

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

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Abstract

Dirofilaria immitis is the causal agent of heartworm, a mosquito-borne parasite that primarily infects domestic and wild canids. The infection is endemic in parts of Canada, and Ontario has been identified as the province where the majority of heart-

worm infections occur. 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 and located in western, southern and eastern Ontario, respectively. 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 directions, east or north, with heartworm infection. The model identified the spatial distribution of HDUs and CMI as positively associated with heartworm infection and therefore important predictors of the infection. In contrast, human population size, increasing northern latitude and drier areas were negatively associated with heartworm infection. The east direction and precipitation were not significant.

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Introduction

The parasite *Dirofilaria immitis*, commonly known as heartworm, primarily infects dogs but can also infect felids, wild canids and, though very rarely, humans (Vezzani and Carbajo, 2006). Domestic dogs and wild canids serve as the primary reservoirs (Ciucă *et al.*, 2016) with mosquitoes 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 and Slocombe, 1984; Slocombe *et al.*, 1989). *Ae. 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). *Ae. stimulans* can be found as early as mid-May and can live into the summer months (Slocombe *et al.*, 1989).

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°C to 34°C (Fortin and Slocombe, 1981). The amount of heat required for larval development to the infective stage is 130 heartworm development units (HDU) (Slocombe *et al.*, 1989), which is defined as the cumulative sum of daily aver-



age degrees centigrade above the 14°C threshold temperature within a period of 30 days (Slocombe *et al.*, 1989; Knight and Lok, 1998). In Canada, warmer temperatures are generally first observed in southern Ontario compared to other areas in the province and country. Based on weather data from 1957-1986 collected from Windsor, Ontario the transmission period for Canada is considered June 1st to October 9th (Slocombe *et al.*, 1989). However, the wide range of climate across Canada means that the true risk period varies geographically. Historically, the start of transmission was 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 and Lok, 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, the drug family used for preventive medication, are marketed as oral (ivermectin and milbemycin oxime), topical (moxidectin and selamectin) and subcutaneous injectable (slow-release moxidectin-impregnated lipid microspheres) products, which all have one month's reach-back activity (Graham *et al.*, 2012).

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). A temperature increase 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). It is possible that climate change has impacted the heartworm infection prevalence among domestic Ontario dogs.

Many factors influence transmission of heartworm including those that impact mosquito populations. Precipitation, vegetation and moisture can impact breeding, consequentially impacting *D. immitis* transmission (Alho *et al.*, 2014). Mosquitoes also 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. It is not known how factors, such as precipitation, which impact the mosquito population, may impact heartworm infection among domestic dogs in Ontario.

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, precipitation, moisture index and human population size and heartworm infection prevalence in companion dogs using a spatial Poisson regression model.

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. SNAP 3Dx tested for *D. immitis*, *Borrelia burgdorferi* and *Ehrlichia canis*. SNAP 4Dx and 4Dx Plus tested for *D. immitis*, *B. burgdorferi*, *E. canis*, *Anaplasma phagocytophilum* and *A. platys*. 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 *e.g.* felines. Test results that had not been recorded as 0 (negative) or 1 (positive) were removed. The age of the dog were reported in months; dogs younger than 6 months of age (minimum age the antigen test would be able to detect heartworm) and older than 240 months (20 years) were removed.

Natural Resource Canada (<https://www.nrcan.gc.ca/home>) supplied HDUs and climate normals for the centroid of all 49 Ontario census divisions based on thin plate spline smoothing algorithms to develop spatially continuous climate models using weather station data (Natural Resources Canada, 2018). The centroid locations were applied to the grid model to provide the climate normals for 1980 to 2010 for that location, including the mean moisture index and mean precipitation. 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 observed recorded daily temperatures using the grid model for the census division centroids and 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. The start of transmission was defined as the accumulation of a minimum of 130 HDUs within 4 weeks (using the weekly data provided), roughly the lifespan of a mosquito (Fortin and Slocombe, 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 (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., 2018). The MEPs were then matched to the corresponding census division number using a postal code to census division translation table (DMTI Spatial Inc., 2018). 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 labelled 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 (2016) for the 2011 census year; 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 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, 2012). 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 (Assunção and Reis, 1999; Waller and 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 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 (number of persons), human population density (per one km²), 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 (Kulldorff *et al.*, 2009). The spatial scan test was applied 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 (ESRI, Redlands, CA, USA) 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 (*smc* 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 $\alpha=0.05$ was applied for all analyses, unless otherwise indicated.

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 partially sampled years.

