Risk factors associated with the carriage of *Ixodes scapularis* relative to other tick species in a population of pet dogs from southeastern Ontario, Canada

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ABSTRACT

In eastern North America, the blacklegged tick, *Ixodes scapularis*, is the vector for *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum*, the causal agents for human and canine Lyme disease and anaplasmosis, respectively. The extensive range expansion of *I. scapularis* in Ontario is a growing veterinary and public health concern. However, there is limited information on the risk factors associated with *I. scapularis* carriage on dogs. Within an emerging area for Lyme disease risk in southeastern Ontario, we identified the tick species carried by dogs; determined the prevalence of *B. burgdorferi* and *A. phagocytophilum* in *I. scapularis*; and examined associations between pet demographics, travel history, and geographical location and the odds of: a dog carrying *I. scapularis* relative to other tick species (i.e., case-case design), and a removed *I. scapularis* being infected with *B. burgdorferi*. Seven species of ticks were collected from 543 companion dogs at 20 participating veterinary hospitals from April to December 2015. *Ixodes scapularis* were detected on 85.6% of parasitized dogs, and 7.5% of these dogs were carrying at least one *B. burgdorferi*-positive tick. Based on a multivariable logistic regression model, the odds of *I. scapularis* infestation relative to other tick species was significantly higher in fall and spring compared to summer, with closer proximity to Lake Ontario, in female compared to male dogs, in dogs weighing over 30 kg compared to lighter dogs, and in dogs that had not visited a farm in the 7 days prior to tick removal. Based on univariable exact logistic regression models, the odds of *B. burgdorferi*-positive *I. scapularis* carriage relative to *B. burgdorferi*-negative *I. scapularis* were significantly higher for dogs that traveled in the 14 days prior to tick removal, for those dogs under 1 year of age, and for those weighing less than 10 kg. This case-case study provides information for veterinarians and public health practitioners to help protect dogs and their owners from Lyme disease in southeastern Ontario.

1. Introduction

Tick-borne diseases pose an ongoing threat to human and animal health throughout the world (Dantas-Torres et al., 2012). In North America, Lyme disease is the most important tick-borne disease affecting humans and dogs, and the clinical manifestations of Lyme disease have been well documented (Borchers et al., 2015; Bouchard et al., 2015; Littman et al., 2018). In eastern North America, the etiologic agent associated with Lyme disease is *Borrelia burgdorferi* sensu stricto (henceforth *B. burgdorferi*), which is vectored by *Ixodes scapularis* (Piesman and Happ, 1997), the blacklegged tick. Adult and nymphal stages of *Ixodes scapularis* acquire infection when they feed on infected

hosts, including small mammals and birds, which serve as the main reservoirs for *B. burgdorferi* in this area (Anderson et al., 1986; Lindsay et al., 1997; Ogden et al., 2008; Kilpatrick et al., 2017). *Ixodes scapularis* can also transmit other pathogens, including *Anaplasma phagocytophilum*, a bacterium that may cause disease in people, dogs and horses (Woldehiwet, 2010; Krakowetz et al., 2014).

From the first discovery of a population of *I. scapularis* in Ontario (Watson and Anderson, 1976) until the early 1990's, the only reproductive populations of *I. scapularis* in Ontario were thought to occur along the north shore of Lake Erie (Barker et al., 1992). However, over the past 25 years, the expanding distribution of *I. scapularis* in Ontario has resulted in an increasing risk of *B. burgdorferi* exposure and incited

widespread public and animal health concern (Ogden et al., 2008; Nelder et al., 2014; Ogden et al., 2014; Clow et al., 2016).

Dogs often live in the home, travel with their owners, and partake in outdoor recreational activities with their families. Dogs can readily acquire ticks from the environment (Bouchard et al., 2015), and have been used in passive tick surveillance (Abdullah et al., 2016; Estrada-Peña et al., 2017). Due to the diversity of tick phenology, data for tick species carriage requires regionally specific information. The passive surveillance of ticks removed from people in Ontario has been on-going for many years, but up-to-date knowledge of the tick species carried by domestic dogs in Ontario is unavailable. Current knowledge of the ticks parasitizing dogs in Ontario will provide veterinarians with a useful reference to guide the diagnosis, treatment, and prevention of tick bites and tick-borne disease. Our objectives were to: 1) describe the tick species parasitizing pet dogs in an emerging area for Lyme disease risk in southeastern Ontario, Canada; 2) identify risk factors (e.g., animal and owner demographics, management, and lifestyle) for *I. scapularis* carriage relative to other tick species (i.e., case-case design) in this population of dogs; 3) determine the prevalence of *B. burgdorferi* and *A. phagocytophilum* present within the collected *I. scapularis*; and 4) identify risk factors associated with the carriage of a *B. burgdorferi*-infected *I. scapularis* relative to *B. burgdorferi*-negative ticks.

2. Methods

2.1. Tick collection

Mixed and companion animal veterinary practices located in an approximately 11,000 km^2 area of Ontario (Fig. 1), where *I. scapularis* is thought to be expanding its range (Clow et al., 2016), participated in the collection of ticks from companion animals. The locations of veterinary clinics were grouped into three regions: 1) the "Lakeshore" region for veterinary clinics within approximately 5 km of Lake Ontario; 2) the "Middle" region for veterinary clinics greater than approximately 5 km north of Lake Ontario and south of Lakefield, Ontario; and 3) the "Highland" region for veterinary clinics north of the city of Peterborough to Minden, Haliburton and Bancroft, Ontario (Fig. 1).

Twenty of the 24 veterinary practices contacted by convenience agreed to collect ticks from companion dogs. Seventeen of the participating veterinary practices were companion animal veterinary clinics and three were mixed animal practices (i.e., companion and food animals).

