Accession" Numbers were removed

6. The dataset included non-canine species, including: alpaca, amphibian, avian, bear, camelid, equine, feline, ferret, mouse, not specified, other, ovine, pinniped, porcine, reptile and unknown

7. Non-canine species were removed

8. The two written versions of canine – "canine" and "Canine" were edited to have the value written one way, "Canine", in the dataset

9. Dogs older than 240 months (20 years) and dogs under 6 months were removed due to biological plausibility and the diagnostic requirements of the test

10. Values not coded as "0" negative, or "1" positive for heartworm test were removed

11. The sex of the dog was edited to code only "Male" and "Female". "Spayed Female" were coded as "Female" and "Neutered Male" coded as "Male". Canines coded as "NA" or "Unknown" were coded to "Not Specified"

12. Province values were edited for consistency. All various forms of province names were re-coded as their two-letter short form e.g. "ON" for Ontario or On

13. Hyphens between the six-digit postal codes were removed to create postal codes in the form X1X 1X1 instead of X1X-1X1.

14. A CSV file of the clean data was written and exported to use for data analysis

Appendix II: Postal Code and Census Division Matching in Ontario

Laboratory data were collected provided with the postal codes for each of the veterinary clinics that submitted seroprevalence samples for diagnostics. The point data available is not representative of where the dogs live, or the range of territory they may cover on walks. Heartworm is also a "rare" disease in Canada, and there are many regions from where samples were not submitted. Aggregating to a higher level of spatial area was necessary to represent a more accurate area that the dogs may live in, and the potential exposure area.

To examine Ontario in more detail the census division level was selected as a mid-level scale. The census sub-division (CSD) level is very fine scale (Ontario has 574 CSDs), and some of the northern regions did not submit samples. Ontario has 36 public health units and so the census division level, with 49 units, was selected for mapping purposes as it is larger than a CSD, yet smaller than a public health unit.

The data were aggregated over time to conduct a spatial analysis. Some of the northern regions in Ontario lacked test results certain years thus, the data were aggregated to provide adequate samples across Ontario to perform a spatial analysis. Temporal patterns were analyzed for an increase or decrease in trend, but a space-time analysis was not conducted.

Postal codes required translation to their corresponding census division unit. A postal code to census translation table is a look-up table between the Multiple Enhanced Postal Code product (MEP) (DMTI, 2014) and the Canadian Census product (DMTI, 2014). The Postal to Census Translation Table contains information regarding the relationships between the six-digit postal code point geometry to different levels of census geography, available for the years 1996, 2001, 2006 and 2011 (DMTI, 2014). The postal codes from the laboratory data did not contain the information to their corresponding MEP unit.

Ontario postal codes were examined for aggregated years 2007 to 2016. There were 21 test results missing postal codes and were excluded from further analysis. It was also determined that there were ten test results with the same Newfoundland postal code listed as being collected in Ontario. These were also excluded. There were 1195 unique postal codes in Ontario (with thousands of observations) to be matched to their unique MEP identification numbers. Any obvious transcription errors based the postal code pattern, e.g. I instead of 1 or O instead of 0, were made to allow for matches.

The Multiple Enhanced Postal Codes (MEP) – 2014 product (DMTI, 2014) was used to match the laboratory postal codes to this document to translate the MEPs to census division units using the Postal to Census Translation Table (DMTI, 2014). The MEP 2014 product contains two files, an active MEP file and a retired MEP file. The files were opened in ArcGIS and the province "Ontario" attribute was selected out, as this was the variable of interest. There are many repeating postal codes in the MEP file, and according to the documentation with the file (DMTI, 2014), it is suggested to use the Single Link Indicator (SLI) = 1 when there are multiple records because there is only one MEP associated with SLI=1 and it is the main postal code record. The SLI= "1" attribute was selected to be used to match postal codes to their MEP identification.

