The Epidemiology of *Ixodes scapularis* and *Borrelia burgdorferi* Collected from Pet Dogs in an Emerging Lyme Disease Risk Area of Southeastern Ontario, Canada

by

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ABSTRACT

THE EPIDEMIOLOGY OF *IXODES SCAPULARIS* AND *BORRELIA BURGDORFERI* COLLECTED FROM PET DOGS IN AN EMERGING LYME DISEASE RISK AREA OF SOUTHEASTERN ONTARIO, CANADA

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The major objectives of this thesis were to identify the tick species parasitizing companion dogs in southeastern Ontario, determine demographic and management factors associated with the carriage of *I. scapularis* and *B. burgdorferi*-positive ticks, and identify clustering of the carriage of these organisms in space, time, and space-time. Seven species of ticks from 543 companion dogs were collected from 20 participating veterinary hospitals. *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. The odds of *I. scapularis* parasitism was significantly greater 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, and in dogs that had not visited a farm. Significant spatial, temporal and space-time clusters were identified that were frequently consistent with surveillance data acquired from active tick surveillance.

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"Unless someone like you cares a whole awful lot,
nothing is going to get better. It's not."

Dr. Seuss (1904 - 1991)

"The question is not what you look at, but what you see?"

Henry David Thoreau (1817 – 1862)

DECLARATION OF WORK

I declare that all of the work reported in this thesis was performed by me, with the exception of the items indicated below.

The tick identification and diagnostic testing for tick pathogens were performed by Dr. Robbin Lindsay's lab at the Public Health Agency of Canada's National Microbiology Laboratory in Winnipeg, Manitoba.

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LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

95% CI	95% Confidence interval
B. burgdorferi	*Borrelia burgdorferi* sensu stricto
ELISA	Enzyme-linked immunosorbent assay
LD	Lyme disease
mg/kg	Milligram per kilogram
O/E	Observed over expected
Osp	Outer surface protein
PCR	Polymerase chain reaction
PHAC	Public Health Agency of Canada
PHO	Public Health Ontario
PO	*per os* (i.e., medicine taken orally)
RR	Relative risk
q24hr	Given once every 24 hours

CHAPTER 1: LITERATURE REVIEW AND RESEARCH OBJECTIVES

1.1 BRIEF HISTORY AND INTRODUCTION TO TICKS

Ticks are obligate blood-feeding ectoparasites. Roughly 10% of all tick species are competent vectors for human and domestic animal pathogens including: bacteria, rickettsia, viruses, protozoa, and parasites (Jongejan and Uilenberg, 2004). The fossil record suggests that ticks arose 65 to 146 million years ago during the Cretaceous period with dispersal and evolution occurring 5-65 million years ago (de la Fuente, 2003). The earliest reports documenting ticks and tick-borne fevers occurred in ancient Egyptian papyrus scrolls (1550 BC) (Obenchain and Galun, 1982). Ticks were first confirmed to be vectors for pathogens, when Smith and Kilborne (1893) determined that *Rhipicephalus (Boophilus) annulatus* transmits *Babesia bigemina*, the causal agent of Texas Cattle Fever in North America. Evidence for the long-standing co-evolution of ticks and pathogens was recently documented when spirochete-like organisms were visualized within the hemocoel and alimentary tract of an *Amblyomma sp.* embedded in Dominican amber and dated from 15 to 20 million years ago (Poinar, 2015).

Ticks are members of a monophyletic class of Arachnida, in the order Parasitiformes which consists of three families: Nuttalliellida, Argasidae (soft ticks) and Ixodidae (hard ticks) (Black and Piesman, 1994).

1.1.1 Nuttalliellidae

The Nuttalliellidae family consists of only one species, *Nuttalliella namaqua* (Horak et al., 2002). Very few specimens have been found in southern Africa and little is known about its life history (Mans et al., 2011). *Nuttalliella namaqua* have a partially

sclerotized pseudo-scutum, leathery integument and apical mouthparts, and likely feed on reptiles (Mans et al., 2011). This tick has not been found outside of the African continent.

1.1.2 Argasidae – Soft Ticks

Soft ticks consist of approximately 180 species; eight of these species are thought to occur in Canada (Horak et al., 2002; Lindquist et al., 2016). They have a leathery integument, ventral anterior mouthparts, and lack a chitinised (hard) scutum (Anderson, 2002). Many soft ticks are nidicolous (nest dwelling) (Vial, 2009), and they can be vectors for agents that can cause relapsing fever in humans and dogs (e.g., *B. hermsii*) (Felsenfeld, 1965; Kelly et al., 2014). Their life cycle consists of egg, larval, several nymphal instars, and an adult stage (Anderson, 2002). During feeding, most nymph and adult soft ticks engorge rapidly over a period of minutes to a few hours (Oliver, 1989). These ticks consume five to twelve times their weight in blood and excess water is secreted by coxal glands (Balashov, 1972). In North America, soft ticks are distributed in western Canada and arid southwestern regions of the USA (Keirans and Durden, 2001; Lindquist et al., 2016). Soft ticks can be imported on domestic animals, but are uncommon in eastern Canada, and due to their rapid feeding, they are rarely found attached to their host (Berrada and Telford, 2009; Vial, 2009). An exception of note is *Otobius megnini*, a soft tick that parasitizes the external ear canal of domestic animals. Unlike most other soft ticks, *O. megnini* is a one host tick; larvae engorge and molt into the two nymphal stages deep within the external ear canal, and can remain there for 200 days (Rich, 1957; Jongejan and Uilenberg, 2004). Nymphs drop into the environment

and molt into adults, mating occurs off the host, and adult *O. megnini* do not feed as they have very poorly developed mouth parts (Rich, 1957; Oliver, 1989). These ticks are not known to transmit pathogens, but can cause significant discomfort and dermatoses within the ear canal (Jongejan and Uilenberg, 2004).

1.1.3 Ixodidae – Hard Ticks

The Ixodidae family accounts for approximately 80% of tick species, and consists of over 700 species distributed worldwide; 32 of these species are thought to occur in Canada (Guglielmone et al., 2014; Lindquist et al., 2016). The etymology of *Ixodes* comes from the ancient Greek word "ixos" which means sticky or mistletoe. In Roman times, the sticky berries from European mistletoe (*Viscus album*) were crushed and used to make adhesive traps for catching birds; Ixodes refers to the "sticky" nature of ticks while embedded in the flesh of their chosen host (Seelig, 2011). Hard ticks are characterized by a sclerotized scutum (dorsal shield) and apical mouthparts. Hard ticks have five development stages namely: eggs, larvae, nymphs and adult males or adult females. Life cycles are defined by the number of hosts required to complete development and range from one-, two- or three-host life cycles (Sonenshine, 1991). Attachment and feeding to repletion can last for a few days to a few weeks, and these ticks can consume more than one hundred times their body weight in blood (Anderson, 2002). This blood meal is concentrated and the excess water (60-70%) is secreted back into the host as saliva (Sauer and Hair, 1986).

The North American Ixodidae of medical and veterinary significance are summarized in Table 1.1. Their distinguishing features, habitat and host preferences,

seasonality (if present in Ontario), general distribution, and potential tick-borne pathogens are summarized. In addition to pathogens, certain tick species have been associated with other diseases or conditions, and where appropriate these have been included in Table 1.1. The saliva of some species of Ixodidae (e.g., *Dermacentor andersoni*) can contain a neurotoxin that produces an acute symmetrical ascending flaccid motor paralysis. In North America, tick removal generally reverses this paralysis within 24 to 48 hours (Grattan-Smith et al., 1997; Eppleston et al., 2013). Historically, tick paralysis caused considerable morbidity and mortality in people, livestock, and dogs in British Columbia (Mail and Gregson, 1938), but it is now a rare occurrence and there is evidence to suggest that cattle can develop immunity to tick paralysis (Gregson, 1957; Schmitt et al., 1969; Rich, 1973; Wilkinson, 1982; Lysyk et al., 2009). Another disquieting sequela is the discovery that in some people the bite of a lone star tick (*Amblyomma americanum*) can result in the development of antibodies to a red meat protein (oligosaccharide galactose-α-1,3-galactose) which can in turn cause a delayed (IgE) anaphylactic reaction upon exposure to red meat (Commins et al., 2011; Wolver et al., 2013). Another North American tick endemic to the southern USA, *Amblyomma maculatum*, is associated with infestations causing a curled or drooping edematous deformed ear ("gotch ear"), which has been reported in cattle, horses, and goats (Edwards, 2011).

Table 1.1. Summary of the Ixodidae of significant veterinary and medical importance in North America. The distinguishing features, habitat and host preferences, month of peak activity (if established in Ontario), distribution, and the vector potential for tick-borne pathogens, or association with disease have been included for comparative purposes.

Scientific Name	Common Name	Distinguishing Features (Veterinary/Field ID)[1]	Habitat Preference	Host Preference (Month of peak activity in Ontario)	General Distribution[2, 17]	Tick-borne Pathogens(s)[3]
Ixodes scapularis (Say)	Eastern Blacklegged or Deer Tick	Solid black oval/round scutum, long palps and mouth parts	Deciduous forest [4, 5]	Larva: small mammals and birds, and in south USA lizards [6, 7] (June and August) [8] Nymph: same hosts as larva (June and July)[8,9] Adult: white-tailed deer and larger/medium mammals[6] (April and October)[4]	Eastern USA to Texas & Midwest, and Southern Ontario, Quebec and Maritimes[10]	*Borrelia burgdorferi* *Borrelia miyamatoi*[11] *Anaplasma phagocytophilum* *Babesia microti* *Francisella tularensis* Powassan virus [12] Tick paralysis*[13]
Ixodes pacificus (Cooley & Kohls)	Western Blacklegged Tick	Solid black oval/round scutum, long palps and mouth parts	Coastal redwood and deciduous forest[14]	Larva & Nymph: lizards and birds[15] Adult: Columbian black-tailed deer, large/medium mammals[15, 16]	British Columbia[17], Westcoast USA, parts of Nevada, Arizona and Utah[18]	*Borrelia burgdorferi* *Borrelia miyamatoi*[19] *Anaplasma phagocytophilum*
Ixodes cookei (Packard)	Groundhog Tick	Solid black angular scutum, short palps	Brushy areas, along trails and roads bordered with long grass, groundhog burrows[20. 21]	Larva: small mammals (August)[20,21] Nymph: small and medium mammals (May – July) [20, 21] Adult: carnivores, medium mammals (May) [20, 21]	East of the Rocky Mountains, Manitoba to Prince Edward Island[22]	Powassan virus [23]
Dermacentor variabilis (Say)	American Dog Tick	Ornate scutum, short rectangular mouth parts, with magnification 300+ small goblets on spiracular plate	Grassy fields, young forests and along roads and trails[24]	Larva: small mammals (June and August)[25] Nymph: small mammals (June) [25] Adult: dog, bear, raccoon, porcupine (May - June) [25]	East of the Rocky Mountains and limited areas along the Pacific Coast[2]	*Rickettsia rickettsii* *Fransciella tularensis* *Anaplasma marginale* *Cytauxzoon felis* (experimental)[26] Tick paralysis*[27]
Dermacentor andersoni (Stiles)	Rocky Mountain Wood Tick	Ornate scutum, short rectangular mouth parts, with magnification fewer & larger goblets on spiracular plates than *D. variabilis*	Grassy fields, young subalpine forests and along roads and trails[28]	Larva & Nymph: small mammals[29] Adult: larger mammals[29]	Cascade Mountains to the Rocky Mountains, Southern British Colombia, Alberta and Saskatchewan[29]	*Rickettsia rickettsii* *Fransciella tularensis* *Anaplasma marginale* Powassan virus Colorado Tick Fever Tick paralysis*[30]

Amblyomma americanum (Linnaeus)	Lone Star Tick	Females: characteristic yellow-white spot on posterior portion of scutum Males: ornate scutum with silvery streaks along margins of scutum, long mouth parts	Young second growth forests with dense underbrush[31]	Larva & Nymph: birds, wild turkeys, medium and large sized mammals [32] Adult: bear, raccoon, porcupine, white tailed deer[32]	Eastern and Midwestern USA	*Ehrlichia chaffeensis* *Ehrlichia ewingii* *Fransciella tularensis* *Theileria cervi*[33] *Cytauxzoon felis*[34] *Rickettsia amblyommii* *Babesia lonstari* STARI†
Amblyomma maculatum (Koch)	Gulf Coast Tick	Ornate scutum with rectangular long mouth parts, silvery streaks over entire scutum	Grassland and savanna[35]	Larva & Nymph: small mammals and birds[35] Adult: large/medium mammals[35]	Within 100 miles of south Atlantic coast, but moving north and west[35]	*Rickettsia rickettsii* *Rickettsia parkeri* *Ehrlichia ruminantium* *Hepatozoon americanum* Tick paralysis* Meat allergy*[36] Gotch ear*[37]
Rhipicephalus sanguineus (Latreille)	Brown Dog Tick	Solid brown scutum, prominent eyes, hexagonal basis capitulum	Indoors, limestone walls, humid environments[38]	All stages prefer dogs, but will engorge on other hosts[39] Unknown seasonality in Ontario (indoor)	North America (warmer climates) & can establish indoors	*Ehrlichia canis* *Anaplasma platys* *Babesia canis* *Babesia vogeli* *Rickettsia rickettsii*[40] *Rickettsia massiliae* *Bartonella henselae* *Hepatozoon canis* Tick paralysis*[41]

Notes: * Tick paralysis, meat allergy and gotch ear are not known to be caused by pathogens; however, they are associated with tick bites.
† STARI (Southern Tick Associated Rash Illness) is a condition for which an etiologic agent has not been identified.

References:
[1] Keirans and Litwak, 1989
[2] Centers for Disease Control and Prevention, 2016
[3] de la Fuente et al., 2008
[4] Lindsay et al., 1999
[5] Guerra et al., 2002
[6] Keirans et al., 1996
[7] Apperson et al., 1993
[8] Lindsay et al., 1999b
[9] Lord, 1995
[10] Ogden et al., 2008b
[11] Dibernardo et al., 2014
[12] Ebel and Kramer, 2004
[13] Gregson, 1973
[14] Eisen et al., 2002
[15] Eisen et al., 2004
[16] Lane et al., 2005
[17] Gregson, 1956
[18] Davis et al., 2015
[19] Padgett et al., 2014
[20] Ko, 1972
[21] Farkas and Surgeoner, 1990
[22] Scholten, 1977
[23] McLean et al., 1964
[24] Dodds et al., 1969 (ref. Benson 1964 unpublished)
[25] Garvie et al., 1978
[26] Blouin et al., 1984
[27] Jessup, 1979
[28] Wilkinson, 1967
[29] Bishopp and Trembley, 1945
[30] Mail and Gregson, 1938
[31] Semtner et al., 1971
[32] Childs and Paddock, 2003
[33] Reichard and Kocan, 2006
[34] Reichard et al., 2010
[35] Paddock and Goddard, 2015
[36] Commins et al., 2011
[37] Edwards, 2011
[38] Dantas-Torres, 2010
[39] Dantas-Torres, 2008
[40] Demma et al., 2005
[41] Otranto et al., 2012

1.1.4 Global changes in tick distribution

Globally the distribution of ticks in the environment is changing (Scharlemann et al., 2008; Jaenson et al., 2012; Nelder et al., 2014). Although ticks are flightless and do not travel large distances on their own (Randolph, 1998), during their prolonged feeding they can be dispersed over a wide area with the movement of wild animals via migration (Hoogstraal and Kaiser, 1961; Scott et al., 2001; Ogden et al., 2008a) or repopulation (i.e., conservation) (Barker et al., 1992; Leo et al., 2014), livestock transportation (Barré and Uilenberg, 2010), exotic species importation (Keirans and Durden, 2001), and travel of companion animals and people (Kock et al., 2010; Leighton et al., 2012). Environmental change, including climate change, is thought to be an important factor leading to changes in tick distribution in North America (Brownstein et al., 2005; Ogden et al., 2014b) and Europe (Süss et al., 2008; Gray et al., 2009; Boeckmann and Joyner, 2014; Jore et al., 2014). In Canada, the distribution of *Ixodes scapularis* ticks has been expanding at an unprecedented rate (Leighton et al., 2012; Nelder et al., 2014), and because it is the vector for *Borrelia burgdorferi* sensu stricto, the causal agent of Lyme disease, it will be discussed in further detail (Section 1.3.2).

Other tick species are also undergoing range expansion. Within Canada, *Dermacentor variabilis* established in southern Saskatchewan are expanding to the northwest and overlapping with *Dermacentor andersoni*, which are also undergoing range expansion in a northeastern direction within Saskatchewan (Wilkinson, 1967; Dodds et al., 1969; Dergousoff et al., 2013). A maximum entropy (Maxent) modelling approach can be used to estimate habitat suitability using georeferenced bioclimatic

variables, and this method was used to determine that elevation and temperature are biologically significant environmental variables that influence the distribution of *D. variabilis* in the United States (James et al., 2015).