Veterinary clinics were asked to submit all ticks removed from companion dogs from April to December 2015, which encompasses the main activity periods for most life stages of ticks in Ontario (Lindquist et al., 2016). Submissions were also accepted if ticks were removed by the dogs' owners. All canine tick submissions were accepted; however, to avoid repeated submissions and limit sample clustering, when two dogs from the same owner had ticks on the same date, only the submission from the dog with the name that came first in the alphabet was included for analyses. When dog owners submitted ticks from the same pet on multiple occasions, only the ticks from first date of submission were used for analyses. Tick submissions were given a unique identifier, and dog owners were asked to fill out a two-page questionnaire. All ticks from individual dogs were placed into a single 1.2 ml polypropylene cryogenic vial (Corning, Inc., Corning, NY) and stored at −20 °C. Ticks and questionnaires were retrieved from veterinary clinics every 10–16 days. The University of Guelph Research Ethics Board approved client participation in this study (REB # 15FEB19).

2.2. Questionnaire

A questionnaire was used to identify risk factors for *I. scapularis* carriage relative to other tick species in companion dogs, and was completed by participating veterinary clients who owned a dog parasitized by a tick(s). Approximately 12 pet owners were used to pre-test the questions to detect ambiguity, estimate the time to completion, and improve data quality. Letters of intent and questionnaires were distributed to participating veterinary clinics. Veterinary clinic staff were briefed on the research project, the criteria for enrolment, and the acquisition of owner consent for questionnaire completion. Nineteen of the 20 veterinary practices agreed to have clients complete the questionnaire. Any dog with a tick was eligible to participate; however, the minimum age for client participation was 18 years. Tick submissions were accepted even if the pet owner declined to complete the
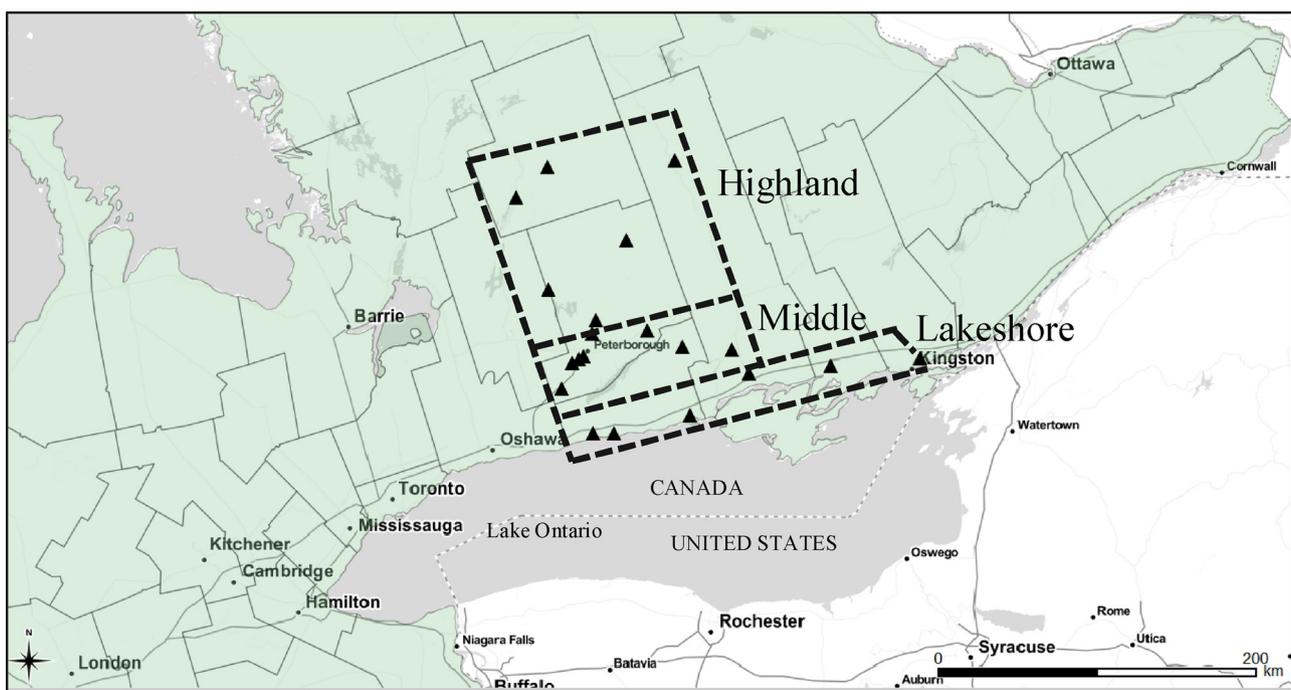

Fig. 1. Map of 20 participating veterinary clinics (▲) in southeastern Ontario where ticks were collected from dogs. The dashed outline represents the three regions used to categorize the location of tick submission: Lakeshore, Middle and Highland regions.

questionnaire.

The questionnaire focused on five main areas of interest: 1) client demographics (e.g., gender, location of primary residence); 2) animal demographics (e.g., breed, age); 3) animal management and lifestyle (e.g., travel history in the previous 14 days); 4) client knowledge of ticks and tick exposure (e.g., previous history of tick infestation); and 5) tick prevention (e.g., use of tick prevention product, date of last application) (see Additional file 1: Table S1).

Information was gathered regarding the dog owner's familiarity and experience with ticks to assess potential bias toward subjects with previous involvement with tick-borne diseases (e.g., Lyme disease). As an incentive for client participation, individuals who completed a questionnaire were offered entry into a draw for an iPad mini (approximate value $300.00 CAD).