Initially 1171 postal codes matched to the MEP 2014 product (active). There were 14 postal codes that matched to the MEP 2014 product (retired). Ten postal codes did not match either file and were further examined. Two were missing their last digit; through searching the first 5-digits using MEP product, the final digit was discovered, and matches were made. Three postal codes did not have the middle space separating the digits and when added they matched to the MEP 2014 product. The final five unmatched postal codes could have been new postal codes and therefore did not match a file from 2014. The GoogleTM search engine was used for the five unmatched postal codes to determine if they existed and were not a typo. The city that came up associated with the address for that postal code was used for postal code matching and matched to the most similar postal code included in the MEP 2014 product from the same city. *E.g. L0H 0E9 does not match to active MEP 2014 product, when searched, that postal code is an actual address in Gormley, Ontario. The closest postal code in the MEP 2014 product with the city Gormley is L0H 1G0, this postal code was used in the place of L0H 0E9 to create a match to the document.*

This procedure was completed for the five unmatched postal codes. After running the join function in ArcGIS between the laboratory postal codes and the active and retired MEP 2014 products, they all matched.

The census translation table also contained an active and retired table. These were each joined to the respective and active MEP/laboratory data join to match the MEP to the census division. The two translation tables were then merged to create one document with the retired

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and the active MEP, postal code and census division unit identifiers to be used for mapping purposes.