Similar to *I. scapularis*, lone star ticks (*A. americanum*) in the eastern and mid-western United States are also undergoing a northern range expansion (Springer et al., 2014). Lone star ticks have been sporadically reported in Canada (Scholten, 1977; Nelder et al., 2014); however, there are no published reports of established populations of *A. americanum* in Canada (Lindquist et al., 2016).

The geographical expansion of ticks and tick-borne diseases has been aided by alterations in land use, reforestation, importation or reintroduction of species, the impacts of climate change and increased travel of people with their pets (Shaw et al., 2001; Ogden et al., 2008c; Keesing et al., 2010; Otranto et al., 2015). The modern pet lives in close proximity with its human caregivers, often sharing the same environment for recreation and travel. Determining the distribution of ticks and associated pathogens in the environment is an essential component of public health services to detect, monitor, and mitigate changes in tick-borne disease risk (Smith et al., 2012; Ogden et al., 2014a).

1.2 IXODIDAE AND COMPANION ANIMALS

1.2.1 Tick Collection

Ticks can be collected by active or passive surveillance. The active collection of ticks from the environment is the gold standard to confirm the establishment of tick populations in new areas (Ogden et al., 2010). Active field surveillance methods for tick

collection include dragging a 1 m^2 white flannel sheet through suspected tick habitat, or the capture of mammals and birds for tick inspection and removal (Ogden et al., 2010). These techniques are labour intensive and require considerable physical and financial resources (Johnson et al., 2004; Nelder et al., 2014).

Passive surveillance involves the collection, identification, and mapping of ticks removed from people and pets as submitted by the public through public health units, physicians, or veterinarians (Ogden et al., 2006; Nelder et al., 2014). Unless animals are intentionally used to collect ticks from a particular region (e.g., Larrousse et al., 1928; Leschnik et al., 2012), all ticks collected from pets are part of the passive surveillance system. Following the identification of *B. burgdorferi*-positive *I. scapularis* ticks at Long Point Provincial Park in 1987 (Barker et al., 1988), the Public Health Agency of Canada (PHAC) initiated a passive surveillance system for tick collection that was eventually adapted across most provinces (Ogden et al., 2006). This long standing tick collection dataset has provided Canadian researchers with information to help measure the changes in Ixodidae tick distribution in Canada (Morshed et al., 2006; Ogden et al., 2006; Leighton et al., 2012; Nelder et al., 2014). The passive surveillance program has focussed on ticks from a variety of hosts including companion animals and humans; however, over time and in the different provinces the program has been modified to meet the demands on resources. For example, in 2009 due to the overwhelming numbers of ticks submitted per annum from pets, Public Health Ontario (PHO) stopped requesting ticks from veterinarians (Nelder et al., 2014). To the author's knowledge, there have been no Canadian studies published limited to the prevalence, risk, and rate of tick infestation in companion animals. Fitzgerald (2012) focused his MSc research on ticks

collected from pet dogs to update historical records for tick species in Alberta. The results from this thesis have not yet been published in an academic journal, but are referenced in a Government of Alberta document from the Surveillance and Assessment Branch of Alberta Health (Government of Alberta, 2014).

Pet focused studies of tick infestation have been undertaken in many countries including: the UK (Ogden et al., 2000; Smith et al., 2011), Belgium (Claerebout et al., 2013), the Netherlands (Nijhof et al., 2007), Germany (Beichel et al., 1996), Austria (Leschnik et al., 2012), Hungary (Földvári and Farkas, 2005), Greece (Papazahariadou et al., 2003), China (Chen et al., 2014), Japan (Shimada et al., 2003; Hiraoka et al., 2007), the USA (Koch, 1982; Trout and Steelman, 2010), and Brazil (Dantas-Torres et al., 2009). Unlike Canada, some countries lack a rigorous tick monitoring program; for example, it was not until 2005 that the Health Protection Agency of Great Britain promoted a national tick surveillance program (Jameson and Medlock, 2011).

Domestic animals have been used for passive tick surveillance to accomplish the following: 1) update historical distribution records and confirm the presence of novel tick species (Földvári and Farkas, 2005; Claerebout et al., 2013); 2) establish rates of tick infestation (Papazahariadou et al., 2003; Shimada et al., 2003; Dantas-Torres et al., 2009); 3) estimate the prevalence of tick-borne pathogens (Beichel et al., 1996; Hiraoka et al., 2007; Smith et al., 2012); 4) investigate tick-borne disease outbreaks (Nijhof et al., 2007; McQuiston et al., 2011); and 5) establish risk factors for tick carriage by dogs (Ogden et al., 2000; Földvári and Farkas, 2005; Smith et al., 2011).

Although passive surveillance may highlight environmental risk areas there are several limitations to this approach. The greatest limitation to passive surveillance is that

adult female ticks may remain attached to their hosts for up to two weeks; though most feed to repletion and drop from the host within seven days (Sonenshine, 1991; Falco et al., 1996). Therefore, ticks may be acquired in one location and discovered (or removed) many days later in a different region. The collection of travel history is essential when analyzing results from passive surveillance (Ogden et al., 2000). This prolonged attachment can also result in adventitious (i.e., accidental or coming from outside) ticks that migrate with their hosts. Research has shown that every year billions of migrating birds have the potential to carry upwards of 50 million ticks 200 km per day (Hoogstraal and Kaiser, 1961; Klich et al., 1996; Ogden et al., 2008a; Hobson et al., 2014). These adventitious ticks may establish autochthonous populations, introduce new foci of *B. burgdorferi* in resident small mammal populations, and/or may bite people or domestic animals and transmit associated pathogens (Ogden et al., 2008a; Scott et al., 2012b).

Leschnik et al. (2012) highlight another significant limitation of passive surveillance, the species and developmental stages of ticks collected are inherently different between active and passive tick collection. In active surveillance, most species of questing ticks are collected; however, some ticks are very host specific and as such, passive surveillance only collects ticks that will bite and engorge on animals. This Austrian study by Leschnik et al. (2012) compared ticks collected via dragging to ticks simultaneously collected from specifically-tick-pathogen-free beagles. All ticks acquired from the dragging technique were accounted for, but only the ticks that bit and engorged for 5 days on the beagles were included in the analysis. Although both techniques yielded similar total tick numbers (dragging n=96 and dogs n=104), the dragging technique retrieved a greater number of species and developmental stages, as well as

twice the number of pathogen infected ticks (Leschnik et al., 2012). In comparison, a recent Polish study found no difference in the prevalence of *B. burgdorferi* spp. in ticks acquired from pets versus those collected from vegetation, which may be the result of regional differences in the prevalence of tick-borne pathogens (Król et al., 2015).

1.2.2 Risk factors for tick infestation

Using passive surveillance data, Nelder et al. (2014) identified that the age of people submitting was significantly different for the different tick species, and that men submitted significantly more *I. scapularis* and *D. variabilis* than women. Reported submission rates for *I. scapularis* in Canada were highest in children under 10 years of age and adults 55-74 years old (Nelder et al., 2014). A similar bimodal age pattern was observed in the United States where the incidence of Lyme disease was highest in boys 5-9 years of age and adults 60-63 years old (Nelson et al., 2015). This age distribution pattern is likely due to greater exposure to ticks or tick habitat in these age groups (Nelson et al., 2015); however, other potential causes should be explored.

Unlike human surveillance, various study designs have been used to investigate the risk factors for tick infestation in pets. Ogden et al. (2000) investigated the species of Ixodidae that had bitten 125 dogs and 67 cats from fifty-six veterinary practices in Great Britain and Ireland. The authors argued that a "true negative" control was impractical because of the time required to ensure an animal was tick-free by the clinic staff. Information about animal travel and exposure was collected for a two-week period prior to tick removal to account for travel-associated ticks. In this Ogden et al., (2000) study, relative to cats, dogs were significantly more likely to be carrying *I. ricinus* and relative

to dogs, cats were significantly more likely to be carrying *I. hexagonus*. Furthermore, exposure to a boarding kennel or cattery was significantly associated with the carriage of *I. canisuga*. In addition, the odds of *I. ricinus* carriage for both dogs and cats was significantly increased with exposure to woodland and moorland habitats, and significantly decreased with exposure to urban parks; however, converse significant associations were noted with *I. hexagonus* carriage (Ogden et al., 2000). In a similar study focusing on 310 dogs from 25 veterinary clinics in Hungary, the researchers found that the odds for tick attachment on dogs decreases from head to legs, but they found no association between living conditions, outdoor activity, and the species of tick; however, there was no description of the statistical methods used for analyses (Földvári and Farkas, 2005).

In an American study regarding ticks on dogs, the researcher focused on rural or suburban outdoor dogs (i.e., the majority of dogs were hounds and no house dogs were examined), and over one year an impressive 75,000 ticks were collected from Oklahoma and northwestern Arkansas (Koch, 1982). During the peak tick season, 30 dogs were chosen at random to record preferences for tick attachment: *R. sanguineus* (head, neck, back, and between toes), *A. americanum* (perianal region and between hind legs), *D. variabilis* (ears, head, and neck), and *I. scapularis* (ears and anterior body). The age, weight, sex, and breeds of the dogs were noted, but there was no discussion regarding associated risk factors between these variables and tick infestation (Koch, 1982). Trout and Steelman (2010) used veterinary clinics in Arkansas to collect ticks and found no difference in species between dogs and cats, but only 16 cats and 156 dogs participated, which may not have provided sufficient statistical power to detect a difference. In this

study, no animal risk factors were discussed, as the pets were solely used as a means for passive tick surveillance to update state records on tick-host interactions (Trout and Steelman, 2010). They did conclude that *A. americanum* infestation in dogs was more severe than had been previously reported by Koch (1982).

Smith et al. (2011) used a more rigorous study design and enrolled 173 veterinary practices in Great Britain to monitor tick infestation in dogs from March to October, 2009. In this study, each veterinary practice participated for 3 months and each week 5 dogs admitted for surgery were randomly selected and thoroughly examined for ticks using a standardized technique; 3534 dogs were examined, 810 of whom were carrying at least one tick. Researchers acknowledged that some veterinary practices may have misunderstood the study protocol and only submitted data from dogs carrying ticks. As a result, those practices were removed from the analysis. Using logistic regression, travel, acaricide treatment within the previous 2 months, age, sex, and size were not significant predictors of tick infestation; however, certain breeds (gundog, terrier, and pastoral breeds), intact dogs, and dogs with medium hair length were significantly more likely to be carrying ticks (Smith et al., 2011). This study also reported that relative to urban areas, a significantly higher proportion of dogs from rural practices were infested with *I. ricinus*, and significant seasonal differences in tick infestation were identified for some tick species (Smith et al., 2011). A potential limitation to this study is that veterinary practices only participated for 3 months at a time, which may have limited seasonal comparison within geographic regions.

In a unique study design, researchers measured the seasonal and spatial distribution and abundance of *I. ricinus* using 1500 blanket drags in a peri-urban park in

south west England, and then two years later, owners visiting the park more than once a month were invited to fill out a short questionnaire and have their dog wear a GPS collar to walk their usual route through the park (Jennett et al., 2013). Ticks were not actually retrieved from the dogs after wearing the tracking collar, but owners were asked about frequency of walks in the park, history of tick infestation, use of tick prevention, and if they could identify a tick. Only owners that could properly identify a tick were included in the study. Using regression models, this study found that owners of shorter haired dogs reported a higher incidence of ticks, and breed was not a significant predictor of tick infestation. Surprisingly, the most significant predictor of tick infestation was the frequency of visits to this peri-urban park, and not the amount of time (nor distance) a dog spent in high-risk woodland and forest edge habitats. The authors noted that their results may be confounded by the wide range of habitats frequented by most of the dogs in the park, and other variables such as grooming protocols and climatic conditions during the walk (Jennett et al., 2013).

The variety of study designs and research techniques used to establish risk factors for tick carriage in companion animals results in a variety of conflicting conclusions. For example, both medium-haired dogs relative to short-haired (Smith et al., 2011) and short-haired dogs relative to long-haired dogs (Jennett et al., 2013) were considered at higher risk for tick bites. This difference may be attributed to the somewhat subjective nature of "hair length" as an explanatory variable. In addition, potential confounding variables (e.g., species of tick, numbers of ticks questing in the environment, grooming habits) may contribute variability to the conclusions.

1.2.3 Tick-borne disease serology

Dogs and cats can act as sentinels for vector-borne disease (Shaw et al., 2001; Nicholson et al., 2010; Mead et al., 2011). When Lyme disease was first discovered in the mid 1970's, serological surveys for antibodies against *Borrelia burgdorferi* were performed on many species (Magnarelli et al., 1984). The interpretations of these early immunofluorescent antibody (IFA) and enzyme linked immunosorbent assay (ELISA) tests were confounded by a lack of standardization and validation, and the inability of IFA to distinguish between natural exposure and a vaccinated animal (Greene, 1990; Banerjee et al., 1996). Current *B. burgdorferi* antibody tests are much more sensitive and specific (Little et al., 2010; Bouchard et al., 2015), and will be discussed in section 1.3.3.

Dogs have been proposed for use as sentinels for Lyme disease risk in people because they: 1) mount a robust and easily measurable antibody response to *B. burgdorferi*; 2) are suitable hosts for *I. scapularis*; 3) are commonly exposed to tick habitat; 4) lack protective clothing; and 5) have limited ability to self-groom (Eng et al., 1988; Rand et al., 1991; Guerra et al., 2001; Duncan et al., 2005; Little et al., 2010). In an early study on seroprevalence, Eng et al., (1988) concluded that dogs were significantly more likely to be seropositive than their owners. No associations were found between positive serological test results and the age, sex, breed, hours spent outdoors, location, and use of flea and tick products in the dogs from this Massachusetts study (Eng et al., 1988), nor were associations found with canine signalment (i.e., age, sex and breed) and positive serology in a seroprevalence study in California (Olson et al., 2000). In the Netherlands, research found that seroprevalence in dogs was a better indication of Lyme disease risk than the use of human incident cases (Goossens et al.,

2001). Dogs are not considered important reservoir hosts for *B. burgdorferi*, and most studies have found no correlation between dog ownership and human seropervalence (Eng et al., 1988; Goossens et al., 2001; Wilking et al., 2015). However, one study used a large random-digit-dialing telephone survey (Foodborne Disease Active Surveillance Network, CDC) to look at risk factors for tick bites in Tennessee, and found that dog owners were at significantly greater odds of tick bites than people who did not own dogs (Jones et al., 2002). The results from this study may be specific to dog ownership in Tennessee, but may also suggest that dog owners visit outdoor tick habitat with their dogs, or dog owners have a knowledge of ticks due to their dogs also getting parasitized.

Guerra et al (2001) identified the following risk factors for canine seroprevalence: 1) dogs that were used for hunting or herding were 1.61 times more likely to be seropositive compared to pet animals; 2) dogs living in forested or urban areas had an approximately two fold increase in the odds of being seropositive compared to dogs living in agricultural communities; 3) dogs vaccinated for Lyme disease were less likely to be seropositive for *B. burgdorferi* at the individual level, but at the county level the percentage of Lyme disease vaccinated dogs was not associated with seropositivity; and 4) dogs with a limited home range were also reported to be less likely to be seropositive regardless of the amount of time spent outdoors (Guerra et al., 2001). This study and others support the notion that the geographical distribution of serologically positive dogs can follow the distribution for Lyme disease in people (Duncan et al., 2005; Little et al., 2010). However, seasonal and yearly trends of Lyme disease in people were not found to be well predicted by serological surveillance in dogs, which is likely due to the duration of seroconversion in dogs which will be addressed in section 1.3.4 (Johnson et al., 2004).

1.2.4 National database surveillance

Electronic medical records can facilitate access to large de-identified databases to answer general questions regarding patient risk factors and seasonality of tick exposures (Glickman et al., 2006). However, the extrapolation to specific tick-borne diseases may be limited since medical records often do not specify tick species and the accuracy of tick identification may be unreliable because not all clinicians can identify ticks to the species level. Travel history may also be missing or unavailable from medical records, and could contribute to overestimation of tick-borne disease in certain regions (Millen et al., 2013). Despite these limitations, research has determined that a spike in tick related medical visits by dogs and humans in North Carolina occurred four weeks before the onset of signs and symptoms of tick-borne disease seen in hospital emergency visits; and this information could be used to alert public health units for when to promote the use of tick prevention (Rhea et al., 2011). In another study using a nation-wide veterinary database from the United States, tick infestations peaked in May and June and younger, male, and sexually intact dogs were at increased risk of infestation; however, due to the statistical power of these large sample sizes (i.e., 8 million dogs) even small changes in risk estimates were statistically significant (Raghavan et al., 2007).