2.3. Laboratory analysis

All ticks were shipped to the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Manitoba) where larvae, nymphs and adult ticks were identified to species (using the keys of Clifford et al., 1961; Keirans and Clifford, 1978; Durden and Keirans, 1996; Lindquist et al., 2016), the level of engorgement was determined (i.e., unfed, slightly, partially or fully engorged) and ticks were tested for infection with pathogens. Adult and nymphal *I. scapularis* were tested for the presence of *B. burgdorferi* sensu stricto, *B. miyamotoi*, *A. phagocytophilum*, and *B. microti*. Due to its potential involvement in the sylvatic cycle of Lyme disease (Dolan et al., 2000), any *Ixodes muris* collected were also tested for these pathogens. Standard polymerase chain reaction (PCR) techniques were used for all pathogen testing (Ogden et al., 2006, 2008; Krakowetz et al., 2014; Werden et al., 2014). Briefly, the DNA from individual *I. scapularis* was extracted with commercial DNA extraction kits (96-well format DNeasy, QIAGEN, Inc., Toronto, ON, Canada) (Ogden et al., 2006). Following amplification, multiplex real-time PCR was used to simultaneously target *Borrelia* spp. (23S rRNA) and *A. phagocytophilum* (*msp2*) genes (Courtney et al., 2004). Samples positive for *Borrelia* spp. were confirmed using primers and probes specific for the *B. burgdorferi ospA* gene, and those ticks negative for *ospA* were subsequently tested using real-time PCR for *B. miyamotoi* targeting the *glpQ* gene (Dibernardo et al., 2014). *Ixodes scapularis* were screened for *B. microti* using primers and probes for the CCTη gene, as previously described by Nakajima et al. (2009). *Amblyomma americanum* were tested for the presence of *Ehrlichia chaffeensis* using a quantitative real-time PCR assay, as described by Loftis et al. (2003). Other tick species were not tested for pathogens.

2.4. Variables

Seasons were defined as spring (April to June), summer (July and August), and fall (September to December). December was included in the fall category because the maximum daily temperature did not stay below 4 °C, the temperature at which *I. scapularis* are thought to remain active (Duffy and Campbell, 1994), until December 26th, 2015 (Government of Canada, 2015). Specific information was collected on the location of each dog's travel history within a 14-day period prior to tick removal. However, due to the uncertainty of travel location and duration, travel was analyzed as a dichotomous variable (i.e., travel or no travel in the 14 days prior to tick removal).

The age of the dog was categorized into puppy (< 1 yr), adult (1 to < 7 yr), and senior ($\geq$ 7 yr). Dog weight was categorized into small dogs ($\leq$ 10 kg), medium dogs (> 10 kg to 30 kg) and large dogs (> 30 kg). Dog breed was an open-ended option on the questionnaire, and yielded many categories. Breed was subsequently categorized to seven breed categories: herding, hound, non-sporting, sporting, terrier, toy, and working dog (Canadian Kennel Club, 2016). We also included an additional category for cross-bred dogs.

2.5. Statistical analyses

A priori causal diagrams were constructed to examine possible confounding and intervening relationships among explanatory variables relative to the outcomes of interest (Dohoo et al., 2009). Logistic regression models, were used to examine canine risk factors for two outcomes: 1) the acquisition of *I. scapularis* versus other tick species; and 2) the exposure to *B. burgdorferi*-positive versus -negative *I. scapularis*. The potential risk factors examined included animal and client demographic factors, pet travel history, and management factors (Table S1). Stata/MP 13.1 (StataCorp, College Station, TX) was used for all statistical analyses.

The linearity of continuous variables relative to the log odds of each outcome was assessed using locally weighted regression (lowess curves). If it appeared to be a quadratic relationship, a quadratic term was included in the model and its significance was tested. If a non-linear relationship could not be modeled as a quadratic relationship, a log transformation was attempted to achieve linearity; if linearity could still not be achieved the variable was categorized based on the median value, quartiles, or biologically relevant categories. The correlation between explanatory variables was assessed using various correlation analyses (i.e., Spearman rank and Phi coefficient). If the correlation was > |0.8|, we only used the most biologically plausible or well answered variable for subsequent analysis to avoid issues associated with collinearity.

2.5.1. Model 1: Risk factors for carriage of I. scapularis versus other tick species

Univariable mixed logistic regression models with a random effect for veterinary clinic were constructed to examine the association between the explanatory variables and the outcome of *I. scapularis* carriage versus carriage of another species of tick. All variables significant with a liberal *p*-value ($p \leq 0.20$) were considered for inclusion in the final multivariable model. Variables that were not statistically significant and not suspected to be a confounder based on a causal diagram were excluded from multivariable analysis.

A mixed multivariable logistic regression model with a random intercept for veterinary clinic was fitted by manual backwards elimination using variables that were statistically significant on univariable analysis based on a significance level of α = 0.05. Subsequently, variables with a *p*-value > 0.05 and $\leq$ 0.20 on univariable analysis were re-introduced to the model using a forward selection process. Variables were left in this main effects model if they were statistically significant (α = 0.05) or acted as a confounding variable. A variable was considered a confounder if it was not an intervening variable, and if its removal resulted in a 25% or greater change in the coefficient of a significant variable. Once the main effects model was fitted, pairwise interactions between all remaining variables were assessed for statistical significance. All categorical variables, including interaction terms, were assessed using Wald's χ^2. All variables in the final model were either statistically significant, part of a significant interaction term, or acted as a confounding variable.

2.5.2. Residuals and diagnostics

If the random effect of veterinary clinic had a statistically significant influence on the mixed-effects logistic regression model, the Pearson and deviance residuals were explored for outlying observations, and the homogeneity of variance and normality of the best linear unbiased predictors were evaluated. If there was no significant clustering of the outcome by clinic, the model was run as an ordinary logistic regression and a Hosmer-Lemeshow goodness of fit test with ten groups was used to assess model fit. Pearson residuals and delta-beta diagnostics were visualized graphically to identify any outlying or highly influential observations. If appropriate, the model was refit without the outlying or highly influential observations to assess their impact on the interpretation of the final model in terms of the direction of association and

statistical significance.