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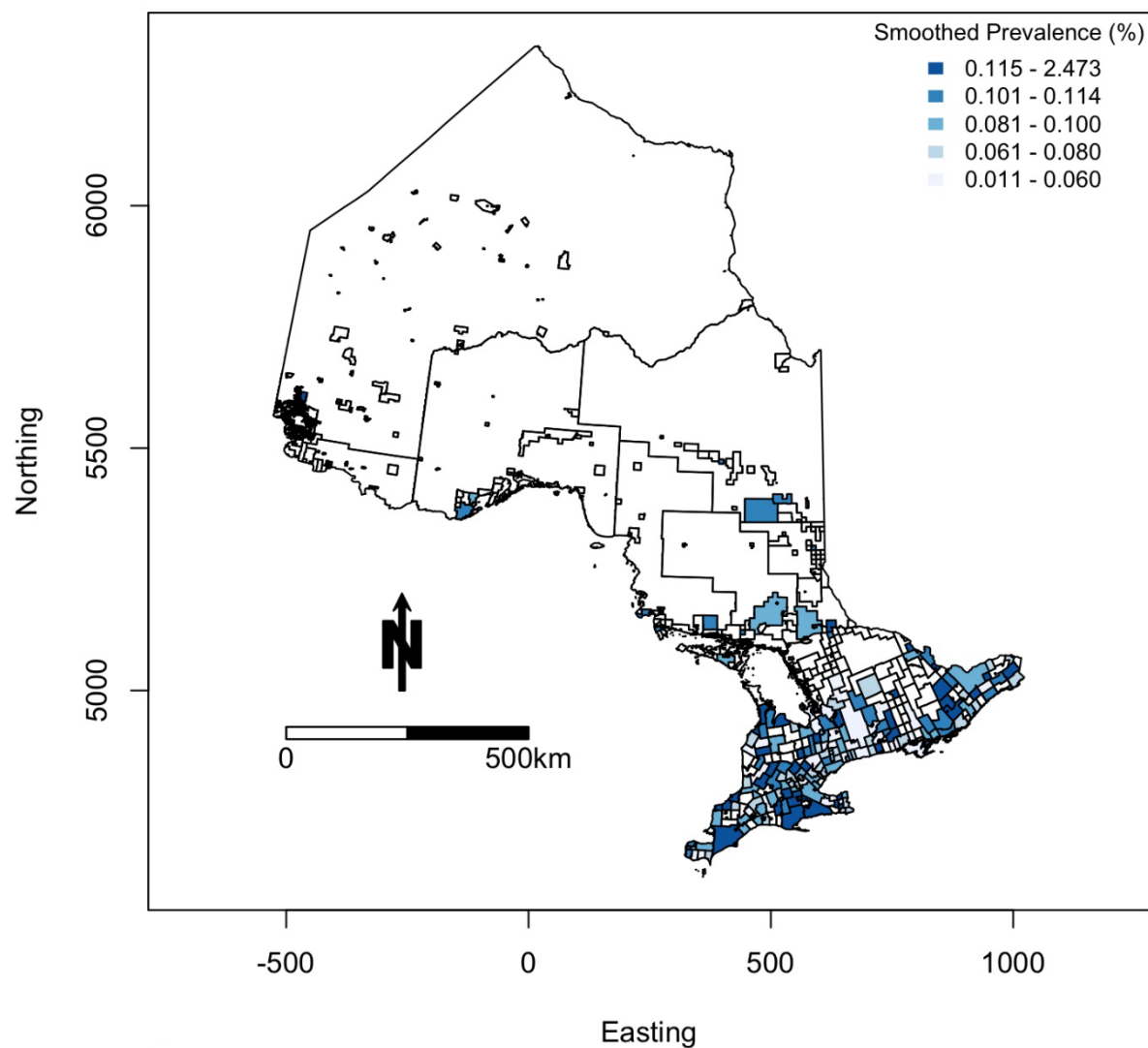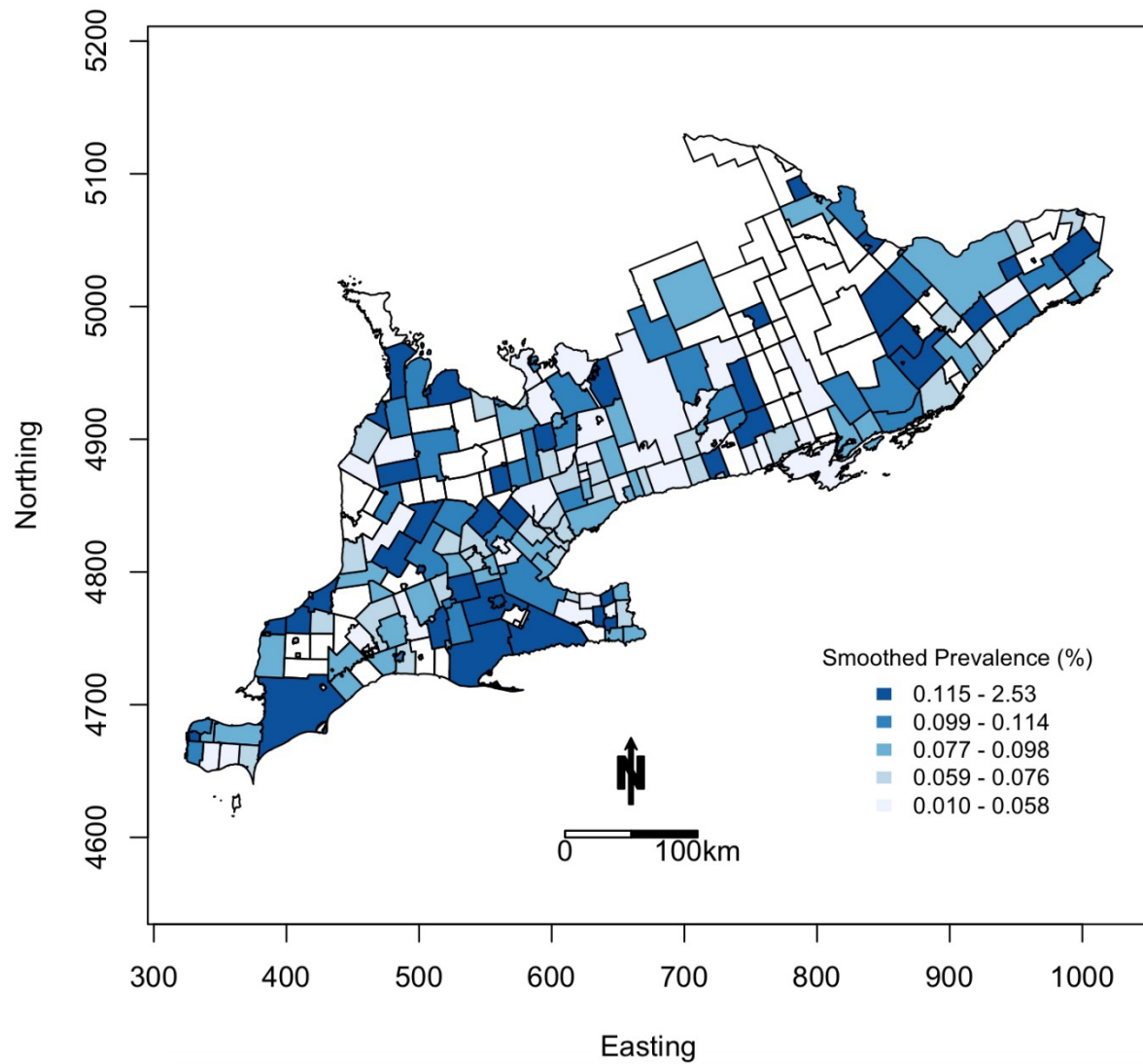
Figure 1. Choropleth map of all 574 Ontario census subdivisions with aggregated smoothed heartworm infection prevalence from 2007 to 2016 using laboratory data for all dogs tested.