Large veterinary datasets of canine vector-borne disease serology have also been used for epidemiological studies (Little et al., 2014; Qurollo et al., 2014; Yancey et al., 2014). In the USA, a greater than 5% canine *B. burgdorferi* seropositivity was associated with a higher than average incidence of human Lyme disease (Mead et al., 2011). In Canada, endemic areas for Lyme disease are defined as geographical regions where active field surveillance has identified the presence of multiple stages of *I. scapularis*

(i.e., larva, nymph and adult ticks) with either ticks or animals positive for *B. burgdorferi* in several seasons in more than one year (Ogden et al., 2006; Government of Canada, 2015). A recent study of the Canadian serology tests submitted to a diagnostic laboratory (i.e., IDEXX Laboratories, Inc., location not specified) in 2013 and 2014, reported that 2.5% of the 115,636 tests performed were positive for *B. burgdorferi* (Herrin et al., 2015). These researchers found that within the province of Ontario, 5.1% of tests in southeastern Ontario, a known endemic focus for *I. scapularis*, were positive for antibodies to *B. burgdorferi* compared to 0.9% for the rest of Ontario. These findings highlight the regional differences for *B. burgdorferi* infection risk within Ontario.

Although serology appears to be a sensitive indicator of regions endemic for *B. burgdorferi*, overestimation is possible in areas of low pathogen prevalence if information regarding the travel history of dogs is lacking. In a non-endemic region of Colorado, researchers found 11 of 12 dogs that seroconverted to *B. burgdorferi* had a travel history to, or resided in, an area endemic for Lyme disease (Millen et al., 2013). Although the ELISA test for *B. burgdorferi* antibodies (i.e., SNAP 4Dx Plus Test, IDEXX Laboratory Inc.,) is very sensitive (94.1%) and "exquisitely specific" (96.2%) (Little et al., 2014), the positive and negative predictive values of a test are influenced by the prevalence of the disease in the population being tested, and when testing in areas of low pathogen prevalence, poor positive predicted values should be anticipated (Peregrine et al., 2007; Millen et al., 2013; Forrester et al., 2015).

In an effort to enhance the accuracy of the predictive models from these large datasets, an expert panel was assembled by the Companion Animal Parasite Council to identify quantitative factors that influence the prevalence of canine tick-borne disease

(Stich et al., 2014). The panel of experts identified five categories of interest: 1) vector (e.g., distribution and abundance); 2) wildlife hosts; 3) abiotic (e.g., temperature and humidity); 4) habitat (e.g., vegetation and land use); and 5) social (e.g., access to preventative care, recreation, socioeconomic status, media coverage). Other variables that were thought to be important included the prevalence of pathogens in vectors, vector abundances, total number of pet dogs per county, and tick control product sales per region. The next phase of the project is to statistically assess these factors for predictive power in statistical models (Stich et al., 2014).

1.3 LYME DISEASE

1.3.1 History of Lyme disease

North American interest in vector-borne disease increased after an epidemiological cluster of juvenile arthritis was attributed to the tick-borne spirochete, *Borrelia burgdorferi,* in the region of Lyme, Connecticut (Steere et al., 1977; Burgdorfer, 1984; Telford and Goethert, 2004). Dr. Willy Burgdorfer (1993) wrote an interesting account of how the etiological agent of Lyme disease (LD) was discovered. Originally, LD was thought to be a newly emerging or introduced disease; however, examination of archival tick collections and ticks recovered from museum specimens confirmed the presence of *B. burgdorferi* positive ticks in endemic regions prior to the 1970's (Persing et al., 1990). In Canada, blacklegged ticks positive for *B. burgdorferi* were first reported in 1987 at the Long Point National Wildlife Area along the northern shore of Lake Erie (Barker et al., 1988; Barker et al., 1992).

1.3.2 The vector

Ixodes scapularis, in eastern regions of North America, and *Ixodes pacificus*, in the west, are known competent vectors for *Borrelia burgdorferi* sensu stricto (henceforth referred to as *Borrelia burgdorferi*), the causal agent of LD (Lane and Burgdorfer, 1987; Piesman and Happ, 1997). Around the world, the causal agents of LD are transmitted by other *Ixodes* species including: 1) *Ixodes ricinus* in Europe, West Asia, and North Africa, and 2) *Ixodes persulcatus* in Asia and Eastern Europe (Margos et al., 2011; Franke et al., 2012). Historically, the blacklegged tick, *I. scapularis*, was considered closely related to a separate northern species, *Ixodes dammini* Spielman (Spielman et al., 1979). However, further studies demonstrated that *I. dammini* and *I. scapularis* were not independent species (Keirans et al., 1996).

The first endemic population of *I. scapularis* in Ontario was identified at Long Point in the early 1970's and was purported to have arrived on migrating birds or imported with deer in 1886 (Watson and Anderson, 1976; Barker et al., 1992). Since their discovery in Canada, much work has been done to determine habitat suitability and the likelihood of survival through the Canadian winter (Lindsay et al., 1995; Lindsay, 1995; Lindsay et al., 1999; Simon et al., 2014; Werden et al., 2014).

Blacklegged ticks have preference for deciduous forests (Lindsay et al., 1998; Guerra et al., 2002), and the abundance of food for key hosts is positively associated with the density of immature stages of *I. scapularis* (Ostfeld et al., 2006). *Ixodes scapularis* have a three-stage life cycle consisting of larva, nymph and adult (Anderson and Magnarelli, 1980). Each stage requires a blood meal, and the lifecycle takes at least 3 years to complete, but may be extended to four years if suitable hosts are not found

(Yuval and Spielman, 1990; Lindsay et al., 1998). *Ixodes scapularis* is a euryxenous (broad host range) tick, and has been reported to feed on at least 125 species of North American vertebrates (Keirans et al., 1996). By accepting a variety of potential hosts, this tick may be easily distributed by migrating birds or mammals into new areas (Keirans et al., 1996; Scott et al., 2012). Research by Ogden and colleagues, suggests there is a 5 year lag between the arrival of *I. scapularis* and the establishment of *B. burgdorferi* in small mammal host populations (Ogden et al., 2013).

Ixodes scapularis ticks transstadially transmit *B. burgdorferi* from larva to nymph, and from nymph to adult (Mukolwe et al., 1992; Piesman and Happ, 1997). Unlike *Borrelia miyamotoi* that cause relapsing fever, *B. burgdorferi* do not appear to be transovarially transmitted, and as such larva are not implicated in *B. burgdorferi* transmission (Rollend et al., 2013). In humans, nymphs are responsible for the majority of *B. burgdorferi* infections (Falco et al., 1999; Diuk-Wasser et al., 2006), but it has been suggested that adult stages may be responsible for exposing dogs to *B. burgdorferi* (Little et al., 2010). Adult and nymphal ticks become infected when they feed on infected hosts, including small mammals and birds, which serve as the main reservoirs for *B. burgdorferi* (Donahue et al., 1987; Lindsay et al., 1997; Richter et al., 2000; Hanincova et al., 2006). In contrast, the main host for larval and nymph stages in the southern USA are small lizards (Apperson et al., 1993). Lizard blood has bactericidal properties for *B. burgdorferi*, and this may explain why there is a reduced prevalence of Lyme disease in the Southern USA (Levin et al., 1996; Duncan et al., 2005). White-tailed deer are the preferred host for adult *I. scapularis* and deer also support both larval and nymphal ticks (Piesman et al., 1979). Deer are not a competent reservoir for *B. burgdorferi* (Telford et

al., 1988). Research has shown that pathogen transmission can occur between ticks when they co-feed in close proximity on the same host (Gern and Rais, 1996; Randolph et al., 1996). Other ticks, including *Amblyomma americanum, Dermacentor variabilis* and *Ixodes cookei* can acquire *B. burgdorferi* when feeding on an infected host, but they do not maintain infection after they moult (Mather and Mather, 1990; Lindsay et al., 1991; Barker et al., 1993).

1.3.3 The pathogen

Pathogenic tick-borne *Borrelia* species can cause 1) Lyme borreliosis (i.e., *Borrelia burgdorferi* sensu lato complex) or 2) relapsing fever (e.g., *B. miyamotoi* and *B. lonestari*) (Scoles et al., 2001; Franke et al., 2012). Each *Borrelia* species has multiple genetic strains (Margos et al., 2011). Up until very recently, the only known species to cause LD in North America was *Borrelia burgdorferi* sensu stricto. In 2016, Pritt et al. (2016) described a novel species, candidatus *Borrelia mayonii*, found in the upper Midwest region of the United States. This novel *Borrelia* species is associated with human LD, and can present with an uncharacteristically high spirochaetaemia (Pritt et al., 2016). *Ixodes scapularis* has been confirmed as a competent vector for this novel pathogen (Dolan et al., 2016).

Borrelia burgdorferi is a small gram-negative bacterial spirochete (0.2 µm to 30 µm), and 21 plasmids have been identified in wild strains; 12 linear and 9 circular (Casjens et al., 2000). The plasmid genome carries 670 potential genes (Strother et al., 2005), many of which code for outer surface proteins (Osp) (Chaconas and Norris, 2013). These proteins aid in the survival and persistence in the vertebrate, and facilitate the

transmission of *B. burgdorferi* from the tick vector to the host (Purser and Norris, 2000; Strother et al., 2005; Chaconas and Norris, 2013). *In-vitro*, *B. burgdorferi* can lose some pathogenic potential due to the loss of plasmid genome, but passage of an attenuated *B. burgdorferi* strain through *I. scapularis* can re-enhance pathogenicity in mice (Adusumilli et al., 2010).

Borrelia burgdorferi is very resilient within the host, and has specific host metabolic product requirements (e.g. N-acetyl-glucosamine), but it does not survive well in the environment (Guerra et al., 2002; Krupka and Straubinger, 2010). In unfavourable *in-vitro* conditions this spirochete can convert to a cystic form (Brorson and Brorson, 1997). Once deposited in the host, *B. burgdorferi* can migrate through the skin and connective tissues with a periplasmic flagella (Straubinger et al., 1997a; Sal et al., 2008). This bacterium does not circulate in peripheral bloodstream in large numbers, and is slow growing and therefore difficult to isolate (Greene, 2012). *Borrelia burgdorferi* requires manganese, zinc, and magnesium for growth and motility, and is unique among bacteria in that it is not inhibited by the host cellular secretion of lactoferrin, which limits the availability of iron to replicating bacteria (Posey and Gherardini, 2000).

Tick saliva is an important component of the immunopathogenesis of Lyme borreliosis (Mason et al., 2013). In experimental mouse models, tick saliva interferes with signaling pathways and has inhibitory effects on the migration of dendritic cells, the antigen presenting cells of the innate immune system (i.e., CD4 T-cells) (Skallová et al., 2008; Slámová et al., 2011). Specifically, the tick salivary protein, Salp15, interferes with CD4 activation and improves the capacity of *B. burgdorferi* to colonize the host (Anguita et al., 2002; Ramamoorthi et al., 2005).

Transmission and infection of *B. burgdorferi* to a susceptible host requires attachment of an infected competent *Ixodes* vector and prolonged attachment (i.e., greater than 24 hours) (Piesman et al., 1987). When *B. burgdorferi* bacteria colonize the midgut of competent vectors, they display OspA (Yang et al., 2004). After 24 to 36 hours of tick feeding, the upregulation of OspC allows the bacteria to migrate from the tick midgut to the salivary gland where they will enter the host with saliva during the blood meal (Pal et al., 2004). If these bacteria enter the host, a VlsE gene codes for both variable surface proteins, which aid to evade host immune response, as well as invariable regions of which the most notable is the C6 peptide (Levy et al., 2008). Antibodies to the C6 peptide form the basis for current serology testing for natural *B. burgdorferi* exposure in dogs allowing differentiation from antibodies formed by Lyme vaccination (Wagner et al., 2012; Barth et al., 2014).

1.3.4 The disease

Borrelia burgdorferi has become the most common vector-borne human pathogen in North America (Kurtenbach et al., 2006). Human cases have increased dramatically over the past 10 and 20 years in Canada and the USA, respectively. The Centers for Disease Control and Prevention (CDC) reported 17,730 cases of LD in 2000, and 33,461 confirmed or probable cases for 2014 (Centers for Disease Control and Prevention, 2015). Nelson et al. (2015) suggest that in the USA incident LD cases may be 10-fold higher than actually reported. In Canada, a similar pattern for LD has emerged with 64 cases reported in 2005 and 917 cases reported in 2015 (Government of Canada, 2015; Kulkarni et al., 2015). Lyme disease became nationally notifiable for Canada in 2009

(Ogden et al., 2009). This may reflect some of the increase in cases; however, after accounting for under reporting, as noted in the USA, the true incidence of Lyme disease is expected to be higher. In 2015, Public Health Ontario updated the 2009 LD case definition for confirmed and probable cases to include travel to an "endemic OR risk area" as defined by their 2015 Ontario Lyme Risk Area Map (Public Health Ontario, 2015). In endemic areas of the US, the risk of human LD after exposure to an infected tick has been estimated to be between 1-3% (Sood et al., 1997; Hojgaard et al., 2008). A thorough discussion of human LD is beyond the scope of this literature review. Borchers et al. (2015) provide a current thorough review of the diagnostic criteria and treatment of LD in people.

In the early days of LD investigation, dogs were found to seroconvert to *B. burgdorferi*, and subsequently 30 years of epidemiology and canine research has ensued (Magnarelli et al., 1984). Lyme disease has been extensively studied in dogs and uncommonly reported in horses and cattle (Magnarelli et al., 2004; Greene, 2012; Divers, 2013). Although cats are bitten by *I. scapularis* and they mount an antibody response to *B. burgdorferi*, clinical LD has not been described in this species (Krupka and Straubinger, 2010; Greene, 2012). Unlike humans, currently there is no formal reporting system for LD in North American dogs (Duncan et al., 2005). It has been be difficult to associate natural *B. burgdorferi* exposure with canine clinical disease due to the lengthy incubation period (2-5 months), prolonged antibody response (seroconversion lasting at least 69 weeks), and plethora (95%) of subclinical exposures (Liang et al., 2000; Littman et al., 2006; Greene, 2012).

In experimental models, LD consists of 1 to 4 days of intermittent and often recurrent shifting leg lameness, anorexia, depression, and pyrexia (39.5ºC to 40ºC) (Appel et al., 1993; Chang et al., 2001; Straubinger et al., 1997b). Experimental *B. burgdorferi* infection requires the transmission of the bacterium to the host via the *I. scapularis* vector; injecting research animals intradermally or intravenously with laboratory strains of *B. burgdorferi* did not result in clinical disease (Appel et al., 1993). Seroconversion is detected 4 to 6 weeks after exposure, but not all dogs seroconvert, nor do all dogs develop clinical disease (Straubinger et al., 1997b; Straubinger et al., 2000). Clinical signs of lameness are self-limiting, and by 6-8 weeks, dogs had fully recovered and did not show any additional signs for the 17 month observation period (Appel et al., 1993). Joint histopathology of acutely lame experimentally infected dogs consisted of a fibrinopurulent arthritis and synovitis (Appel et al., 1993). In another study (Summers et al., 2005), lameness was observed in 63% (39/62 dogs) of specific-pathogen-free beagles who seroconverted to *B. burgdorferi*; lameness developed 2-5 months after exposure to *B. burgdorferi*-infected ticks, most dogs had more than one episode, and the 3-4 days of lameness resolved without treatment. In the above study, of the remaining 23 dogs that showed no signs of LD, 19 of them had evidence of lymphoplasmacytic arthritis in at least one joint, but the researchers could not exclude other causes of canine arthritis in these animals. Susta et al. (2012) confirmed that in experimental models, LD manifestations of disease are limited to a range of non-specific clinical and pathological changes; complete blood counts, biochemistry, urine protein creatinine ratios, synovial and lymph node cytology, and renal histopathology are not useful to identify subclinical *B. burgdorferi* infections.

One of greatest challenges of studying LD in dogs is that the disease is multifactorial and Koch's postulates have not been completely satisfied; some sequelae attributed to *B. burgdorferi* are not reproducible in experimental models (Littman et al., 2006; Littman, 2013). A Lyme-associated protein-losing nephropathy (PLN) consisting of an immune-mediated glomerulonephritis with diffuse tubular necrosis and interstitial nephritis has been described (Dambach et al., 1997; Littman, 2013). Labrador and golden retrievers were found to be at increased risk for Lyme nephritis (Dambach et al., 1997; Hutton et al., 2008). Currently, there are no predictive tests for this disease and seropositive dogs should be monitored for proteinuria (Littman, 2013). Cardiac manifestations and ocular inflammation (e.g., conjunctivitis, anterior uveitis, retinal detachment and optic neuritis) have also been attributed to *B. burgdorferi* infection in the field (Levy and Duray, 1988; Littman et al., 2006; pers. comm. Dr. H. Gray, OVMA Conference 2016).