2.5.3. Model 2: Risk factors for carriage of a B. burgdorferi-positive I. scapularis

Univariable exact logistic regression (*p*-values were calculated using the score method) was used to examine the association between owner demographics, dog demographics, management factors and season, and carriage of *I. scapularis* positive for *B. burgdorferi* relative to *I. scapularis* that tested negative for this bacterium. Because of the small effective sample size, multivariable modelling was not possible. Lyme disease risk areas were defined as localities where blacklegged ticks infected with *B. burgdorferi* have become established (Public Health Ontario, 2016); travel to these areas could increase the risk of positive tick carriage. Consequently, univariable exact logistic regression analyses were also examined using only dogs with no history of travel in the 14 days prior to removal of *I. scapularis*.

3. Results

3.1. Tick collection

In total, 973 ticks were collected from 588 dogs at the 20 veterinary clinics between April and December 2015 (Table 1). Tick submissions per clinic ranged from 9 to 60 with a median of 26 submissions per clinic. Seven species of ticks were identified (Table 1). However, 98.2% (95% CI = 95.7–98.5) of dogs were parasitized by one of three species: *I. scapularis* (87.2%; 95% CI = 84.1–90.0), *D. variabilis* (9.8%; 95% CI = 7.4–12.6) and *I. cookei* (3.6%; 95% CI = 2.2–5.5). Three dogs used in the analyses carried 2 species of ticks: *I. scapularis* and *D. variabilis* (Table 1). The number of ticks per dog ranged from 1 to 100, however 83.2% of dogs were carrying a single tick. The majority (96.1%) of dogs were infested with adult ticks while only 2.4% of dogs were carrying immature life stages. A single *I. cookei* larva was removed from a dog that was concurrently carrying an *I. cookei* nymph. Dogs parasitized by a combination of adult and immature stages accounted for 1.5% of dogs. Thirty-seven of the 588 dogs were excluded from subsequent analyses as they represented a 2nd or 3rd occurrence of tick carriage from a dog already enrolled in the study. However, only 5 of these families submitted ticks from the same dog in different seasons. Another eight dogs were excluded because they were additional dogs from the same owner with the same date of submission. There was seasonal variation in the tick species submitted and in the numbers of tick submissions; *Ixodes scapularis* was always the most common tick species recovered, with peak recovery in the fall season (Fig. 2).

3.2. Client participation and questionnaires

The responses from questionnaires are summarized and provided in a supplemental table (Table S1). The questionnaire response rate was 92.6%, but the response rate for individual questions was lower (range 72 to 100%; median 90%) (Table S1). One veterinary clinic made the a priori decision to not give their clients the opportunity to complete the questionnaire which accounted for 2% of canine tick submissions. Client participation varied between clinics (range: 40 to 100%; median 97%).

3.3. Pathogen testing

Borrelia burgdorferi and *A. phagocytophilum* were detected in 12.6% (95% CI = 10.1–15.2) and 0.6% (95% CI = 0.2–1.5) of the 685 collected adult *I. scapularis* ticks, respectively. Male *I. scapularis* accounted for 5.8% of those positive for *B. burgdorferi*, and all *I. scapularis* positive for *A. phagocytophilum* were female. Overall, 6.8% of 543 dogs used in the risk factor analysis were carrying at least one *I. scapularis* positive for *B. burgdorferi* (35 dogs) or *A. phagocytophilum* (2 dogs). The sole *I. muris* tick collected was negative for pathogens. No co-infections were detected and no ticks were positive for *B. miyamotoi* or *B. microti*. None of the *A. americanum* recovered (n = 4) tested positive for *E. chaffeensis*.

3.4. Logistic regression model

3.4.1. Model 1: I. scapularis carriage relative to carriage of all other tick species

Results from univariable mixed logistic regression analysis are summarized and available in a supplemental table (Table S2). Variables considered for inclusion in the multivariable model based on a *p*-value ≤ 0.20 included: season, region, time spent on a farm in the 7 days prior to tick removal, travel within 14 days of tick removal, number of dogs in the home, location of tick attachment on the body of the dog, sex of the dog, fur length, reproductive status, and time spent on a

Table 1

The species, life stage, and level of engorgement for ticks parasitizing domestic dogs collected from April through to December 2015 from 20 participating veterinary hospitals in southeastern Ontario, Canada.

Tick species	*Ixodes scapularis*	*Dermacentor variabilis*	*Ixodes cookei*	*Amblyomma americanum*	*Rhipicephalus sanguineus* sensu lato	*Dermacentor albipictus*	*Ixodes muris*	Co-infested with *I. scapularis* & *D. variabilis*	Total
Common name	Blacklegged tick	American dog tick	Groundhog tick	Lone star tick	Brown dog tick	Winter tick	Mouse tick		
Life stage									
Female	653	40	10	1	2		1		707
Male	32	16		3		1			52
Nymph			17		100	2			119
Larva			1						1
Various[a]		94							94
Engorgement									
Unfed	70	58	2	3		1			134
Slightly	104	16	2	1	1				124
Partially	459	31	24		101				615
Fully	52	5				2	1		60
Various[a]		40							40
Total ticks	685	150	28	4	102	3	1		973
Total dogs	503	51	20	3	3	3	1	4	588
Total dogs in study[†]	462	49	19	3	3	3	1	3	543

[a] Various life stages or levels of engorgement.