Figure 2. Choropleth map of all 288 southern Ontario census subdivisions with aggregated smoothed heartworm infection prevalence from 2007 to 2016 using laboratory data for all dogs tested
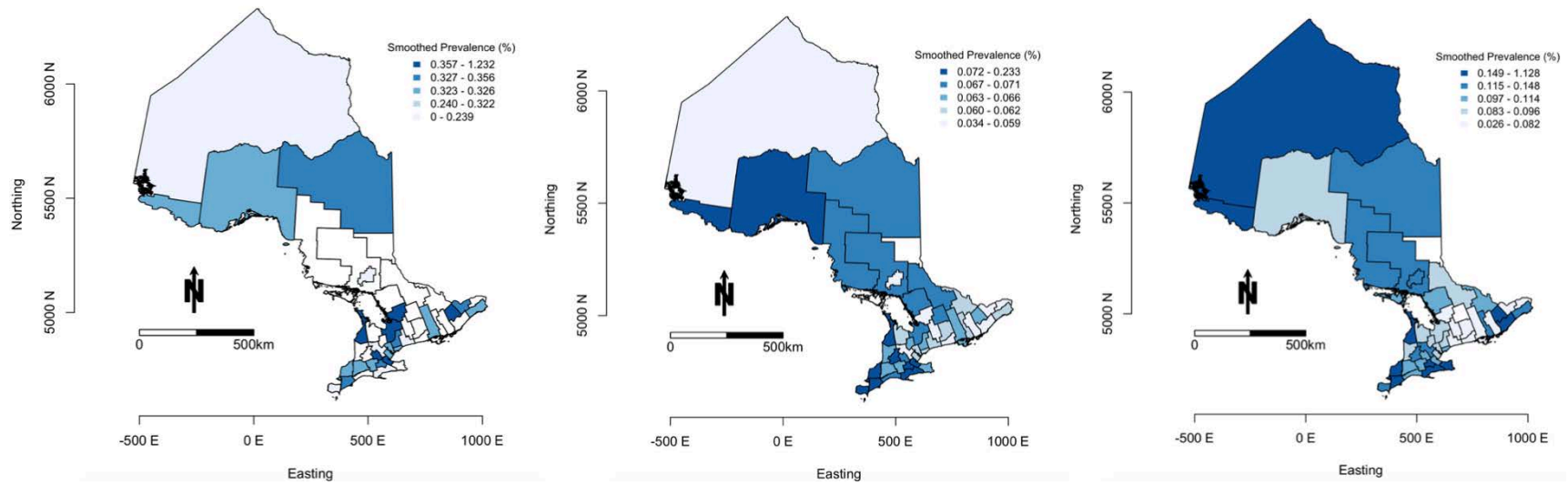
Figure 3. A comparison of heartworm infection prevalence distribution in domestic dogs for all 49 census divisions in Ontario over the study period. Left panel - 2008; middle panel - 2012; right panel - 2015.