In private practice, the diagnosis of canine LD is challenging in regions of low disease prevalence (Peregrine et al., 2007). As previously described, the current serological tests measures antibodies to the C6 peptide, generated 4-6 weeks after exposure to *B. burgdorferi* and is very sensitive (SNAP 4Dx Plus 98.5%; AccuPlex4 78.5%) and specific (SNAP 4Dx Plus 95.7%; AccuPlex4 72.9%) (Littman et al., 2006; Goldstein et al., 2014). However, it is estimated that only 5% of exposures will result in disease and the current recommendation is to not treat clinically normal animals (i.e., only positive for C6 antibodies) unless there is reason to believe they are proteinuric due to LD (Littman et al., 2006; Littman, 2013). Bouchard et al. (2015) summarize the key criteria for LD diagnosis in dogs: 1) clinical signs consistent with LD (fever, anorexia,

depression, and lameness); 2) credible exposure to a competent vector or travel to an *I. scapularis* risk area; 3) positive laboratory tests; 4) elimination of other differential diagnoses; and 5) response to treatment. The risk area for *I. scapularis* bites in dogs may be much greater than that published for humans, as dogs are very proficient at acquiring adventitious ticks. However, the areas for *B. burgdorferi* exposure should be similar to those for people (Bouchard et al., 2015).

Treatment is recommended for dogs clinically ill with Lyme disease (Littman et al., 2006). Current antimicrobial therapy for LD includes doxycycline (10 mg/kg PO q24hr for 4 weeks), or in young animals amoxicillin (20 mg/kg PO q8hr for 4 weeks), or recently cefovecin (two subcutaneous injections 14 days apart at 8 mg/kg) has been proposed to offer better owner compliance for the treatment of LD (Greene, 2012; Littman et al., 2006; Wagner et al., 2015). Despite treatment with antibiotics, research suggests that many dogs remain persistently infected with *B. burgdorferi* (Appel et al., 1993; Straubinger et al., 1997b ; Straubinger et al., 2000), and relapse may be possible (Littman, 2003; Krupka and Straubinger, 2010). Occasionally dogs remain PCR positive after treatment for *B. burgdorferi*, but in most cases antibody levels declined, joint lesions were prevented or resolved, and dogs remained free of clinical signs for the duration of the study period (Straubinger et al., 1997b; Straubinger et al., 2000). It is yet to be determined if the slowly replicating *B. burgdorferi* bacteria persist in the poorly vascularized connective tissues or hide intracellularly (Georgilis et al., 1992; Girschick et al., 1996; Straubinger et al., 2000).

1.3.5 Current distribution of *Ixodes scapularis* and *Borrelia burgdorferi* in Ontario

Since *I. scapularis* were first documented at Long Point National Wildlife Area in 1976, the vector has gradually become endemic in at least seven areas within Ontario: 1) Point Pelee National Park (Leamington); 2) Prince Edward Point National Wildlife Area (Prince Edward Country); 3) St. Lawrence Islands National Park (Brockville); 4) Rondeau Provincial Park (Chatham); 5) Turkey Point Provincial Park (Port Rowan); 6) Wainfleet Bog Conservation Area (Colborne); and 7) Rainy River (Sider et al., 2012). Geographical risk areas for the potential exposure to *I. scapularis* tick bites have been predicted through the use of active and passive surveillance data combined with mathematical models (Ogden et al., 2006; Koffi et al., 2012; Leighton et al., 2012). In addition, Public Health Ontario published an on-line map of identified Lyme disease risk areas in Ontario including: 1) Kingston and surrounding area; 2) Rouge Valley region (eastern Toronto); 3) St. Lawrence valley to the Quebec border and northeast towards Ottawa; 4) Pinery Park along the shore of Lake Huron; and 5) Lake of the Woods region (south of Kenora) (Ogden et al., 2014a; Public Health Ontario, 2015). Within these risk areas, *I. scapularis* will likely only be found in appropriate tick habitat (i.e., woody and brushy areas). The identification of risk areas helps inform clinicians and veterinarians in regards to appropriate testing, diagnosis, and treatment of LD (Ogden et al., 2014a). The public health units are vital components for the ongoing surveillance of *I. scapularis* and *B. burgdorferi* expansion in Ontario. In the summer of 2015, the Peterborough County City Health Unit established a link (no longer active) on their website to request information from local veterinarians regarding the confirmation of *I. scapularis* ticks on their patients.

1.3.6 Other tick-borne pathogens of *Ixodes scapularis* and co-infections

Ixodes scapularis are also competent vectors for human babesiosis (*Babesia microti*), canine and human anaplasmosis (*Anaplasma phagocytophilum*), relapsing fever (*Borrelia miyamotoi*) and Powassan virus (Flaviviridae) (Thompson et al., 2001; Ebel, 2010; Dibernardo et al., 2014). In correspondence with the geographic expansion of their primary vector, *I. scapularis*, the incidence of confirmed human cases and asymptomatic exposures to *Babesia microti* and *Anaplasma phagocytophilum* has shown a steady increase in North America (Centers for Disease Control and Prevention, 2012; Jin et al., 2012; Dahlgren et al., 2015). Likewise, Little et al. (2014) documented an expansion of positive *Anaplasma* spp. serology in dogs from Northeastern USA in 2010-2012 compared to that previously reported from 2001-2006 by Bowman et al. (2009). However, there is currently a very low risk of exposure to these pathogens in Ontario (Nelder et al., 2014; Werden et al., 2015).

Ixodes scapularis ticks can become co-infected with multiple pathogens by taking a meal from a co-infected host, or from exposure to infected hosts at different life stages (i.e., as a larva and nymph) (Levin and Fish, 2000; Swanson et al., 2006). In a New York state study examining questing *I. scapularis* nymphs, co-infection with *Babesia microti* and *Borrelia burgdorferi* was 83% greater than that predicted by chance, and as such physicians and veterinarians should be aware of the increased risk of co-infection in that region (Hersh et al., 2014). The existence of co-infections can make the diagnosis and treatment of tick-borne infections challenging and complex (Swanson et al., 2006; Sperling and Sperling, 2009). In humans, co-infections can result in a longer duration of illness, greater severity of symptoms and surprisingly fewer cases of erythema migrans

(i.e., migrating rash or bullseye lesion at the site of a tick bite) (Edlow, 1999). At present, blacklegged ticks in Canada are tested for evidence of a suite of pathogens; however, co-infections are very rare and most involve *B. burgdorferi* with either *B. miyamotoi* or *A. phagocytophilum* (Dibernardo et al., 2014). Clinical manifestations of co-infections in domestic animals are not well characterized or understood (Day, 2011).

1.3.7 Prevention

Two types of Lyme vaccinations (i.e., monovalent and multivalent) are licensed for use in dogs in Canada. The monovalent vaccine is a purified OspA of *B. burgdorferi* derived from a recombinant vector and is designed to act within the tick vector to prevent mobilization of *B. burgdorferi* from the midgut to the salivary gland thereby preventing infection to the host (Sadziene and Barbour, 1996; Conlon Rice et al., 2000). Over a 5 year period, this vaccination has been shown to reduce the incidence of seroconversion to *B. burgdorferi* in dogs living in an endemic area in Maine, USA (Eschner and Mugnai, 2015). The second type is a multivalent vaccine which uses a combination of subunits (e.g., OspA, OspB, and/or OspC) which act both inside the tick to prevent migration of *B. burgdorferi* and within the canine host by inducing bactericidal antibody (Earnhart and Marconi, 2007; LaFleur et al., 2009). Vaccine-induced antibodies do not cross-react with the C6 serology test, which identifies animals naturally exposed to *B. burgdorferi* (O'Connor et al., 2004). However, vaccination only protects against Lyme disease, and as previously discussed, depending on travel history dogs, can be exposed to several different species of ticks and tick pathogens.

Since the 1940s, more that 60% of the emerging infectious diseases identified are zoonotic (Jones et al., 2008). Strategies for the prevention and control of tick-borne diseases have historically relied on the use acaricides, on hosts or in the environment, for vector control (George et al., 2004; Graf et al., 2004). With the recognition of multiple acaricide resistance occurring in some tick species, namely *Rhipicephalus microplus* (Miller et al., 2013), veterinarians and animal producers need to be strategic and judicious with their continued use of these products. The cattle tick, *Rhipicephalus (Boophilus) microplus* (as well as *Rhipicephalus annulatus*) are the vectors for cattle fever (*Babesia bovis* and *Babesia bigemina*), and were successfully eradicated from most of the United States between 1906 and 1943 (Cooley, 1946). The Cattle-Tick Fever Eradication Program was successful because all of the life stages occur on the host, therefore by quarantine and treatment these ticks could be controlled (Cooley, 1946). However, because all life-stages (i.e., the entire population of ticks) occur on cattle, as producers aimed to mitigate their economic losses from cattle fever, there was a strong selection towards strains of *Rhipicephalus microplus* that could survive treatment and thereby foster acaricide resistance (George et al., 2004).

The tick-species of domestic cats and dogs have complex life-cycles often involving multiple hosts, and as such there are ticks in refugia (i.e., portions of the tick population exist off of the host). Therefore, unlike *Rhipicephalus microplus*, the rise of acaricide resistance in these ticks is less likely to occur (Graf et al., 2004; Coles and Dryden, 2014). In companion animal medicine, lack of acaricide efficiency (or treatment failure) often stems from non-compliance with application (i.e., at incorrect intervals) (Coles and Dryden, 2014). Resistance has been reported to the Arthropod Pesticide

Resistance Database (ARPD) for *Amblyomma americanum* (1 report in 1971), *Dermacentor variabilis* (2 reports in 1960) and *Rhipicephalus sanguineus* (23 reports from 1971 to 2015) (APRD, 2016; Coles and Dryden, 2014). *Rhipicephalus sanguineus* is a three-host tick in which all life stages will feed on dogs, and they are also well adapted to living and infesting indoor environments (e.g., dog kennels) (Dantas-Torres, 2008). As a result, the repeated sub-lethal applications of acaricides to an *R. sanguineus* infested environment likely resulted in populations of *R. sanguineus* with resistance to permethrin and tolerance to fipronil (Miller et al., 2001; Eiden et al., 2015).

In Canada, the veterinary approved acaricide products available for dogs consist of pesticides and systemic pharmaceutical products. The pesticides include permethrin based products found in spot-on treatments (e.g., K9 Advantix®, Bayer) and amitraz found in preventative collars (e.g., Preventic® Tick Collar for Dogs, Virbac). Both of these products contain neuromodulators that cause hyperexcitability of the tick nervous system and prevent successful tick bites; however, these products are very toxic to cats. Systemic products available in Canada for the treatment and control (or aid in the treatment and control) of ticks on dogs include the oral tablets afoxolaner (i.e., NexGard®, Merial), fluralaner (i.e., Bravecto®, Merck), and sarolaner (Simparica®, Zoetis). These systemic pharmaceuticals of the isoxazoline family inhibit ligand-gated chloride channels (e.g., neurotransmitter gamma-aminobutyric acid) resulting in the uncontrolled activity of the tick central nervous system, and will kill most ticks within 48 hours of attachment and feeding (Compendium of Veterinary Products, 2016).

In Canada, selamectin (Revolution®, Zoetis) has a label claim for the treatment and control of *Rhipicephalus sanguineus* and an aid in the control for *Dermacentor*

variabilis. However, in the USA this product does not have a label claim for *R. sanguineus*, and is not considered effective for the treatment of tick infestations, nor for the control of other tick species. Of cautionary note, some of the above acaricide products have different durations of effectiveness for different tick species, and the product label claims may vary by country. For example, in the United States, the product insert for Bravecto® (Merck) declares that the product has a 3 month effectiveness for *Ixodes scapularis*, but only 8 weeks for *Amblyomma americanum*. Likewise, in the United Kingdom, K9 Advantix® (Bayer) has a 4 week effectiveness for *Ixodes ricinus*, but only 3 weeks for *Dermacentor reticulatus* (DEFRA, 2016). These differences in product effectiveness are important to consider for tick prevention in dogs that travel.

Tick prevention for cats consists of over-the-counter pyrethroid insecticide based tick collars (e.g., Vet-Kem® Ovitrol® BreakAway® Flea and Tick collar, Wellmark) and veterinary approved topical spot-on treatments. In the United States, the veterinary approved spot-on treatment Frontline® for cats (Merial) and the Seresto® Tick Collar for cats (Bayer) are available. In Canada, currently Bravecto® (Merck) topical solution is the only veterinary licensed product approved for the treatment and control of ticks on feline patients.

The use of acaricides to treat the environment and wildlife tick-hosts have also been explored (Daniels et al., 2009; Fish and Childs, 2009). Gaff et al. (2015) experimented with a "TickBot", a robotic device fitted with a CO_2 sensor to attract ticks and permethrin treated cloth to reduce tick densities. The device proved effective at reducing tick numbers for several hours, and may have a role for the temporary reduction

of tick numbers where people want to spend time outdoors, or in certain seasons (Gaff et al., 2015).

Environmental stewardship and toxicity concerns propel researchers to explore non-chemical options of vector control. Vaccination against tick vectors has been proposed as an environmentally friendly method of interrupting tick feeding and reproduction, and has been shown to reduce the duration of feeding, infestation rates, and oviposition in some research trials (Reviewed by de La Fuente et al., 2015). Other "green" tick control strategies considered for vector and disease-risk reduction include the use of tick pheromones (Sonenshine, 2006), parasitic wasps (Knipling and Steelman, 2000; Mather et al., 1987), entomopathogenic fungi (Benjamin et al., 2002; Kurtti and Keyhani, 2008; Fernandes et al., 2011), landscape design (Jackson et al., 2006; Connally et al., 2009), and vegetation management (Piesman, 2006; Allan et al., 2010). Most of these interventions provide only modest reductions in local tick populations and most lack the residual activity necessary for more long-term tick prevention and substantive tick population reduction.

The prevention of tick bites in people and dogs requires an integrated pest management approach combined with avoidance of tick habitat during periods of tick activity, and responsibility for personal protection (e.g., thorough tick checks) (Ogden et al., 2015).

1.4 STUDY RATIONALE AND OBJECTIVES

The ecology and distribution of ticks and their associated diseases are complex and important for the health, welfare, and protection of all animals (Jaenson et al., 2012; Nelder et al., 2014; Ogden et al., 2006; Scharlemann et al., 2008; Werden et al., 2015). With the expanding range of *I. scapularis* and *B. burgdorferi*, Canadians have benefited from a robust tick monitoring program that was initiated in the 1990's (Barker et al., 1992; Ogden et al., 2006). Public health agencies at both the provincial and federal levels are working together to identify tick species and their associated pathogens including *B. burgdorferi*, *A. phagocytophilum*, *Babesia microti*, *Ehrlichia chaffeensis*, and Powassan virus (Ogden et al., 2014a). Although extrapolation of these data to pets is possible, unquestionably the mandate of the Public Health Agency of Canada is for human health, and in some jurisdictions like Ontario, they no longer accept tick submissions from pets (Nelder et al., 2014). Limited information is available regarding the current diversity of tick species that bite dogs in Ontario. Dogs readily acquire ticks and are susceptible to Lyme disease (Littman et al., 2006; Bouchard et al., 2015). Veterinarians will benefit from specific knowledge regarding the tick species and distribution of pathogens in combination with thorough patient demographics and travel history. An important consideration for our analysis will be that our data are limited to assessing the ticks that bite domestic pets in southeastern Ontario.

Specific research objectives include:

1) Identifying the species of ticks that bite dogs in an emerging risk area for *Ixodes scapularis* by enrolling veterinary clinics to collect ticks in an emerging area of Lyme disease risk in Ontario (Chapter 2).
2) Assessing the prevalence of *Borrelia burgdorferi* and other pathogens (i.e., *Anaplasma phagocytophilum*, *Babesia microti*, and *Borrelia miyamotoi*) of *Ixodes scapularis* ticks collected from pets in the study region (Chapter 2).
3) Identifying risk factors for *I. scapularis* carriage relative to all other tick species on Ontario dogs using a client survey and a case-case study design (Chapter 2).
4) Identifying risk factors for *B. burgdorferi*-positive *I. scapularis* carriage relative to *B. burgdorferi*-negative *I. scapularis* on Ontario dogs using a client survey and a case-case study design (Chapter 2).
5) Determining the rate of tick infestation in the study region by collecting data on the number of dog visits per participating veterinary clinic in each week of the study period (Chapter 3).
6) Identifying statistically significant spatial, temporal and space-time clusters of tick infestations and clusters of *B. burgdorferi*-positive *I. scapularis* carriage on companion dogs using spatial scan statistics, and to determine the impact of travel history and geolocation data quality on the results of spatio-temporal analyses (Chapter 3).

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CHAPTER 2: A CASE-CASE STUDY EXAMINING RISK FACTORS FOR *IXODES SCAPULARIS* CARRIAGE RELATIVE TO OTHER TICK SPECIES; INCLUDING RISK FACTORS FOR *BORRELIA BURGDORFERI*-POSITIVE TICK CARRIAGE IN A POPULATION OF PET DOGS FROM SOUTHEASTERN ONTARIO.