[†] Only the first incidence of tick attachment was included in the regression analyses. Likewise, only one dog per date from each household was included in the analyses.
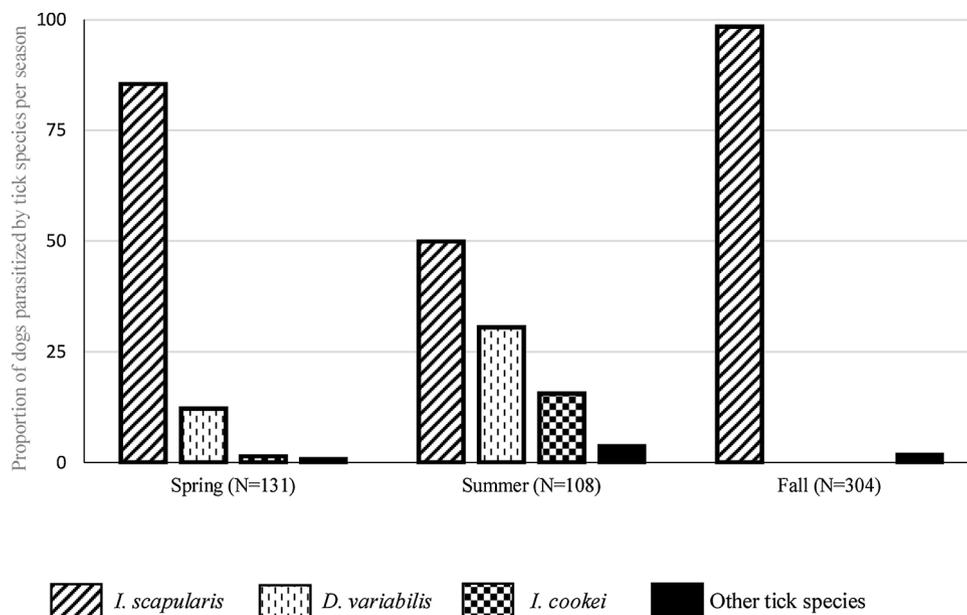
Fig. 2. Seasonal variation in tick species, including nymphs and larvae, submitted from April to December 2015 among 543 parasitized dogs from 20 veterinary clinics in southeastern Ontario. Other tick species included: *Amblyomma americanum, Rhipicephalus sanguineus* sensu lato, *Dermacentor albipictus* and *Ixodes muris*.

sidewalk in the 7 days prior to tick removal (Table S2).

In the final multivariable mixed-effects model, the season and region of tick submission, spending time on a farm in the 7 days prior to tick removal, and the sex and weight category of the dog had a significant influence on the odds of *I. scapularis* carriage relative to other tick species in this population of pet dogs (Table 2). No significant interactions were found between variables in the multivariable mixed-effects model.

In the fall, the odds of removing an *I. scapularis* relative to other tick species were significantly greater than in the spring (Table 2) and the summer season (OR = 53.22; 95% CI = 19.45–145.67; *p*-value < 0.001). The odds of removing an *I. scapularis* relative to other tick species were significantly higher in the Lakeshore region compared to the Highland region (Table 2), but no significant difference was found

between the Middle region compared to the Lakeshore region (OR = 0.52; 95% CI = 0.21–1.31; *p*-value = 0.17).

Although larger dogs were at significantly greater odds of *I. scapularis* carriage relative to other tick species compared to medium and smaller dogs (Table 2), no significant difference was detected between medium and small dogs (OR = 0.82; 95% CI = 0.37–1.81; *p*-value = 0.62). Female dogs were at significantly greater odds of *I. scapularis* carriage compared to male dogs, but spending time on a farm in the 7 days prior to tick submission significantly reduced the odds of *I. scapularis* carriage compared to other tick species (Table 2).

The variance at the clinic level was negligible, 9.95×10^{-35}, and no differences were found in coefficients or 95% confidence intervals between the model with and without this random effect. Consequently, model diagnostics were also performed on an ordinary logistic

Table 2

Descriptive statistics and results from the final mixed multivariable logistic regression model[a,b] with a random effect for veterinary clinic[c] estimating the risk factors for *I. scapularis* carriage versus the carriage of other tick species in a population of pet dogs from southeastern Ontario, Canada.

Variable description	Number of dogs (%)	% I. scapularis (95% CI[d])	OR (95% CI)	p-value[e]
Season	543 (100.0)			
Spring	131 (24.1)	85.5 (78.3 - 91.0)	Referent	
Summer	108 (19.9)	50.0 (40.2 - 59.8)	0.19 (0.10 - 0.39)	**< 0.001**
Fall	304 (56.0)	98.4 (96.2 - 99.5)	10.23 (3.60 - 29.10)	**< 0.001**
Region	543 (100.0)			
Highlands	153 (28.2)	73.2 (65.5 - 80.0)	Referent	
Middle	205 (37.8)	86.3 (80.9 - 90.7)	1.73 (0.88 - 3.43)	0.115
Lakeshore	185 (34.1)	95.1 (91.0 - 97.8)	3.32 (1.32 - 8.38)	**0.011**
Visit farm in previous 7 days	497 (91.5)			
No	417 (83.9)	86.3 (82.7 - 89.5)	Referent	
Yes	80 (16.1)	76.3 (65.4 - 85.1)	0.40 (0.18 - 0.87)	**0.021**
Sex of dog	536 (98.7)			
Female	252 (47.0)	88.1 (83.4 - 91.8)	Referent	
Male	284 (53.0)	83.1 (78.2 - 87.3)	0.50 (0.26 - 0.96)	**0.038**
Dog weight category	531 (97.8)			
Large > 30 kg	185 (34.8)	88.1 (82.6 - 92.4)	Referent	
Medium 10 – 30 kg	220 (41.4)	83.6 (78.1 - 88.3)	0.34 (0.16 - 0.71)	**0.004**
Small < 10 kg	126 (23.7)	84.2 (77.5 - 90.7)	0.41 (0.18 - 0.96)	**0.041**

[a] 484 dogs had complete information for inclusion in the final model.
[b] Overall significance of the mixed multivariable model based on a Wald's χ^2 was $p < 0.001$.
[c] Variance component for random effect of veterinary clinic = 9.95×10^{-35} (95% CI was not estimated).
[d] 95% Confidence Interval.
[e] Significant *p*-values in bold.

Table 3

Descriptive statistics and results of univariable exact logistic regression analyses concerning variables significant[a] associated with the carriage of a *Borrelia burgdorferi*-positive versus -negative *Ixodes scapularis* collected from 465 parasitized dogs from 20 veterinary clinics in southeastern Ontario between April and December 2015.