The choropleth map of the smoothed heartworm prevalence for the 49 Ontario census divisions from 2007 to 2016 is shown in Figure 1; southern Ontario data are presented at higher resolution in Figure 2. There were three hotspots 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) (Figures 1 and 2). Each hotspot is outlined in a different colour for identification and was significant at the $\alpha=0.05$ 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 hotspot 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 1. Human population size had a protective effect on heartworm infection prevalence; as the number of persons within a census division 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 5 mm 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 (*per* one kilometer²) 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) 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).

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 in addition to the cluster in southern Ontario, there was also a cluster in northern Ontario, in Rainy River (Figure 1). Historically, northern Ontario did not have a heartworm cluster (Slocombe, 2011; Herrin *et al.*, 2017). It is possible that veterinarians in Rainy River did not expect heartworm to occur that far 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).

Climate change has been implicated in the changing tick distribution, including *Ixodes scapularis* the vector of *B. burgdorferi*; thus, Lyme disease has become a growing concern in Ontario (Greer *et al.*, 2008; McPherson *et al.*, 2017). 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, the proportion of tests occurring after heartworm season testing (April to June) increased from 24% in 2008 to 30% in 2015 (2007 and 2016 were partial years); suggesting the *D. immitis* positive results detected later in the year may have been accidental in testing for tick-borne pathogens.

The historic HDU 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

Table 1. Summary of the output from a spatial Generalized Linear Mixed Model Penalized Quasi-likelihood model for heartworm infection prevalence among dogs in southern Ontario over 2007 to 2016.

Variable	Coefficient Value	Variable Range*	P-value	PRR
East (km)	-0.0030	352.8-975.7	0.1967	0.15
North (km)	0.0088	4669.4-5058.0	0.0866	30.55
Precipitation (mm)	0.0976	74.5-94.6	0.0926	7.08
CMI (cm)	31.97	-0.73-1.96	0.0100	2.23×10^{37}
HDU	0.0101	211.3-816.8	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

PRR, prevalence risk ratio for the total variable; CMI, Climate moisture index; HDU, Heartworm development units (the amount of heat required for larval development to the infective stage (130 HDU). *Minimum and maximum value for that variable.

supplied by Natural Resource Canada (2018) (based on the historic model) accurately predicted the heartworm prevalence observed from the data, which illustrated the validity of the HDU model.

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 for detecting *D. immitis* 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 using the overall Ontario prevalence of 0.11% for the study years 2007 to 2016, and 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 and 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*