2.1 ABSTRACT

The blacklegged tick, *Ixodes scapularis*, is the vector for *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum*, the causal agents of Lyme disease and anaplasmosis, in humans and dogs in eastern North America. The extensive range expansion of this tick is a growing health concern. 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, the objectives of this study were to: i) identify the tick species carried by dogs; ii) determine the prevalence of *B. burgdorferi* and *A. phagocytophilum* in *I. scapularis* collected; iii) use logistic regression models to examine the associations between pet demographics, travel history, and location and the odds of the following: a dog carrying *I. scapularis* relative to other tick species, and a removed *I. scapularis* being infected with *B. burgdorferi*. Seven species of ticks from 543 companion dogs were collected and analyzed from 20 participating veterinary hospitals in southeastern Ontario. *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* parasitism 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 who traveled in the 14 days prior to tick removal, and for those dogs under 1 year of age and weighing less than 10 kg. This study provides evidence-based information for veterinarians and public health practitioners to protect dogs and their owners from Lyme disease in southeastern Ontario.

2.2 INTRODUCTION

In many regions of the world, humans and dogs can become seriously ill with Lyme borreliosis, and the clinical manifestations of Lyme disease have been well documented (Borchers et al., 2015; Bouchard et al., 2015). The etiologic agent associated with Lyme disease is a bacterial spirochete from the *Borrelia burgdorferi* sensu lato complex. Until recently, *B. burgdorferi* sensu stricto (hereafter termed *B. burgdorferi*) was the only confirmed spirochete associated with Lyme disease in North America. However, with recent molecular and epidemiological discoveries, other *Borrelia* species (e.g., candidatus *B. mayonii*) are being considered as agents in human clinical disease (Dolan et al., 2016; Pritt et al., 2016). Within Ontario the only known competent vector for *B. burgdorferi* transmission is *Ixodes scapularis* (Piesman and Happ, 1997), commonly known as the blacklegged or deer tick. *Ixodes scapularis* can also transmit other human pathogens (e.g., *B. miyamotoi*, Powassan virus and *Babesia microti*) (Ebel and Kramer, 2004; Centers for Disease Control and Prevention, 2012; Krause et al., 2014), and *Anaplasma phagocytophilum*, a bacterium that can cause disease

in people, dogs and horses (Woldehiwet, 2010; Greene, 2012; Krakowetz et al., 2014; Dahlgren et al., 2015).

From the first discovery 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). Over the past 25 years, the distribution of *I. scapularis* in Ontario has undergone unprecedented northward expansion, increasing the risk of *B. burgdorferi* exposure and inciting widespread public and animal health concern (Ogden et al., 2008a; Nelder et al., 2014; Ogden et al., 2014; Clow et al., 2016). Within the last 10 years, and in response to the establishment of populations of *Ixodes scapularis* infected with *B. burgdorferi* within parts of Ontario including eastern Ontario, Public Health Ontario and the Public Health Agency of Canada intensified their passive tick surveillance programs. Passive tick surveillance includes the submission and identification of ticks from public health units, physicians, veterinarians, and private citizens (Ogden et al., 2006). Knowledge of tick species and bacterial prevalence can help direct public health interventions and guide diagnostics for medical and veterinary health professionals, aiding in the successful treatment of tick-borne diseases.

Among Canadian households, 33% own a pet dog (Perrin, 2009). These dogs often live in the home, travel with their owners, and partake in outdoor recreational activities with their families. Dogs readily acquire ticks from the environment (Bouchard et al., 2015), and by sharing the same environment as their owners, pet dogs have been shown to acts as sentinels for human tick-borne disease risk (Mead et al., 2011; Smith et al., 2011). Furthermore, since dogs are susceptible to Lyme disease and anaplasmosis

(Greene, 2012), regional knowledge of the tick species that parasitize dogs are similarly important for the diagnosis, treatment, and prevention of canine tick-borne disease.

Globally, dogs are used as a means of passive tick collection, and risk factors for tick carriage have been identified. In a case-case study from the United Kingdom, the location of the pet owner's home in a rural area was significantly associated with *Ixodes ricinus* carriage, while residence in a non-rural area was associated with *Ixodes hexagonus* (Ogden et al., 2000). These researchers also determined that exposure to a boarding kennel was significantly associated with *Ixodes canisuga* carriage (Ogden et al., 2000). Alternatively, a Hungarian study showed no associations between tick species, living conditions or outdoor activities, but the statistical methods were not described (Földvári and Farkas, 2005). In Arkansas and Oklahoma, USA, dogs have been used for passive tick collection studies, but identifying risk factors for tick carriage was not a study objective (Koch, 1982; Trout and Steelman, 2010). Overall, the methods used for data collection and analysis vary widely between studies and no consistent risk factors for tick carriage have emerged. Notwithstanding, risk factors for tick species carriage likely require regionally specific information as tick species will vary with geographic location, tick habitat preferences, changes in host abundance, seasonal and environmental conditions, and owner and pet lifestyle habits (Ogden et al., 2000; Ostfeld et al., 2006; Ogden et al., 2008b; Simon et al., 2014; Werden et al., 2014; Trout Fryxell et al., 2015).

Although patterns of tick infestation of humans in Ontario have been examined in detail, the Public Health Ontario Laboratory stopped encouraging submission of ticks from domestic animals in 2009 (Nelder et al, 2014). 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 in this population of dogs; 3) determine the prevalence of *B. burgdorferi, A. phagocytophilum, and B. microti*, 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.

2.3 METHODS

Tick Collection

Mixed and companion animal veterinary practices located in an approximately 11, 000 km^2 area of Ontario (Figure 2.1), 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 distinct regions: 1) the "Lakeshore" region for veterinary clinics within approximately 5 km of Lake Ontario; 2) the "Middle" region for veterinary clinics approximately greater than 5km 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 (Figure 2.1). Twenty of the 24 veterinary practices contacted by convenience agreed to participate: 5 of 5 clinics participated that had been previous locations of locum veterinary employment of C. James; 8 of 9 clinics participated that expressed interest in the study after a recruitment talk at a Kawartha Veterinary Association meeting in Peterborough, Ontario; and 7 of 11 veterinary clinics that were invited by phone agreed

to voluntarily collect ticks from pet 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. Submissions were also accepted if ticks were removed by the dogs' owners. All 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^{o}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).

Questionnaire

A questionnaire was completed by participating owners to identify risk factors for tick attachment in companion dogs. 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 (Appendix A) and questionnaires (Appendix B) were distributed to participating veterinary clinics and completed by clients submitting a tick(s) from their dog. 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 old. Tick submissions were accepted even if the pet owner declined to complete the questionnaire.

The questionnaire focused on five main areas of interest: 1) client demographics (i.e., gender, age category, postal code, location of primary residence, number of dogs, children and cats in the home); 2) animal demographics (i.e., breed, age, sex, neuter status, body mass, coat length and colour); 3) animal management and lifestyle (e.g., distance and frequency of walks, time spent on leash, locations visited and travel history in the previous 14 days); 4) client knowledge of ticks and tick exposure (e.g., could the client recognize a tick, did the dog have a previous history of tick parasitism, were ticks ever found in the home); and 5) tick prevention (e.g., tick avoidance, frequency of tick checks, use of prevention product, type/brand of tick prevention product, and time of last application) (Table 2.1).

Information was gathered regarding the dog owner's familiarity and experience with ticks to assess potential bias towards subjects with previous involvement with tick-borne diseases (e.g., Lyme disease). Signed consent was requested on the questionnaire for future access to an animal's medical records. 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).

Laboratory Analysis

Adult ticks were identified to species using a standard dichotomous key (Keirans and Litwak, 1989) and given an engorgement score (unfed, slightly, partially or fully engorged). Ticks were then shipped to the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Manitoba) for confirmation of species identification for most species of adult ticks (Keirans and Clifford, 1978), species determinations for larvae or nymphs (Clifford et al., 1961; Durden and Keirans, 1996), level of engorgement, and pathogen testing. Adult and nymphal stages of *I. scapularis* were tested for the presence of *Borrelia burgdorferi* sensu stricto, *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, and *Babesia 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; Ogden et al., 2008a; 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.

Variables and Statistical Analysis

Causal diagrams were constructed to examine possible confounding and intervening relationships among explanatory variables relative to the outcomes of interest (Figures 2.2 and 2.3). Using logistic regression models, we examined canine risk factors for two outcomes: 1) the acquisition of *I. scapularis* versus other tick species (Figure 2.2); and 2) the exposure to *B. burgdorferi*-positive versus -negative *I. scapularis* (Figure 2.3). The potential risk factors examined included animal and client demographic factors, pet travel history, and management factors (Table 2.1). Survey data were entered into a Microsoft Access Database (Microsoft Corp. Redmond, WA) and were merged with a Microsoft Excel spreadsheet (Microsoft Corp. Redmond, WA) containing tick testing results. Stata/MP 13.1 (StataCorp, College Station, TX) was used for all statistical analyses.

Temporal and spatial explanatory 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^{o}C for more than 72 hours 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 route and duration, this was analyzed as a dichotomous variable (i.e., travel or no travel in the 14 days prior to tick removal) (Table 2.1).

Animal explanatory variables

Age of the dog was categorized into puppy (<1 yr), adult (1 to < 7 yr), and senior (≥ 7 yr). Dog weight was categorized into small dogs (≤ 10kg), medium dogs (10.1 – 30kg) and large dogs (> 30kg). 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. Dog haircoat length was categorized as short, medium and long. Owners were asked to check all of the coat colours that were appropriate, and were given the choice of black, brown, yellow, white, grey, and an "other" category that allowed for a description of a dog's coat colour. However, due to great diversity of reported coat colours, this variable was re-categorized to light (white, golden, yellow, tan, grey, sandy, apricot, tawny), dark (brown, black, red, black/tan), and mixed (light and dark colour, roan, merle, brindle, sable, dapple) coat colours (Table 2.1).

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.

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 (Figure 2.2) 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 $\alpha = 0.05$. Subsequently, variables with a *p*-value > 0.05 and ≤ 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 ($\alpha = 0.05$) 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.

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.

Model 2: Risk factors for carriage of a *Borrelia 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 are defined as localities where blacklegged ticks infected with *B. burgdorferi* have become established (Public Health Ontario, 2016), and 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*.

2.4 RESULTS

Tick collection

In total, 973 ticks were collected from 588 dogs which participated in the 20 veterinary clinics between April and December 2015 (Table 2.2). Tick submissions per clinic ranged from 9 to 60 with a median of 26 submissions per clinic. Seven species of ticks were identified; 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 2.2). The majority of the ticks removed from dogs were adults (96.1%) while nymphal ticks accounted for only 2.4% of the ticks collected. A single *I. cookei* larva instar 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. Another eight dogs were excluded because they were additional dogs from the same owner submitting a tick sample on the same date.

There was seasonal variation in the tick species submitted and in the numbers of tick submissions: spring = 131 (24.1%), summer = 108 (19.9%) and fall = 304 (56.0%) (Figure 2.4). *Ixodes scapularis* parasitized 85.1% of dog participants; however, only 11.3% of these dogs were infested during the summer months, compared to the 24.0% in the spring and 64.7% in the fall. Of the remaining 81 dogs carrying another species of

tick, 93.8% were infested in the spring or summer months (Figure 2.4). In the fall season, other tick species only accounted for 1.6% of parasitized dogs.

Client participation and questionnaires

The responses from questionnaires are summarized in Table 2.1. The generous commitment by veterinary clinic staff to introduce the questionnaire and the enthusiasm from the pet owners to fill out the two-page survey, resulted in a 92.6% questionnaire response rate for tick submissions. However, some questionnaires were not fully completed (Table 2.1). One veterinary clinic made the a priori decision to not give their clients the opportunity to complete the questionnaire, and that accounted for 2% of canine tick submissions. Client participation varied between clinics (range: 40 to 100%; median 97.0%). Of the 493 clients who responded, 68.2% of the owners reported previously detecting ticks on their dog (Table 2.1).

Pathogen testing

All 685 of the *I. scapularis* removed from domestic dogs were adults. *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 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* or *A. phagocytophilum*. *Ixodes scapularis* positive for *B. burgdorferi* or *A. phagocytophilum* were removed from 35 and 2 dogs, respectively. 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*.

Logistic regression model

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

Results from univariable analysis are summarized in Table 2.3. Variables considered for inclusion in the multivariable model based on a *p*-value $\leq$ 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 sidewalk in the 7 days prior to tick removal (Table 2.3).

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 in this population of pet dogs (Table 2.4). With spring as the referent category, the odds of removing an *I. scapularis* relative to other tick species were significantly increased in the fall and significantly decreased in the summer (Table 2.4). Likewise, the odds of removing an *I. scapularis* relative to other tick species were significantly greater in the fall than in 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 2.4). However, no significant difference was found between the Middle region and the Highland region, or the Middle region compared to the Lakeshore region (OR=0.52; 95% CI=0.21 - 1.31; *p*-value=0.17).

Larger dogs were at significantly greater odds of *I. scapularis* carriage compared to medium and smaller dogs (Table 2.4). However, there was no significant difference

detected in the odds of removing *I. scapularis* relative to other tick species 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 previous 7 days prior to tick submission significantly reduced the odds of *I. scapularis* carriage compared to other tick species (Table 2.4). No significant interactions were found between variables in the multivariable mixed-effects model.

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 regression model. A Hosmer-Lemeshow goodness of fit test was not significant ($p = 0.852$), suggesting the model fit 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.

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 four variables as significant ($p < 0.05$) predictors for carriage of an *I. scapularis* positive for *B. burgdorferi* (Table 2.5). 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 2.5). Puppies (< 1 year of age) were at significantly greater odds of carrying a tick testing positive for *B. burgdorferi* compared to adult and senior dogs (Table 2.5). No significant difference in the odds were observed between adult and senior dogs (OR = 1.12; 95% CI = 0.46 - 2.84; *p*-value = 0.839). Finally, 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 2.5).

Table 2.6 summarizes the results of univariable exact logistic regression using the 331 dogs parasitized with *I. scapulari*s with no history of travel within 14 days of tick removal. Only 16 of these dogs were parasitized with at least one *I. scapularis* positive for *B. burgdorferi*, and region did not emerge as a significant predictor of positive *I. scapularis* carriage. The odds of *B. burgdorferi* exposure were significantly greater in the summer and the fall relative to the spring (Table 2.6). No significant difference was found between the odds of *B. burgdorferi*-positive tick carriage in the 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 2.6). However, 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).

2.5 DISCUSSION

Ticks and owner demographics

Although passive surveillance for ticks in Ontario has been on-going for many years, much of the surveillance effort has targeted human hosts and as a result current knowledge of the tick species carried by domestic dogs is unavailable. We collected a total of 973 ticks from dogs that constituted seven different tick species. However, in keeping with recent human passive surveillance studies in Canada, three species

accounted for 98.2% of submissions: *I. scapularis*, *D. variabilis*, and *I. cookei* (Gasmi et al., 2016; Nelder et al., 2014). Other passive surveillance studies in Canada have reported greater tick species richness and yielded substantially more ticks (Gasmi et al., 2016; Nelder et al., 2014; Scholten, 1977), but their analyses included more host species, a greater geographical area, and a longer time period for submission. Around the world *Rhipicephalus sanguineus* is considered the most ubiquitous vector-borne tick parasitizing 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* 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 with approximately 100 ticks, and two other dogs were carrying a single *R. sanguineus*. Adult Ixodidea ticks have a prolonged feeding period, lasting a couple of weeks, and can be transported considerable distances while attached to their host (Anderson, 2002; Balashov, 1972; Hoogstraal and Kaiser, 1961), and this heavily parasitized puppy highlights 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 represent adventitious ticks, or ticks acquired during travel. 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; Burroughs et al., 2016), and/or the immature stages generally having a feeding preference for smaller mammals and birds (Piesman and Spielman, 1979; Morshed et al., 2005). All *I. scapularis* collected from pet dogs in this study were adult ticks and highlights the

potential that adult blacklegged ticks may play in the transmission of *B. burgdorferi* to companion animals.

The majority of dog owners that participated in this study were older than 55 years of age and living in a rural environment. This may be due to the demographics of the study area, but could also reflect a response bias for this age category of people having both the time to submit ticks and complete a questionnaire. The age category for pet owners and information on children in the home (Table 2.1) was requested because previous research has identified age as a significant variable for *I. scapularis* carriage in people (Nelder et al., 2014; Rand et al., 2007). These owner demographic variables were not significant on univariable analysis, nor did not they act as confounding variables in the final model for *I. scapularis* carriage.