Variable description	N (%)	% *B. burgdorferi* positive ticks (95% CI[b])	Odds Ratio (95% CI)	*p*-value[f]
Travel in previous 14 days	414 (89.0)			
No	331 (80.0)	4.8 (2.8 - 7.7)	Referent	
Yes	83 (20.1)	18.1 (10.5 - 28.0)	4.32 (1.89 - 9.85)	**< 0.001**
Age of dog	460 (98.9)			**0.007c**
Puppy (< 1 yr)	46 (10.0)	19.6 (9.3 - 33.9)	Referent	
Adult (1-7 yr)	244 (53.0)	6.6 (3.8 - 10.4)	0.29 (0.11 - 0.80)	**0.008**
Senior (> 7 yr)	170 (37.0)	5.9 (2.8 - 10.5)	0.26 (0.09 - 0.78)	**0.007**
Engorgement	465 (100.0)			
Fed[d]	429 (92.3)	6.8 (4.5 - 9.6)	Referent	
Unfed	36 (7.7)	16.7 (6.3 - 32.8)	2.75 (0.87 - 7.47)	**0.043**
Dog weight category	454 (97.6)			0.090[c]
Large (> 30 kg)	163 (35.9)	4.3 (1.7 - 8.7)	Referent	
Medium (10 - 30 kg)	184 (40.5)	8.7 (5.1 - 13.7)	2.11 (0.80 - 6.26)	0.130
Small (< 10 kg)	107 (23.6)	11.2 (5.9 - 18.8)	2.80 (0.98 - 8.72)	0.050
Reproductive status	457 (98.3)			
Intact	93 (20.35)	11.8 (6.1 - 20.2)	Referent	
Neutered	364 (79.7)	6.0 (3.8 - 9.0)	0.48 (0.21 - 1.14)	0.071
Visited a park[e] in previous 7 days	424 (91.2)			
No	312 (73.6)	5.8 (3.4 - 9.0)	Referent	
Yes	112 (26.4)	10.7 (5.7 - 18.0)	1.96 (0.83 - 4.47)	0.088
Visited a field in previous 7 days	424 (91.2)			
No	235 (55.4)	5.5 (3.0 - 9.3)	Referent	
Yes	189 (44.6)	9.0 (5.3 - 14.0)	1.69 (0.75 - 3.89)	0.185

[a] Only variables with a p-value $\leq$ 0.20 are presented.
[b] 95% Confidence Interval.
[c] Overall significance for variables with more than 2 categories; all *p*-values estimated using the score method whereas 95% confidence intervals for odds ratios were estimated using the sufficient statistic.
[d] Fed includes all submission of ticks that were fully, partially, and/or slightly engorged.
[e] From collapsed categories of dogs that visited national, provincial, public, or dog parks and/or took their dog to a beach.
[f] Significant *p*-values in bold.

regression model. A Hosmer-Lemeshow goodness of fit test was not significant ($p = 0.852$), indicating that we failed to reject the null-hypothesis that the model fits the data. No observations or covariate patterns were identified with a large measure of influence or large Pearson residual whose removal changed the interpretation of the final model.

3.4.2. Model 2: Exposure to Borrelia burgdorferi-positive versus -negative I. scapularis

Using the subset of 465 dogs parasitized with *I. scapularis*, univariable exact logistic regression analysis identified three variables as significant ($p < 0.05$) predictors for carriage of an *I. scapularis* positive for *B. burgdorferi*: travel within the 14 days prior to tick removal, age of the dog, and tick engorgement (Table 3). The odds of a tick testing positive for *B. burgdorferi* was significantly greater in dogs who travelled in the 14 days prior to tick removal (Table 3). Although puppies were at significantly greater odds of carrying a tick positive for *B. burgdorferi*, there was no significant difference in the odds observed between adult and senior dogs (OR = 1.12; 95% CI = 0.46–2.84; *p*-value = 0.839). The odds of an unfed tick testing positive for *B. burgdorferi* were significantly greater relative to the category of fed ticks, which included fully, partially, and slightly engorged ticks (Table 3).

The results of univariable exact logistic regression using the 331 dogs parasitized with *I. scapularis* with no history of travel within 14 days of tick removal are summarized in Table 4. Only 16 of these dogs were parasitized with at least one *I. scapularis* positive for *B. burgdorferi*. In univariable analysis, the season, age of dog, and dog weight category were significantly associated with the odds of *B. burgdorfer*-positive tick carriage. The odds of *B. burgdorferi*-positive tick carriage were significantly greater in the summer and fall relative to the spring (Table 4), but no significant difference was found between summer relative to the fall (OR = 2.01; 95% CI = 0.45–7.18; *p*-value = 0.269). The odds of

carriage of an infected tick were significantly greater in dogs under 1 year of age and weighing less than 10 kg (Table 4). Significant differences were not found in the odds of *B. burgdorferi*-positive tick carriage between seniors relative to adult dogs (OR = 0.83; 95% CI = 0.17–3.34; *p*-value > 0.99), nor medium dogs relative to small dogs (OR = 0.33; 95% CI = 0.08–1.22; *p*-value = 0.07).