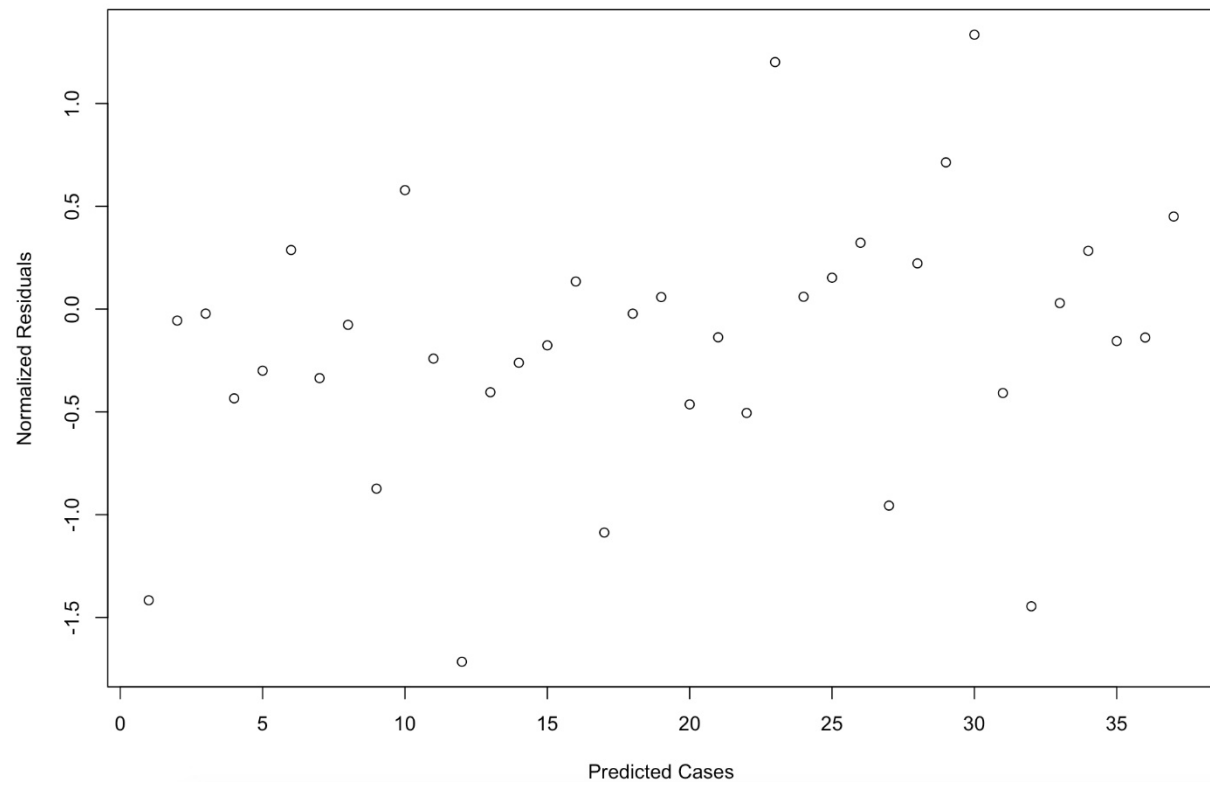
Figure 1. Plot of the normalized residuals from the spatial Poisson regression model for heartworm infection in dogs in southern Ontario against the predicted case prevalence based on the risk factors included.
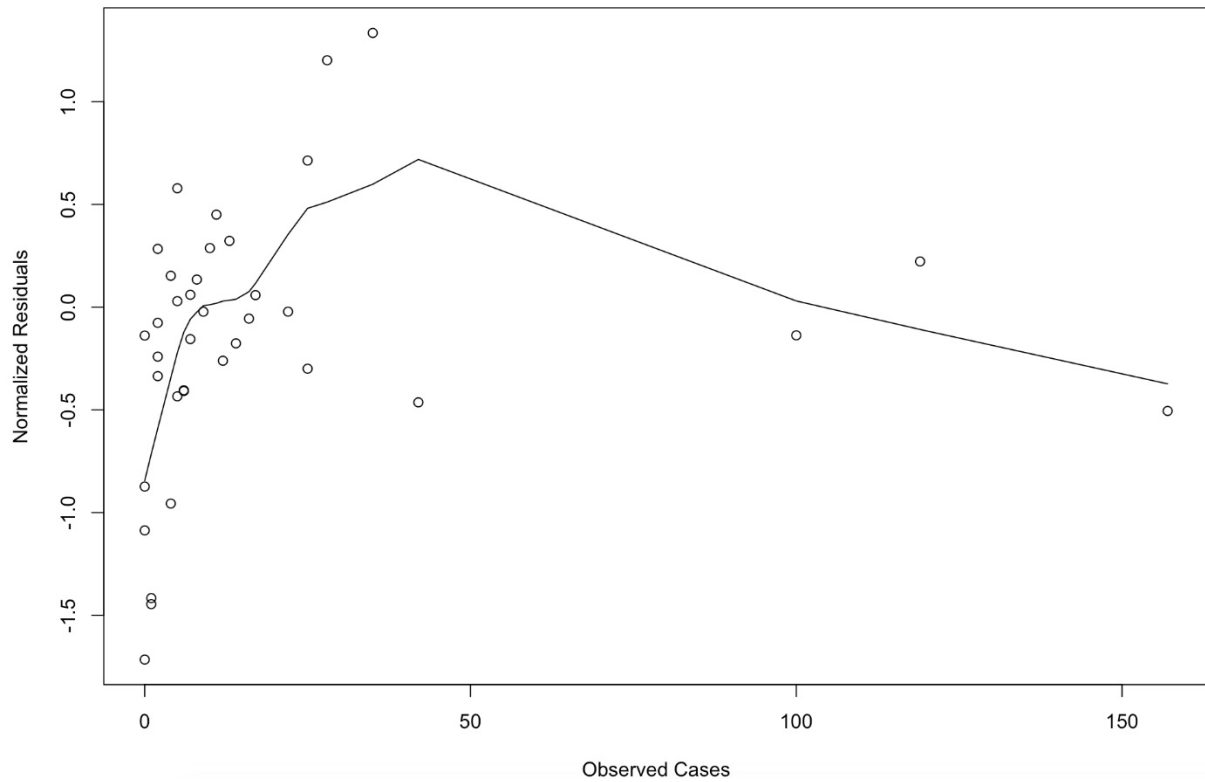