Information was gathered on the reason for the veterinary visit, and of the 72.2% of people who responded, 65% included "tick" (or drop off tick) as their reason for visit to their veterinarian (Table 2.1). Therefore, there may have been a bias towards people interested in ticks; 95% of respondents claimed to know what a tick looked like, 68% of dogs in the study had previously been infested with ticks, few individuals reported finding ticks in their home (18%), and a third (32%) reported that they had removed ticks from themselves or a family member while in Ontario. No effort was made to verify (i.e., with photo identification) the client statement regarding knowledge of tick identification versus other arthropods common to companion animals like fleas or lice.

Risk factors for *I. scapularis* carriage

Using multivariable mixed-effects logistic regression, the following variables were found to have a significant effect on *I. scapularis* tick carriage relative to other tick species: the season and region of tick collection, visiting a farm in the previous 7 days, and the sex and weight-category of the dog.

The seasonal influence on tick carriage is consistent with passive surveillance studies (Rand et al., 2007; Nelder et al., 2014; Gasmi et al., 2016) and in keeping with *I. scapularis* ecology (Spielman et al., 1985). *Ixodes scapularis* are vulnerable to desiccation in the hot dry summer (Bertrand and Wilson, 1996; Lindsay et al., 1998). Nelder et al. (2014) also showed season to have a profound effect on the tick species collected from passive surveillance. We found that the odds of *I. scapularis* carriage in the fall was significantly greater than the odds in the spring and summer seasons.

The first known population of *I. scapularis* was discovered in Ontario on the Long Point peninsula in the early 1970's (Watson and Anderson, 1976), and an exceptional effort was undertaken between 1987 and 1991 to determine if *I. scapularis* ticks were establishing elsewhere in the province (Barker et al., 1992). Since that time, *I. scapularis* has gradually and steadily established in other areas along the north shore of Lake Erie and Lake Ontario. Furthermore, reproducing populations of *I. scapularis* are also expanding in other parts of southern Ontario (Morshed et al., 2006; Ogden et al., 2006; Scott et al., 2012; Werden et al., 2014; Clow et al., 2016). In our study, region had a significant influence on the risk of carriage of *I. scapularis* in pet dogs. 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* 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). Spending time on a farm was identified as having a sparing effect 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), and in the United States *D. variabilis* have been found to cluster in agricultural areas (Trout Fryxell et al., 2015). In univariable analysis, travel within the previous 14 days had a significant sparing effect on the risk of carriage of *I. scapularis*. This may be explained if the location of travel was to more northern areas, or to habitats less favourable for *I. scapularis*, but it was not possible to confirm this information from our study. However, a history of travel did not have a significant influence on *I. scapularis* carriage when the effects of other independent variables (e.g., season and region) were controlled in the multivariable analysis.

Interestingly, the sex and the size of the dog had a significant influence on the odds of *I. scapularis* carriage relative to other tick species. In Ontario, 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 to 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 "taller" dogs may be more likely to come in contact with blacklegged ticks than lighter "shorter" dogs.

Information regarding tick prevention and control was collected (Table 2.1), but widespread client misunderstanding was evident regarding which prescription 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 option on the questionnaire, and we anticipate that additional clients started prevention on the day of tick submission (Table 2.1). For these reasons, variables relating 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.

Borrelia burgdorferi testing and risk factors for exposure

Currently in Ontario, *I. scapularis* is the only known competent vector for *B. burgdorferi* sensu stricto. In total, 12.6 % (95% CI: 10.1 – 15.2) of *I. scapularis* collected from dogs were positive for *B. burgdorferi*, which is similar to previously reported passive surveillance prevalence data (14.1 - 15.0%) in Canada (Dibernardo et al., 2014; Nelder et al., 2014). The prevalence of *A. phagocytophilum* in *I. scapularis* from dogs in this study was 0.6%, which is similar to other Canadian passive surveillance studies (0.3% – 0.8%) (Dibernardo et al., 2014; Nelder et al., 2014). We did not detect

any co-infections nor ticks positive for *B. miyamotoi*, *B. microti*, or *E. chaffeensis*, which is 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 submitting ticks in the Lakeshore region of our study area, which includes areas of emerging Lyme disease risk 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 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* 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*. There is some experimental evidence to support a lower questing height in adult *I. scapularis* infected with *B. burgdorferi* (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 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).

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 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 appear 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.

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Table 2.1. List of owner, pet, and management related variables collected by questionnaire from 543 pet owners submitting ticks removed from their dogs from April to December 2015 in southeastern Ontario, Canada.

Season (n=543)
- Spring (n=131; 24%)
- Summer (n=108; 20%)
- Fall (n=304; 56%)

Region (n=543)
- Highlands (n=153; 28%)
- Middle (n=205; 38%)
- Lakeshore (n=185; 34%)

Owner demographic information

Questionnaires (n=503; 93%)

Gender M/F (n = 496; 69%/31%)

Age Category (n = 478)
- 18 – 25 (6%)
- 26 – 35 (15%)
- 36 – 45 (16%)
- 46 – 55 (19%)
- 56 – 65 (27%)
- 65 + (18%)

City (n = 529)

Postal code (n = 525)

Area type based on postal code (n = 525)
- Rural (67%)
- Urban (33%)

Primary residence (n = 490)
- Village (9%)
- Town (14%)
- Suburb (4%)
- City (13%)
- Rural area (51%)
- Farm (9%)
- Other (1%)

Number of dogs in the home (n = 486)
- 1 (63%)
- 2+ (37%)

Any cats in the home? Y/N (n = 466; 31%/69%)

Any children <12 years of age in the home? Y/N (n = 454; 20%/80%)

Dog demographic information

Breed (n = 539)
- Herding (12%)
- Hound (5%)
- Non-sporting (8%)
- Sporting (23%)
- Terrier (4%)
- Toy (5%)
- Working (9%)
- Crossbreed (35%)

Age (n = 537; mean = 5.4 yr; range: 0.17 – 15)
- Puppy (11%)
- Adult (52%)
- Senior (37%)

Sex M/F (n = 536; 53% / 47%)

Spayed or neutered Y/N (n = 535; 78%/22%)

Weight (kg) (n = 531; mean = 24.5; range: 0.9 – 104.6 kg)
- Small (24%)
- Medium (41%)
- Large (35%)

Hair length (n = 487)
- Short (45%)
- Medium (36%)
- Long (19%)

Colour (n = 486)
- Light (31%)
- Dark (31%)
- Mixed (39%)

Reason for veterinary appointment (n = 392)
- Tick (65%)
- Annual exam/vaccine (15%)
- Exam (12%)
- Employee (3%)

Other (5%)

Lifestyle

Time spent of leash (n = 489)
- Always (80% of time) (36%)
- Sometimes (20-80% of time) (21%)
- Rarely (< 20% of time) (44%)

Distance walked per week (n = 484)
- Less than 1 km (12%)
- From 1 – 5 km (40%)
- More than 5 km (48%)

In last 7 days where has your dog spent time?
(Please check all that apply) (n = 497)
- In the house (84%)
- In the yard (87%)
- Boarding kennel (1%)
- Park/Beach (26%)
- Country road (45%)
- Sidewalk (21%)
- Forest (54%)
- Field (45%)
- Farm (16%)

How many hours does your dog spend outside? (n = 476)
- Spring (Less than 1 hr/1-3 hrs/3+ hrs) (10%/50%/40%)
- Summer (Less than 1 hr/1-3 hrs/3+ hrs) (4%/34%/62%)
- Fall (Less than 1 hr/1-3 hrs/3+ hrs) (12%/48%/40%)

Do you camp with your dog (Y/N) (n = 498; 27%/73%)

In the past 14 days have you traveled with your dog? (Y/N)
(n = 490; 22%/78%)

Tick Exposure

Would you recognize a tick? (Y/N/ I don't know) (n = 484; 95%/3%/2%)

Has this dog ever previously had a tick? Y/N (n = 493; 68%/32%)

Number of ticks removed (n=543; median=1; range 1-100)

Do you know where the tick was acquired? (Y/N) (n = 437; 51%/49%)

Where on the body was the tick removed? (n= 489)
- Head / Neck (66%)
- Not attached (2%)
- Other (27%)

Have you ever found a tick in your home? (Y/N) (n = 485; 18%/82%)

Have you removed a tick from yourself or a family member while in Ontario? (Y/N) (n = 489; 32%/68%)

Tick prevention

Do you avoid taking your dogs to areas if ticks are present?
(Y/N/Occasionally) (n=481; 44%/42%/14%)

Do you regularly check your dog for ticks? (Y/N/Occasionally)
(n = 496; 63%/16%/21%)

If you answered "Yes" how often do you check your dog? (n = 288)
Please specify: _____

Do you use tick prevention products for your dog?
(Y/N/Occasionally) (n = 469; 53%/36%/11%)

Where you purchase your tick prevention? (n = 341)
- Veterinarian (92%)
- Other (8%)

What type of prevention do you use? (n = 481)*
- Topical medication Y/N (57%/43%)
- Oral tablet Y/N (12%/88%)
- Collar Y/N (1%/99%)
- Other (e.g., spray, shampoo, natural product etc. 8%)

Which veterinary prescribed tick prevention product do you use? (n=460)*†
- Revolution ® (Topical) (41%)
- K9 Advantix II ® (Topical) (15%)
- Bravecto ® (Chewable tablet) (7%)
- NexGard ® (Chewable tablet) (3%)
- Preventic ® Tick Collar (0%)
- Seresto ® Flea/Tick Collar (0%)

When was the last time this product last administered or applied? (n = 319)
- Within the last 30 days (48%)
- More than 1 month ago (20%)
- More than 6 months ago (19%)
- Day of survey (13%)

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* We excluded responses of non-tick prevention products (e.g., Sentinel®, Advantage®, Advantage Multi®, and Lyme vaccination)

†One response included a prescription product not available in Canada (Frontline®).

Table 2.2. The species, instar, 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*	*Dermacentor albipictus*	*Ixodes muris*	*I. scapularis & D. variabilis*	Total
Common name	Blacklegged tick	American dog tick	Groundhog tick	Lone star tick	Brown dog tick	Winter tick	Mouse tick		
Instar									
Female	653	40	10	1	2		1		707
Male	32	16		3		1			52
Nymph			17		100	2			119
Larva			1						1
Various*		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*		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

* Various instars 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.

Table 2.3. Results[a] of univariable mixed logistic regression analysis concerning variables associated with *Ixodes scapularis* parasitism relative to parasitism with all other tick species collected from 543 dogs from 20 veterinary clinics in southeastern Ontario between April and December 2015.

Variable description	% *Ixodes scapularis* (95% CI)[b]	Odds Ratio (95% CI)	*p*-value[d]	Variance component (95% CI)
Season			**< 0.001**[c]	0.04 (0.00 – 24.95)
Spring	85.5 (78.3 – 91.0)	Referent		
Summer	50.0 (40.2 – 59.8)	0.17 (0.09 – 0.33)	**< 0.001**	
Fall	98.4 (96.2 – 99.5)	10.06 (3.66 – 27.67)	**< 0.001**	
Region			**< 0.001**[c]	0.13 (0.01 – 1.33)
Highlands	73.2 (65.5 – 80.0)	Referent		
Middle	86.3 (80.9 – 90.7)	2.14 (1.07- 4.26)	**0.031**	
Lakeshore	95.1 (91.0 – 97.8)	6.93 (2.85 - 16.86)	**< 0.001**	
Visit farm in previous 7 days				0.69 (0.23 – 2.01)
No	86.3 (82.7 – 89.5)	Referent		
Yes	76.3 (65.4 – 85.1)	0.40 (0.21 - 0.77)	**0.006**	
Travel in previous 14 days				0.59 (0.19 – 1.78)
No	86.9 (83.1 – 90.1)	Referent		
Yes	76.2 (67.0 – 83.8)	0.47 (0.27 - 0.83)	**0.009**	
Number of dogs in the home				0.60 (0.20 – 1.78)
1	87.6 (83.3 – 91.1)	Referent		
2+	80.0 (73.4 – 85.6)	0.52 (0.31 - 0.88)	**0.015**	
Where was tick attached?				0.58 (0.19 – 1.77)
Other body location	77.6 (69.6 – 84.4)	Referent	**0.028**	
Head and/or neck	87.0 (82.9 – 90.3)	1.84 (1.07 – 3.16)		
Sex of dog (sex)				0.74 (0.26 – 2.18)
Female	88.1 (83.4 – 91.8)	Referent		
Male	83.1 (78.2 – 87.3)	0.69 (0.41 - 1.17)	0.169	
Fur length			0.122[c]	0.63 (0.21 – 1.91)
Long	87.1 (78.6 – 93.2)	Referent		
Short	81.2 (75.4 – 86.2)	0.60 (0.29 - 1.24)	0.168	
Medium	88.6 (83.0 – 92.1)	1.06 (0.48 - 2.37)	0.885	
Reproductive status				0.78 (0.27 – 2.26)
Intact	80.2 (71.8 – 87.0)	Referent		
Neutered or Spayed	86.9 (83.3 – 90.0)	1.62 (0.91 - 2.89)	0.103	
Sidewalk used in previous 7 days				0.51 (0.16 – 1.62)
No	82.8 (78.7 – 86.4)			

Yes	92.2 (85.1 – 96.6)	1.99 (0.89 - 4.45)	0.092	
Dog weight category[a]			0.240 [c]	0.80 (0.28 – 2.29)
Large >30 kg	88.1 (82.6 – 92.4)	Referent		
Medium 10 – 30 kg	83.6 (78.1 – 88.3)	0.60 (0.32 – 1.10)	0.095	
Small <10 kg	84.2 (77.5 – 90.7)	0.68 (0.33 – 1.37)	0.275	

[a] Only variables with a p-value $\leq$ 0.20 are presented unless they were subsequently included in the final multivariable logistic regression model

[b] 95% Confidence Interval

[c] Overall significance based on Wald's χ^2, for variables that included more than two categories

[d] Significant *p*-values in bold

Table 2.4. Results from the final mixed multivariable logistic regression model[ab] with a random effect for veterinary clinic[c] estimating the risk factors for *I. scapularis* carriage versus the carriage other tick species in a population of pet dogs from southeastern Ontario, Canada.

Variable description	Odds Ratio	95% CI[d]	*p*-value[e]
Season			
Spring	Referent		
Summer	0.19	0.10 - 0.39	**< 0.001**
Fall	10.23	3.60 - 29.10	**< 0.001**
Region			
Highlands	Referent		
Middle	1.73	0.88 – 3.43	0.115
Lakeshore	3.32	1.32 – 8.38	**0.011**
Visit farm in previous 7 days			
No	Referent		
Yes	0.40	0.18 – 0.87	**0.021**
Sex of dog			
Female	Referent		
Male	0.50	0.26 – 0.96	**0.038**
Dog weight category			
Large >30 kg	Referent		
Medium 10 – 30 kg	0.34	0.16 – 0.71	**0.004**
Small <10 kg	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 2.5. Descriptive statistics and results of univariable exact logistic regression analyses concerning variables significantly[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 tick (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)			**0.007** [c]
Puppy < 1 year	46 (10.0)	19.6 (9.3 – 33.9)	Referent	
Adult 1-7 years	244 (53.0)	6.6 (3.8 – 10.4)	0.29 (0.11 - 0.80)	**0.008**
Senior > 7 years	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 based on the score method for variables with more than 2 categories

[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

Table 2.6. 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 tick (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 $\leq$ 0.20 are presented

[b] 95% Confidence Interval

[c] Overall significance based on the score method for variables with more than 2 categories

[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

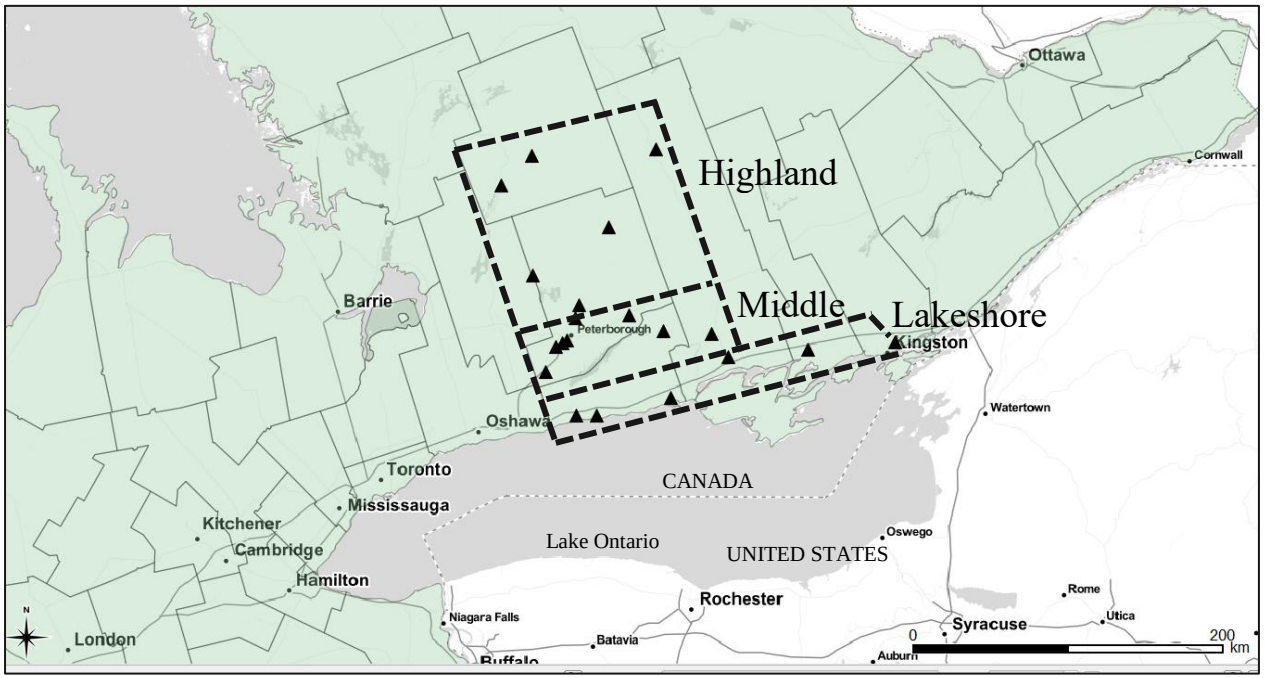

Figure 2.1. Map of 20 participating veterinary clinics (▲) in southeastern Ontario where ticks were collected from dogs. The dashed outline represents the three regions used to categorize the location of tick submission: Lakeshore, Middle and Highland regions.