4. Discussion

Our study collected a total of 973 ticks from pet dogs that constituted seven different tick species. In keeping with recent Canadian human passive surveillance studies, three tick species accounted for 98.2% of submissions: *I. scapularis*, *D. variabilis* and *I. cookei* (Nelder et al., 2014; Gasmi et al., 2016). Other passive surveillance studies in Canada have reported greater tick species richness and yielded substantially more ticks (Scholten, 1977; Ogden et al., 2006; Nelder et al., 2014; Gasmi et al., 2018), but their analyses included more host species, a greater geographical area, and a longer time period for submission. Around the world *Rhipicephalus sanguineus* sensu lato is considered the most ubiquitous vector-borne tick infesting dogs, and in Ontario it occasionally establishes within indoor environments (e.g., kennels or boarding facilities) (Dantas-Torres, 2010; Lindquist et al., 2016). Although *R. sanguineus* sensu lato ticks accounted for 10.5% of the total ticks collected, only 0.55% of dogs were parasitized by this tick. One puppy imported from the southern USA was heavily parasitized, and two additional dogs were carrying a single *R. sanguineus* sensu lato. Adult ixodid ticks have a prolonged feeding period, and can be transported considerable distances while attached to their host (Hoogstraal and Kaiser, 1961; Anderson, 2002); the heavily parasitized puppy emphasizes the importance of tick control in preventing the importation of ticks. At the time of writing, *A. americanum* ticks are not known to have established in Ontario, and those recovered likely

Table 4

Descriptive statistics and results of univariable exact logistic regression analysis concerning variables significantly[a] associated with the carriage of a *Borrelia burgdorferi*-positive versus-negative *Ixodes scapularis* collected from 331 parasitized dogs with no history of travel within 14 days prior to tick removal from 20 veterinary clinics in southeastern Ontario between April and December 2015.

Variable description	N (%)	% *B. burgdorferi* positive ticks (95% CI[b])	Odds Ratio (95% CI)	*p*-value[f]
Season	331 (100.0)			**0.021[c]**
Spring	82 (24.8)	0 (0 - 4.4)	Referent	
Summer	37 (11.2)	10.8 (3.0 - 25.4)	12.62[d] (1.11 - ∞)	**0.042**
Fall	212 (64.0)	5.7 (3.0 - 9.7)	6.82[d] (1.53 - ∞)	**0.008**
Age of dog	328 (99.1)			**0.008**
Puppy (< 1 year)	28 (8.5)	17.9 (6.1 - 36.9)	Referent	
Adult (1-7 years)	178 (54.3)	3.9 (1.6 - 7.9)	0.19 (0.05 - 0.83)	**0.013**
Senior (> 7 years)	122 (37.2)	3.3 (1.0 - 8.2)	0.16 (0.03 - 0.80)	**0.012**
Dog weight category	326 (98.5)			**0.020**
Large (> 30 kg)	126 (38.7)	2.4 (0.5 - 6.8)	Referent	
Medium (10 – 30 kg)	127 (39.0)	3.9 (1.3 - 9.0)	1.68 (0.32 - 11.03)	0.722
Small (< 10 kg)	73 (22.4)	11.0 (4.9 - 20.5)	5.00 (1.15 - 30.27)	**0.020**
Engorgement	331 (100.0)			
Fed[e]	309 (93.4)	4.2 (2.3 - 7.1)	Referent	
Unfed	22 (6.6)	13.6 (2.9 - 34.9)	3.57 (0.60 - 14.67)	0.081

[a] Only variables with a p-value ≤ 0.20 are presented.

[b] 95% Confidence Interval.

[c] Overall significance for variables with more than 2 categories; all *p*-values estimated using the score method whereas 95% confidence intervals for odds ratios were estimated using the sufficient statistic.

[d] Median unbiased estimates.

[e] Fed includes all submission of ticks that were fully, partially, and/or slightly engorged.

[f] Significant *p*-values in bold.

represent adventitious ticks, or ticks acquired during travel outside of Canada. The majority (96.1%) of ticks removed from dogs were adults. This is consistent with similar animal studies, and may be due to a combination of the smaller larval and nymphal stages being overlooked on dogs (Földvári and Farkas, 2005; Beck et al., 2014; Abdullah et al., 2016; Burroughs et al., 2016), and/or the immature stages generally having a feeding preference for smaller mammals and birds (Piesman and Spielman, 1979; Lindsay et al., 1999a; Ogden et al., 2008; Kilpatrick et al., 2017). All *I. scapularis* collected from pet dogs in this study were adult ticks, and highlights the potential importance of adult ticks in the transmission of *B. burgdorferi* to companion dogs.

The odds of *I. scapularis* carriage relative to other tick species was greatest in the fall relative to the spring and summer seasons, and also significantly greater in the spring relative to the summer. This seasonal influence on tick carriage is consistent with human passive surveillance studies (Rand et al., 2007; Nelder et al., 2014; Gasmi et al., 2016) and in keeping with *I. scapularis* ecology in Ontario (Lindsay et al., 1999b). The geographic distribution of *I. scapularis* has been expanding in southern Ontario (Barker et al., 1992; Morshed et al., 2006; Ogden et al., 2006; Scott et al., 2012; Werden et al., 2014; Clow et al., 2016). As hypothesized, dogs from veterinary clinics within approximately 5 km of the north shore of Lake Ontario had a significantly greater odds of carrying *I. scapularis* over other tick species compared to dogs from the Highland region, the most northern part of our study area.

Environment and habitat have important influences on tick bite risk (Piesman and Eisen, 2008). Dogs spending time on a farm had a lower odds for *I. scapularis* carriage relative to other tick species. Although we did not specify agricultural farm, this finding may be expected as *I. scapularis* prefer forested areas over field habitat (Keirans et al., 1996; Lindsay et al., 1998). The sex and size of the dog had a significant influence on the odds of *I. scapularis* carriage relative to other tick species. In a study of Ontario residents, Nelder et al. (2014) found that men submitted significantly more *I. scapularis* and *D. variabilis* than women, which may reflect differences in tick habitat exposure for people. In our study, female dogs had a two-fold increase compared to male dogs in the odds of *I. scapularis* carriage relative to other tick species. Female and male dogs may exhibit behavioural differences while moving through tick habitat; however, further research is required to explain this finding. Large dogs, weighing over 30 kg, were at significantly

greater odds of *I. scapularis* carriage relative to carriage of other tick species when compared to both medium and small dogs. It is possible that the weight of the dog is a proxy for dog height, and questing heights may differ between tick species. The questing height of adult hard ticks is approximately 50–80 cm (Leonovich, 2015), and has been reported to be up to a metre off the ground for *I. scapularis* (formerly *I. dammini*) (Spielman et al., 1985). Based on our results, heavier or possibly "taller" dogs may be more likely to come in contact with blacklegged ticks relative to lighter "shorter" dogs. Alternatively, it is possible that dogs over 30 kg in this study were more likely to frequent suitable *I. scapularis* habitat relative to smaller dogs.