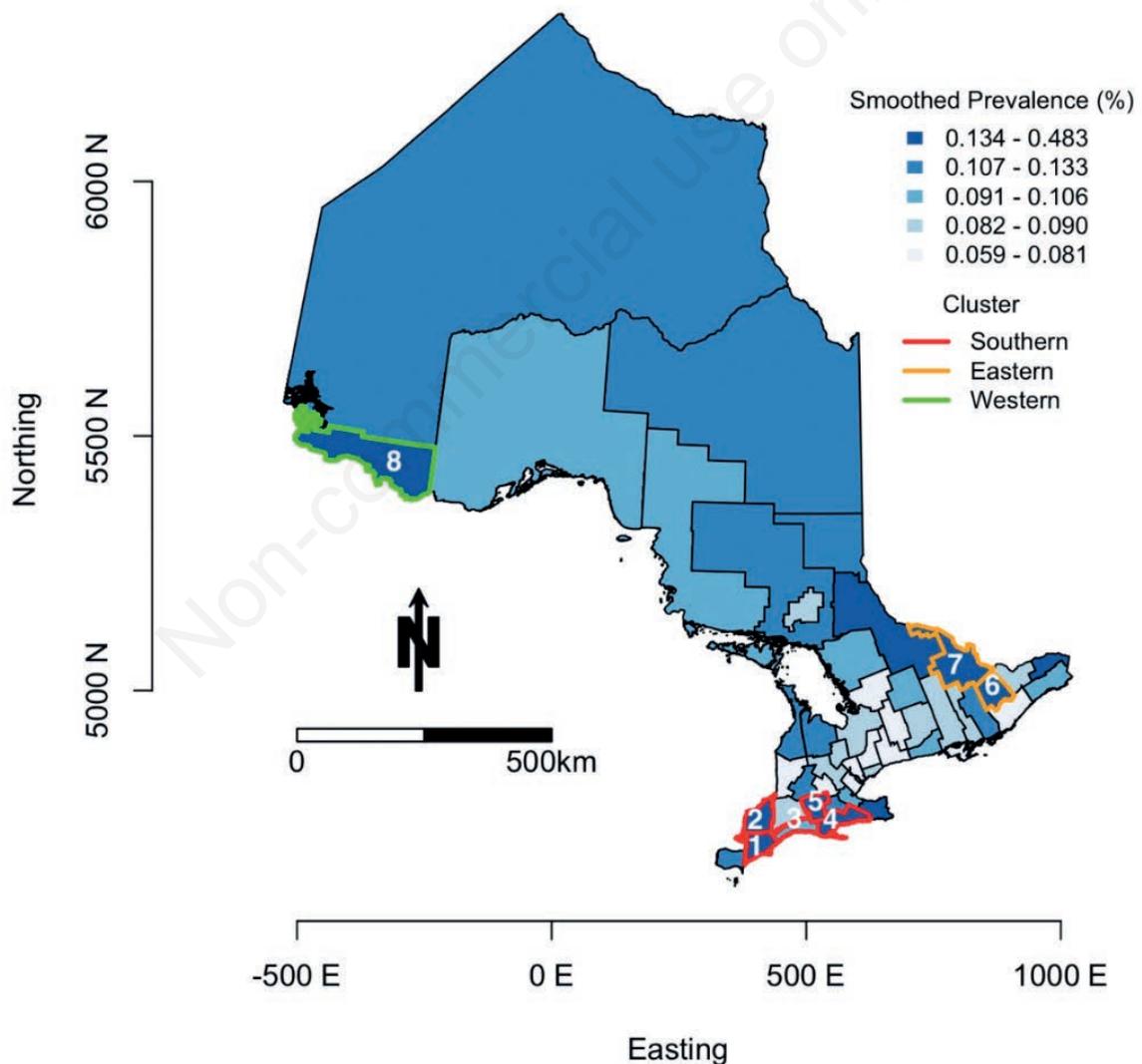


Figure 1. Choropleth map of all 49 Ontario census divisions with aggregated smoothed heartworm infection prevalence from 2007 to 2016 using laboratory data for all dogs tested. Red = primary cluster in southern Ontario (1- Chatham-Kent, 2- Lambton, 3- Elgin, 4- Haldimand-Norfolk, 5- Oxford); Orange = secondary cluster in eastern Ontario (6- Lanark, 7- Renfrew); Green = other secondary cluster in western Ontario (8- Rainy River).

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 for individual dogs. The dataset included a postal code, but that was for the veterinary clinic location, not the residence of the dog. Lastly, travel history outside the province or the country, was not available for this study. To avoid spatial misclas-

sification 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 extrinsic development 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; Slocombe, 2011). 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 hotspot 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.