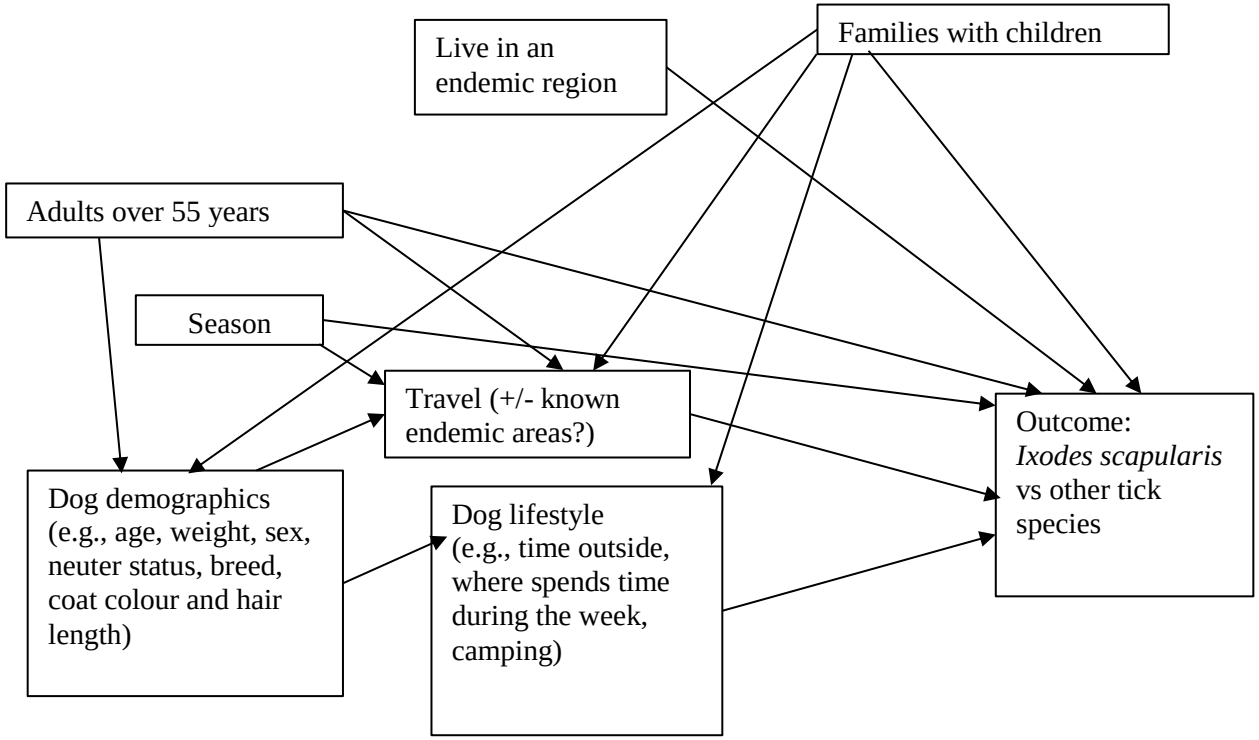

Figure 2.2. Causal diagram depicting the potential relationships between independent variables and the outcome of *Ixodes scapularis* versus other tick species carriage.

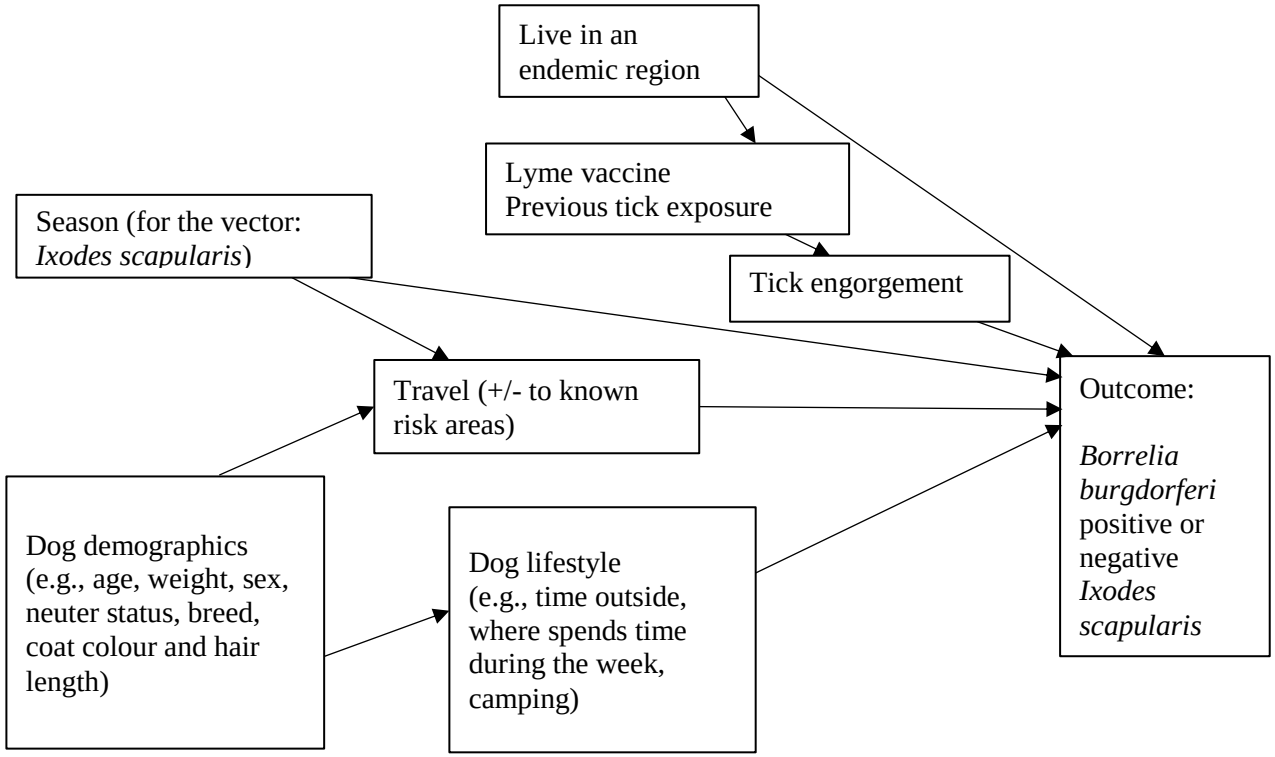

Figure 2.3. Causal diagram representing the potential relationships between independent variables and the outcome of exposure to a *Borrelia burgdorferi*-positive *Ixodes scapularis* versus a *B. burgdorferi*-negative *I. scapularis*.

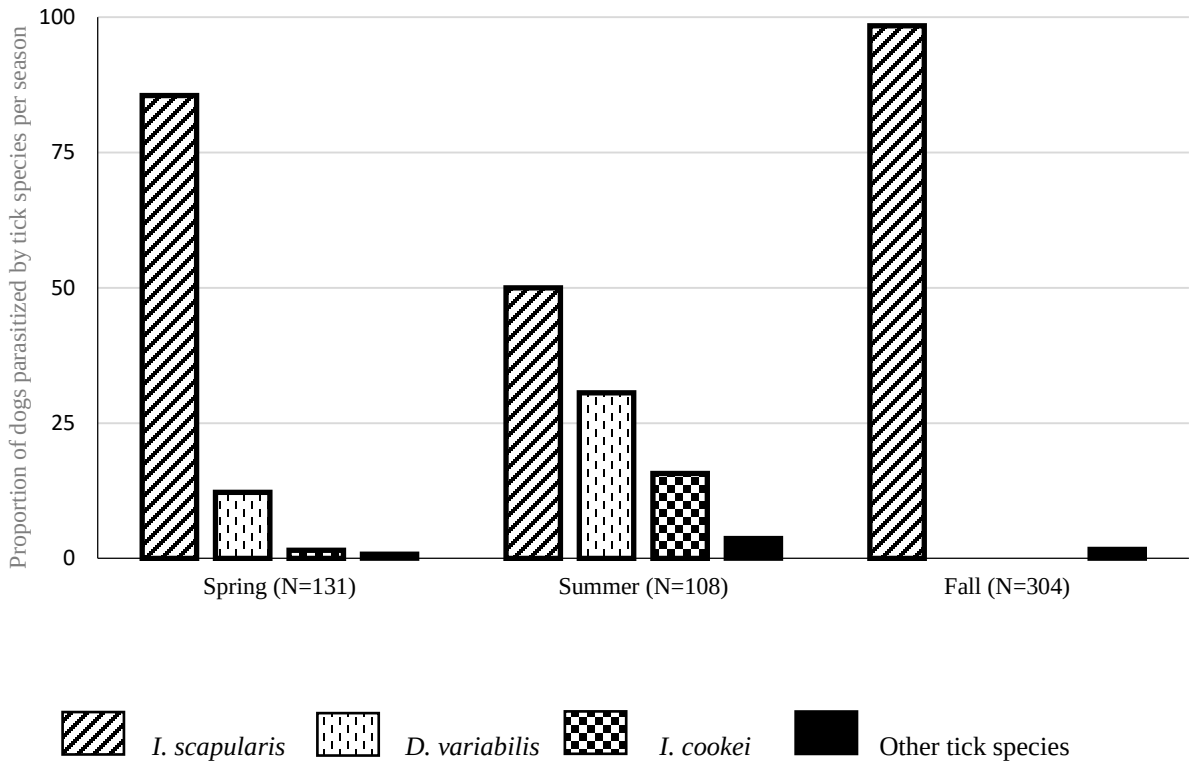

Figure 2.4. Seasonal variation in tick species, including instars, and sample submissions from April to December 2015 among 543 parasitized dogs from 20 veterinary clinics in southeastern Ontario. Other tick species included: *Amblyomma americanum, Rhipicephalus sanguineus, Dermacentor albipictus* and *Ixodes muris.*

CHAPTER 3: SPATIAL, TEMPORAL, AND SPACE-TIME CLUSTERING OF *IXODES SCAPULARIS* PARASITISM IN COMPANION DOGS FROM SOUTHEASTERN ONTARIO, CANADA.

3.1 ABSTRACT

The expanding distribution of *Ixodes scapularis*, the vector for *Borrelia burgdorferi* sensu stricto in eastern North America, and the rising incidence of Lyme disease in southern Ontario are current animal and public health concerns. The objectives of this study were to investigate the spatio-temporal patterns of high-incidence (i.e., higher counts beyond what is expected by chance) carriage of *I. scapularis* and *B. burgdorferi*-positive *I. scapularis* in a population of companion dogs from an emerging Lyme disease risk area in southeastern Ontario. Using the spatial scan statistic, we identified significant temporal clusters, as well as spatial and space-time clusters of *I. scapularis* carriage and/or *B. burgdorferi*-positive tick carriage using Bernoulli, space-time permutation and Poisson models that were georeferenced to the submitting veterinary clinic or the centroid of each dog owner's postal code. In addition, analyses were conducted using all data collected and only data from dogs with no known history of travel. Most of the clusters identified were consistent in space and time with a known high risk area along the north shore of Lake Ontario and the seasonal pattern of *I. scapularis*, respectively. Notably, the space-time permutation model identified an unusual cluster north of current known risk areas that may signal a new area of increasing risk for *I. scapularis* carriage for Ontario dogs. While the choice of statistical model, georeferencing framework, and exclusion criteria impacted the detection, size and location of the clusters identified, the spatio-temporal locations of most of the high incidence clusters were consistent with the known epidemiology of *I. scapularis* and *B.*

burgdorferi in this area. The use of spatial scan statistics may provide a supplementary cost effective method for monitoring the distributions of medically important ticks in Ontario.

3.2 INTRODUCTION

In eastern North America, blacklegged ticks, *Ixodes scapularis*, are the primary vector for *Borrelia burgdorferi* sensu stricto (hereafter referred to as *B. burgdorferi*), the causal agent of Lyme disease (Lane et al., 1991). It has been four decades since *I. scapularis* was first discovered at Long Point Provincial Park in Ontario, Canada (Watson and Anderson, 1976), and the dispersal and expansion of *B. burgdorferi* and its vector, as well as the increasing number of human Lyme disease cases in Canada, is an ongoing public health concern (Ogden et al., 2008a; Ogden et al., 2008b). In addition to human Lyme disease, *B. burgdorferi*, a gram-negative spirochete, can cause arthritis, arthralgia and protein-losing nephropathy in dogs (Appel et al., 1993; Littman et al., 2006; Bouchard et al., 2015).

Climate change is thought to play a role in the geographic expansion of ticks, but increased human and animal travel, as well as changes in land-use over the past century are also considered important factors (Kilpatrick and Randolph, 2012). Various surveillance strategies have been employed to monitor the distribution of ticks and tick-borne diseases in Canada, including using ticks collected from companion dogs (Ogden et al., 2006; Fitzgerald, 2012). However, no clear guidelines exist for the use of animals as sentinels for human disease surveillance (Rabinowitz et al., 2005). The potential for domestic animals to be used for this purpose should not be underestimated (Halliday et

al., 2007); companion dogs share the same environment as their owners and are susceptible to several tick-borne zoonotic diseases (Chomel, 2011). Furthermore, around the world, canine serological studies of tick-borne diseases (Lindenmayer et al., 1991; Rand et al., 1991; Walker et al., 1998; Duncan et al., 2005; Bowman et al., 2009; Carrade et al., 2011; Wagner and Erb, 2012; Little et al., 2014) and studies focused on the ticks removed from companion animals (Ogden et al., 2000; Hamer et al., 2009; Smith et al., 2011; Claerebout et al., 2013; Chen et al., 2014; Iwakami et al., 2014) have been used for the geographical mapping of tick-borne disease risk. The inclusion of animals with a travel history in spatial epidemiology studies could potentially confound results, misrepresent areas of increased risk, and misdirect strategies used by public health officials to prevent and mitigate these diseases. As a result, previous Canadian studies have excluded all people or animals with a known history of travel (Ogden et al., 2006; Koffi et al., 2012; Leighton et al., 2012), but there are no reports estimating the potential impact of including/excluding these data for tick surveillance purposes. This is particularly important to address when the viability of a long-term surveillance program may depend on the epidemiological validity of collecting anonymized exposure data with little supporting epidemiological information beyond host species and the date and location of diagnosis.

Although geographic mapping studies provide a visual illustration of the relative risk of parasitism (or pathogen exposure) across a geographical area, they rarely employ cluster analysis. Cluster analysis is a component of spatial epidemiology that is used in surveillance to evaluate if events are aggregated in space, time, or space-time (Dohoo et al., 2009). Various methods exist for spatial analysis (Ward and Carpenter, 2000; Waller

and Gotway, 2004), but the spatial scan statistic has been widely used for the detection of spatial, temporal and space-time clusters in veterinary medicine and public health (Moore et al., 2005; Pearl et al., 2006; Beroll et al., 2007; Alton et al., 2013).

Spatio-temporal cluster analyses of *I. scapularis* collected exclusively from dogs have not been undertaken in southern Ontario. The main objectives of this study were therefore to: i) identify significant spatial, temporal and space-time clusters of *I. scapularis* carriage and *B. burgdorferi*-positive *I. scapularis* carriage on companion dogs in an emerging area for Lyme disease risk in southeastern Ontario; ii) assess whether the spatial, temporal, and space-time clusters are consistent with the known biology of *I. scapularis* in this region; iii) determine the variation in location and size of significant clusters when data are analysed with the geocoded centroid of the owner postal code or the submitting veterinary clinic; iv) evaluate the effect of inclusion of dogs with a history of travel in the two weeks prior to tick removal on the location, size, and significance of spatial, temporal and space-time clusters; and v) assess what impact various statistical models have on the outcome of the spatial scan statistic, and their relevance and potential usefulness in tick-borne disease surveillance.