Information regarding tick prevention and control was collected (Table S1), but widespread client misunderstanding was evident regarding which products were appropriate for tick control. Thirteen percent of clients reported starting a veterinary prescribed tick product on the day of tick submission, but "starting today" was not offered as an answer on the questionnaire, and we anticipate that additional clients started prevention on the day of tick submission (Table S1). For these reasons, variables pertaining to tick prevention lacked intrinsic validity and were not appropriate for statistical modelling. However, this does identify an area where further client education is imperative.

The prevalence of *I. scapularis* positive for *B. burgdorferi* and *A. phagocytophilum* collected from dogs in this study are similar to previously reported passive surveillance prevalence data in Canada (Dibernardo et al., 2014; Nelder et al., 2014). The lack of co-infections and ticks positive for *B. miyamotoi*, *B. microti*, or *E. chaffeensis* was not surprising given the rarity of these pathogens in Ontario (Dibernardo et al., 2014; Werden et al., 2014; Clow et al., 2016).

Traveling in the 14 days prior to tick removal was significantly associated with an increased odds of *B. burgdorferi*-positive *I. scapularis* carriage. The duration and distance of travel was not accounted for, and a more precise definition for travel would be beneficial for future studies (e.g., out of county, more than 30 km from your home or veterinary clinic, etc.). We hypothesized that the odds of encountering a tick positive for *B. burgdorferi* would be greatest for the dogs residing in the Lakeshore region of our study area, which includes areas of emerging Lyme disease risk recently identified by Public Health Ontario (Public Health Ontario, 2016). Surprisingly, even when dogs with a history of travel within the 14 days prior to tick removal were excluded, region

was not significantly associated with carriage of a *B. burgdorferi*-positive *I. scapularis*. However, our small sample size may have provided insufficient power to detect differences in the odds of carriage of *B. burgdorferi*-positive *I. scapularis* between regions.

While the odds of *I. scapularis* carriage relative to other tick species were significantly higher in the spring and fall, relative to the summer, season was not a significant predictor for the odds of carrying an *I. scapularis* positive for *B. burgdorferi* versus *I. scapularis* negative for this pathogen when all dogs were analyzed. Conversely, when dogs who had travelled were excluded, the likelihood of *I. scapularis* positive for *B. burgdorferi* was significantly greater in the summer and fall relative to the spring. Although the reader is cautioned to the small sample size for theses analyses, our study suggests there may be a seasonal association with the odds of *B. burgdorferi*-positive *I. scapularis* tick carriage on dogs and further research is warranted.

In univariable analyses, dogs under one year of age or weighing less than 10 kg were at significantly increased odds of carrying an *I. scapularis* positive for *B. burgdorferi* versus *B. burgdorferi*-negative ticks. There is some experimental evidence to support a lower questing height in adult *I. scapularis* infected with *B. burgdorferi* compared to uninfected *I. scapularis* (Lefcort and Durden, 1996). As such, our weight variable may be a proxy for dog height, and shorter (i.e., lighter) dogs may be at increased risk of exposure to *B. burgdorferi*-positive *I. scapularis* in the appropriate habitat. Young dogs and/or small dogs may be less likely to be on tick prevention and may not have been previously vaccinated for *B. burgdorferi* due to a presumed reduced risk of exposure to tick bites. Information was not collected on the study questionnaire regarding a history of Lyme vaccination, or previous *B. burgdorferi* exposure. Lyme vaccination with an outer surface protein A subunit is known to reduce spirochete numbers in the tick (Ornstein and Barbour, 2006; Lafleur et al., 2015), and future research into the influence of vaccination history on the prevalence of *B. burgdorferi* in PCR testing is warranted. Similar to other Canadian studies, the odds of a tick testing positive for *B. burgdorferi* were significantly greater in unfed ticks (Dibernardo et al., 2014; Nelder et al., 2014). In early PCR testing methods, the presence of inhibitory substances in fully engorged ticks prevented successful DNA amplification, however this limitation should have been circumvented with the current DNA extraction techniques (Schwartz et al., 1997; Courtney et al., 2004; Dibernardo et al., 2014).

Globally dogs have been used as a means of passive tick surveillance (Smith et al., 2011; Beck et al., 2014; Burroughs et al., 2016; Estrada-Peña et al., 2017), and case-case studies designs have been used to identify risk factors for tick species carriage (Ogden et al., 2000). A limitation to the case-case study design is that risk factors for tick carriage may be have been identical between tick species, and we may have overlooked significant factors that were the same between comparison groups (McCarthy and Giesecke, 1999). However, knowledge of the relative importance of these factors to different tick species and their pathogens is useful information for animal and public health.

This study increases our understanding of the tick species and pathogen prevalence of the ticks parasitizing a population of domestic dogs in southeastern Ontario. *Ixodes scapularis* carriage on these dogs relative to other tick species was significantly influenced by the season and region of tick collection, the sex and weight of the dog, and whether time was spent on a farm in the 7 days prior to tick removal. Dogs under 1 year of age and/or weighing less than 10 kg appeared to be at increased odds of *B. burgdorferi*-positive tick carriage, and where appropriate, tick prevention measures in these dogs should be considered. Tick prevention recommendations should always include a discussion regarding the risk areas for *B. burgdorferi*, however our study provides evidence that irrespective of location, traveling with your dog significantly increases the odds of *B. burgdorferi*-positive *I. scapularis* carriage in southeastern Ontario.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2018.10.004.

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