Figure 2. A plot assessing the linearity of the normalized residuals from the spatial Poisson regression model for heartworm infection in dogs in southern Ontario against the observed heartworm infection cases.
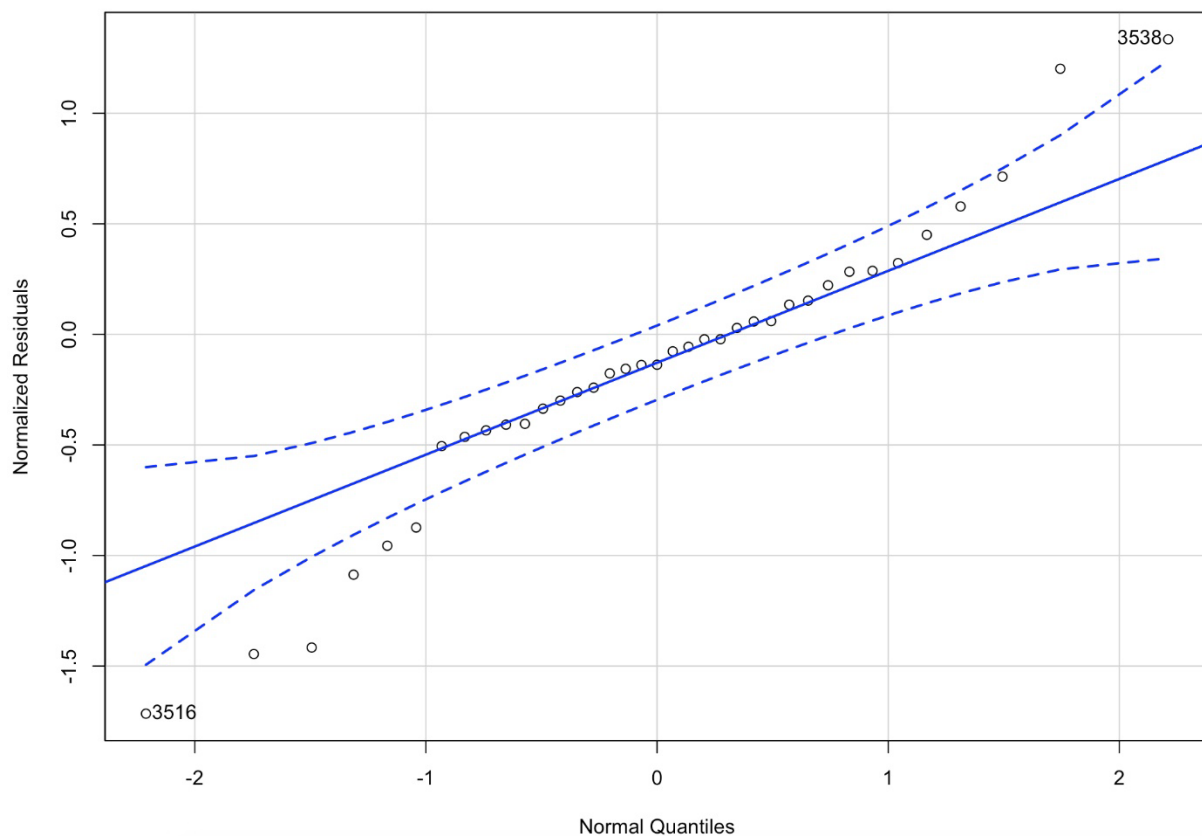
Figure 3. A QQ-plot to assess the normality of the normalized residuals from the spatial Poisson regression model for heartworm infection in dogs in southern Ontario.