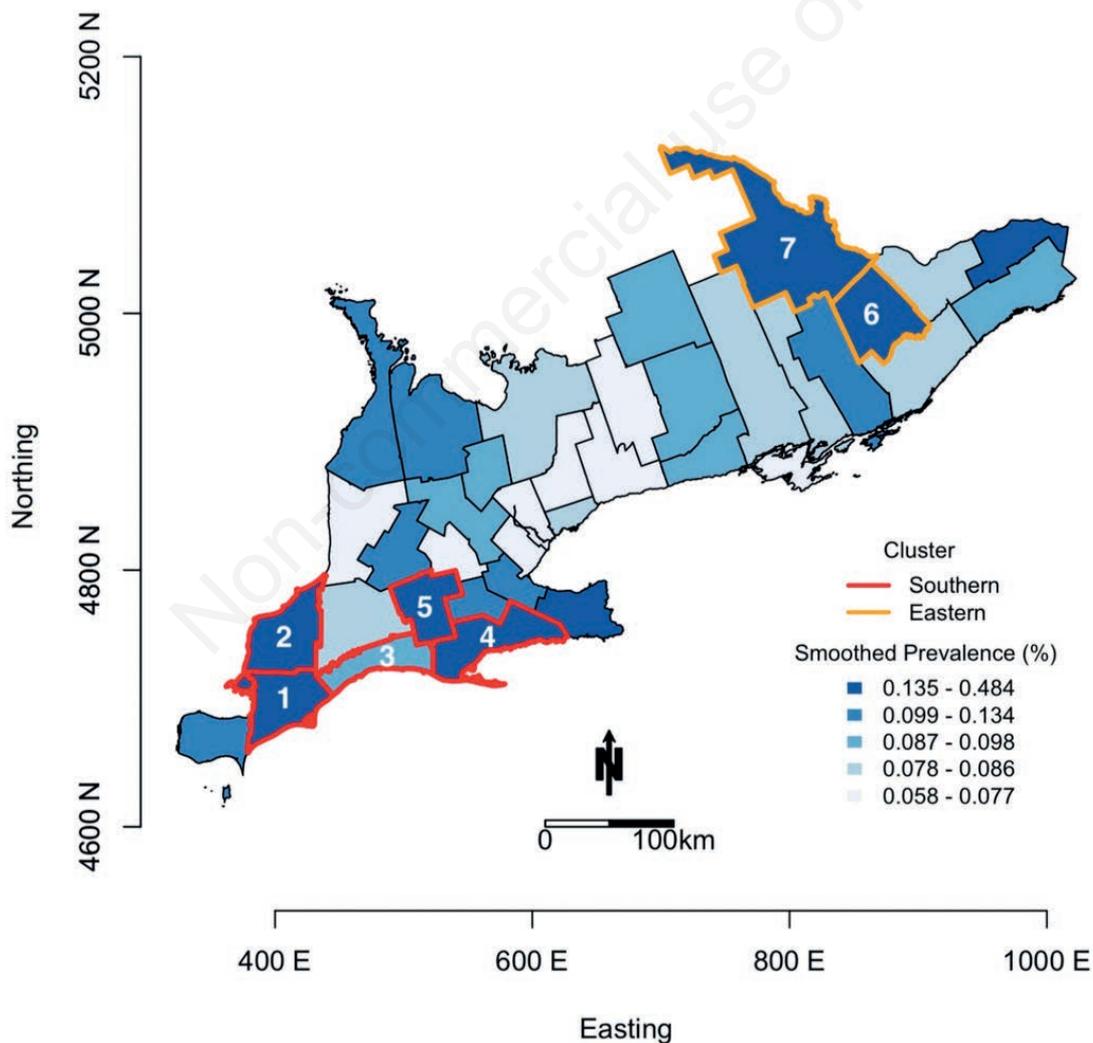


Figure 2. Choropleth map of the 37 census divisions in southern Ontario with aggregated smoothed heartworm infection prevalence from 2007 to 2016, using laboratory data for all dogs tested. Red = primary cluster in southern Ontario (1- Chatham-Kent, 2- Lambton, 3- Elgin, 4- Haldimand-Norfolk, 5- Oxford); Orange = secondary cluster in eastern Ontario (6- Lanark, 7- Renfrew).

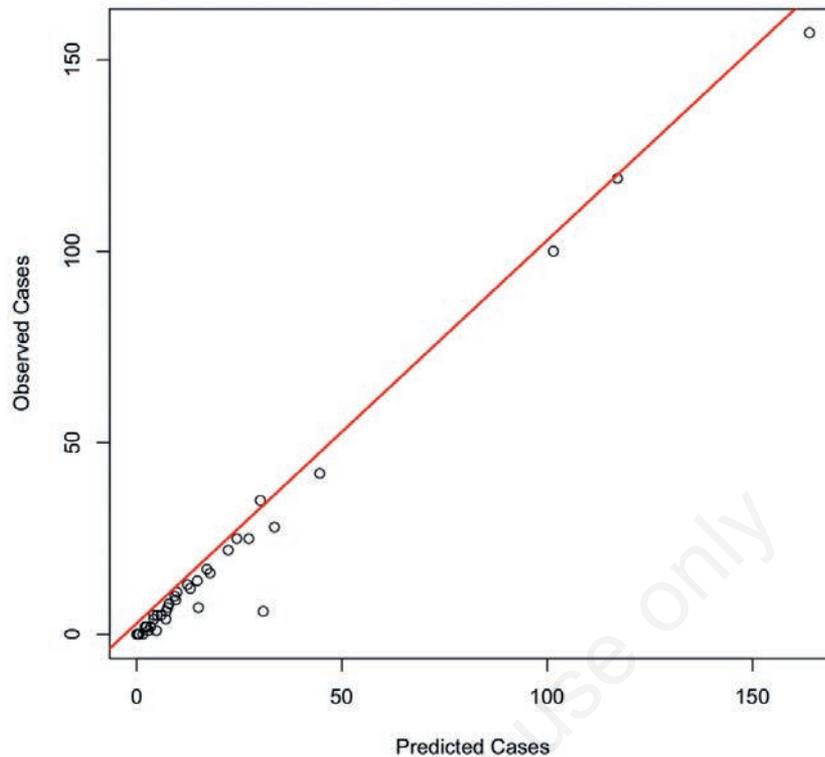


Figure 3. Plot of the observed heartworm infection cases in dogs from the laboratory data for the 37 southern Ontario census divisions against the predicted cases based on variables included in the spatial Generalized Linear Mixed Model Penalized Quasi-likelihood model.

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.

Conclusions

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.

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