3.3 METHODS

Study area and tick collection

Twenty veterinary practices in southeastern Ontario were enrolled to collect all tick species from companion dogs from April to December 2015. Participating veterinary practices were located between Kingston and Port Hope along the north shore of Lake Ontario (76.44^{o} to 78.72^{o} West), and northwards from the city of Belleville towards Bancroft and Haliburton, Ontario (43.96^{o} to 45.06^{o} North) (Figure 3.1). In the

area north and east of Belleville, there is one veterinary clinic that declined to participate in the study. Details of veterinary clinic enrollment and tick collection are summarized in Chapter 2. Briefly, ticks were removed from dogs at each veterinary clinic, frozen at -20^{o}C, and collected approximately every 2 weeks. Ticks were also accepted if the owner removed the tick from their dog, and dropped it off at their veterinary clinic. Owners completed a two-page questionnaire (Appendix B) regarding animal and owner demographics, recreational habits, lifestyle, and history of travel in the two-week period prior to tick removal (Chapter 2). In all analyses, dogs were enrolled once in the study (on the first tick submission), and to control for clustering if two dogs from the same family were parasitized on the same date, only the dog with the name that came first in the alphabet was enrolled. Ticks were still accepted if the owner declined to fill out the questionnaire.

Tick identification and PCR testing for *Borrelia burgdorferi*

Ticks and instars were identified to species using standard keys at the Public Health Agency of Canada's National Microbiology Laboratory in Winnipeg, Manitoba (Clifford et al., 1961; Keirans and Litwak, 1989; Durden and Keirans, 1996; Keirans et al., 1996). This federal laboratory tested *I. scapularis* for *B. burgdorferi* sensu stricto, *Borrelia miyamotoi, Anaplasma phagocytophilum*, and *Babesia microti* using standard PCR techniques previously described (Chapter 2).

Spatial, temporal, and space-time cluster analyses

Using SaTScan™ Version 9.4.2 software (Martin Kulldorff and Information Management Services Inc., Boston, MA, USA), retrospective scan statistics were used to identify statistically significant spatial, temporal and space-time clusters for high-incidence of *I. scapularis* tick carriage on companion dogs. The scan statistic can be

used to search for groups of events in space, time, or space-time that are higher and/or lower than expected by chance alone (H_0 = spatial and/or temporal randomness). Using a flexible scanning window in space, time and space-time with the maximum window size set by the investigator, the scan statistic applies Monte Carlo hypothesis testing to determine if the number of cases inside the window are significantly greater and/or smaller than expected (Kulldorff, 2015). All of the analyses for this study identified higher than expected counts based on the specified statistical model.

Bernoulli models were used to identify clusters of: 1) *I. scapularis* carriage relative to carriage of other tick species, and 2) carriage of *I. scapularis* positive for *B. burgdorferi* relative to *I. scapularis* negative for *B. burgdorferi*. Poisson models were used to identify significant clusters in space, time, and space-time for high rates of *I. scapularis* carriage and carriage of *B. burgdorferi*-positive *I. scapularis*. The population denominator for the Poisson models was the total number of canine veterinary appointments per week in each participating veterinary hospital. For this denominator, it was not possible to obtain data to differentiate between dogs with and without a history of travel, nor dogs with and without a history of tick attachment. A space-time permutation model was used to identify clusters with significant interactions in space and time for: 1) *I. scapularis* carriage, and 2) clusters of *B. burgdorferi*-positive *I. scapularis* carriage on pet dogs. The space-time permutation model uses only cases and adjusts for both purely spatial and purely temporal clusters, thereby identifying clusters that are not independent in space and time and have a space-time interaction (H_0: cases are independent in space and time) (Kulldorff, 2015).

Separate analyses were performed using all dogs and for those dogs with no history of travel in the fourteen days prior to tick removal. Two spatial reference frameworks were used for geographical co-ordinates: 1) the latitude and longitude of each participating veterinary clinic was used for all analyses; and 2) the latitude and longitude of each centroid of 221 unique pet owner postal codes were used for the Bernoulli and space-time permutation models (Geocoder, 2017). The owner postal code was not provided for 18 dogs; for these submissions, the centroid of the submitting veterinary clinic postal code was used as a proxy for this spatial information. Monte-Carlo hypothesis testing was performed using 9999 random replications of the dataset for all analyses using veterinary clinic co-ordinates. To reduce computation time, *p*-values were estimated using 999 Monte Carlo random replications for analyses geocoded to the latitude and longitude of each client postal code centroid. A circular search window was used for all analyses. The ratio of observed over expected (O/E), estimates of relative risk (RR), and *p*-values were reported for the most likely spatial, temporal, and space-time clusters. Secondary clusters were only reported for purely spatial analyses if there was no geographical overlap between clusters. For space-time analyses, the criteria for reporting secondary clusters was initially set so they were only reported if they did not centre on a previously reported cluster. Following manual review, these secondary clusters were only reported if they did not overlap with a more likely cluster in space-time. The maximum scanning window size was limited to 50% of the study population and 50% of the study period. A temporal precision of one day was used for all spatial-temporal and temporal analyses, except for the Poisson models where the time precision was set to the week of tick submissions. For Bernoulli models, iterative analyses were

performed for the identification of purely temporal clusters. The statistical significance level was set at $\alpha = 0.05$ for all analyses. Maps were generated in ArcGIS (ArcGIS 10.4.1, ESRI, Redlands, CA) to visualize all statistically significant space and space-time clusters.

3.4 RESULTS

Tick collection

A total of 543 individual dogs were enrolled in the study from 20 participating veterinary practices in southeastern Ontario between April 4 and December 31, 2015 (Chapter 2). Three dogs had incomplete dates for tick submission and were excluded from all subsequent spatio-temporal analyses. In total, 463 dogs were parasitized with *I. scapularis* and the remaining 77 dogs were carrying one of the following tick species: *Dermacentor variabilis, Ixodes cookei, Amblyomma americanum, Dermacentor albipictus, Rhipicephalus sanguineus* or *Ixodes muris*. A complete description of the tick species collected is available in Chapter 2. Three dogs were parasitized with both *I. scapularis* and *D. variabilis*, and these dogs were included as *I. scapularis* carriage for analyses. All *I. scapularis* removed from dogs were adult ticks. In total, 7.6% of dogs carrying *I. scapularis* had at least one tick positive for *B. burgdorferi* (95% CI: 5.3 - 10.4%). Table 3.1 summarizes the number of dogs with *I. scapularis* or other ticks, dog travel history, and testing results for *B. burgdorferi*.

Population

For rate-based analyses, population data were collected on the number of dog appointments per week in 18 of the 20 participating veterinary clinics; complete data were available for 13 weeks. In total 18,130 canine veterinary appointments among the

18 practices were conducted between May 10 and August 8, 2015. The average number of veterinary appointments per clinic per week was 77 (range: 14 to 176). During this 13-week period, *I. scapularis* were removed from 110 dogs; seven of these dogs were carrying at least one tick positive for *B. burgdorferi*. Upon removal of dogs with a history of travel, there were 80 dogs carrying *I. scapularis* of which three were carrying an *I. scapularis* positive for *B. burgdorferi*.

Scan statistics

I. *Ixodes scapularis* carriage versus carriage of all other tick species

Bernoulli model

Purely spatial clusters of *I. scapularis* carriage were identified using both the veterinary clinic location and the centroid of the client postal code when all dogs were used in the analyses, but no significant clusters were found in the separate analyses using only dogs with no history of travel (Table 3.2; Figure 3.2). Significant space-time clusters were identified using all dogs and only dogs with no history of travel, for both veterinary clinic and client postal code geocoded analyses (Table 3.2; Figure 3.3). With the veterinary clinic as a geographical reference, there was no difference in the spatial location or size of the space-time cluster between all dogs and those with no history of travel (Table 3.2). However, when the centroid of the owner postal code was used, the radius of the space-time cluster was much larger for dogs with no history of travel, and it was located further to the east of the study area in comparison to the significant cluster identified when all of the dogs were used in the analysis (Table 3.2; Figure 3.3). All significant space-time clusters for *I. scapularis* carriage identified with Bernoulli models covered the same time period from September 22nd to December 30th, 2015 (Table 3.2).

Two significant temporal clusters, one in the fall and one in the spring, were identified for *I. scapularis* carriage in the analysis with all dogs, and also in the analysis using only dogs with no history of travel (Table 3.3).

Space-time permutation model

Using the space-time permutation model, we only identified significant space-time clusters when the veterinary clinic location was used for a geographical reference; the time interval of these clusters occurred from June to September, which is much earlier than the significant space-time clusters identified using Bernoulli models (Table 3.2; Figure 3.4). The cluster locations and sizes were the same when separate analyses using all dogs or those with no history of travel were performed. The time precision was very similar; only one day longer (June 2nd to September 25th) when all dogs were used in the analysis compared to the time precision when only the dogs with no history of travel were included in the analysis (June 2nd to September 24th) (Table 3.2).

Poisson model

A significant spatial cluster was identified when all dogs were included in the analysis using a Poisson model (Table 3.4; Figure 3.5). Significant temporal clusters were found between May 10th and June 13th, 2015 when all dogs were used in the analysis; the same temporal cluster was identified when only those dogs with no history of travel were used in the analysis (Table 3.4). Significant space-time clusters with the same spatial location and similar time period were identified when all dogs and only dogs with no history of travel were included in the analyses (Table 3.4; Figure 3.5).

II. *B. burgdorferi*-positive *I. scapularis* versus *B. burgdorferi*-negative carriage

Bernoulli and space-time permutation models

When the participating veterinary clinic was used for georeferencing, the analyses did not identify any significant clusters of *I. scapularis* positive for *B. burgdorferi* carriage on dogs (Table 3.5). Significant spatial and space-time clusters, based on Bernoulli models, were identified using the centroid of each client's postal code when only the dogs with no history of travel were included in the analyses (Table 3.5; Figure 3.6). Space-time clusters, based on space-time permutation models, were not identified for carriage of *B. burgdorferi*-positive *I. scapularis* (Table 3.5). No significant purely temporal cluster were identified (Table 3.6).

Poisson model

Among dogs with no history of travel, a significant temporal cluster of dogs carrying *I. scapularis* positive for *B. burgdorferi* was identified between May 31 and June 13, 2015 (Table 3.7). Significant space-time clusters for high rates of dogs carrying *I. scapularis* positive for *B. burgdorferi* were centred on Peterborough, Ontario when all dogs and those dogs with no travel history were used in the analyses (Table 3.7; Figure 3.7). The temporal window of the significant space-time and significant purely temporal clusters were similar for the analyses involving Poisson models (Table 3.7).

3.5 DISCUSSION

Analyses of *I. scapularis* carriage on companion dogs

Using Bernoulli models, when all of the dogs in the study were analyzed we identified significant spatial clusters where there was a high proportion of *I. scapularis* carriage relative to other tick species. The significant spatial cluster georeferenced to the

dog owners' postal code was centred on an expected location based on the *I. scapularis* risk map published by Public Health Ontario for 2016 (Public Health Ontario, 2016). However, the location of the significant spatial cluster when georeferenced to the veterinary clinic is in a similar location to a statistically significant cluster identified in 2016 for *I. scapularis* collected from the environment across three ecoregions of Ontario (Clow et al., 2016). Due to limitations of our tick collection method (i.e., visiting participating clinics every fourteen days), our study could not encompass all of southern Ontario, which is approximately 137,000 km^2. As a result, this study is not directly comparable to Clow et al. (2016).

The inclusion of dogs with a history of travel was expected to add uncertainty to the data and obscure the evidence of clustering for *I. scapularis* carriage. Contrary to our expectation, no significant spatial cluster was detected when only the dogs with no history of travel were included in the Bernoulli model. This may be due to reduced statistical power; the sample size was reduced by 28.7% when dogs with a history of travel (or unknown travel) were excluded from the analyses. Bernoulli models did identify significant temporal and space-time clusters within this group of non-traveled dogs, which may confirm the considerable temporal influence on tick species parasitism in this region (Chapter 2). Almost 20% of participants in this study responded "yes" to travelling with their dog within the fourteen days prior to tick removal (Chapter 2). However, the interpretation of "travel" may have varied between participants. Although we asked for specific information on travel location, uncertainty regarding the duration and route of travel, as well as some participants' unwillingness to disclose this information, did not allow for further investigation (Chapter 2).

Two significant purely temporal clusters for *I. scapularis* carriage were identified in the analyses using all dogs in the study as well using dogs with no history of travel. The primary cluster occurred in the fall, starting September 22, for both the analyses with all dogs as well as for those with no history of travel and extended into December; the secondary cluster occurred for both groups in the spring (Table 3.3). The average daily temperature in the study area did not drop below 4^{o}C until December 26, 2015 (Government of Canada, 2015). Ticks were therefore likely active throughout most of this month (Duffy and Campbell, 1994). The significant temporal clusters fit very well with the known biology and activity of established populations of adult *I. scapularis* in southern Ontario (Lindsay et al., 1999).

Significant space-time clusters of *I. scapularis* carriage relative to other tick species were identified with Bernoulli models when georeferenced to the veterinary clinic and postal code centroid for both analyses using all dogs and those with no history of travel in the 14 days prior to tick removal (Figure 3.3). These space-time clusters have a similar temporal portion (September 22 to December 30, 2015) to the significant purely temporal cluster identified; of the ticks collected in the fall, 98.4% of dogs (95% confidence interval = 96.2 to 99.5) were parasitized by *I. scapularis* (Chapter 2). The space-time cluster of *I. scapularis* carriage is also consistent with the purely spatial clusters identified, and fits with the biology and current distribution of established populations of *I. scapularis* in our study area (Clow et al., 2016). The large radius of the significant space-time cluster, using dogs with no history of travel, illustrates a limitation to analyzing spatial information georeferenced to the centroid of the owner's postal code; within this cluster, there were postal code centroids that were more than 240 km east of

our most eastern participating veterinary hospital. This implies that in this study, some dog owners, who had not traveled, were a considerable distance from their home and visited a local veterinary clinic when their dog was parasitized by *I. scapularis*. The use of veterinary clinic location may have been a better indicator of where a tick encounter occurred in this population of dogs. Ultimately, accurate knowledge of where the dog encountered the tick would provide the most reliable information for spatial analyses, but this information is rarely available. Georeferencing to the submitting veterinary clinic would also remove any privacy issues associated with the use of owner postal code information (Kulldorff et al., 2005).

Space-time clusters of *I. scapularis* carriage, identified using space-time permutation models, revealed an unusual cluster from June 2 to September 24, 2015 using data from all dogs and dogs with no history of travel. This is an interesting finding because the centroid of this significant space-time cluster is in Bancroft, Ontario, which is approximately 70 km north of the currently identified risk area (Public Health Ontario, 2016). However, space-time permutation models only use case data; if the background population increases or decreases faster in certain locations relative to others, a population shift bias may occur (Kulldorff and Hjalmars, 1999; Mack et al., 2012). The Bancroft area is a popular recreational destination, and although the census population in 2016 was 3881 people (Statistics Canada, 2016) and the regional population is estimated to be 40,000, this area is reported to receive 150,000 visitors per year (www.Bancroft.ca, Retrieved March 25, 2017). Within this significant cluster, there were 23 dogs with no history of travel, and only two of these dogs had a postal code with a home location greater than 250 km (Unionville and Cookstown, Ontario) from the cluster's centroid.

This suggests that the majority of dogs in this cluster resided within the local region. However, the design of the questionnaire did not allow us to differentiate between year round residents and seasonal residents with a local postal code. The significant cluster identified in Bancroft is in a location and at a time that does not fit well with the known biology of adult *I. scapularis* (i.e., no confirmed populations of reproducing ticks in Bancroft (Public Health Ontario, 2016) and seasonal activity of adults in Ontario should be in the spring and the fall (Lindsay et al., 1999)). Thus, follow-up investigations should be carried out to differentiate whether this cluster was the result of population shift bias or the emergence of *I. scapularis* activity in this area.

To compare the rate of *I. scapularis* carriage on companion dogs between the different clinic locations, spatio-temporal analyses based on Poisson models were used. Some participating veterinary clinics did not have a computerised system in place to record the number of canine appointments per week. Furthermore, access to this information proved challenging, and complete data were only available for a 13-week period between May 10 and August 8, 2015. Despite the small dataset, both spatially and temporally, these analyses offered an interesting comparison with the Bernoulli models. Significant spatial and space-time clusters for *I. scapularis* carriage using the Poisson models are very similar in size and shape to the significant space and space-time clusters of *I. scapularis* carriage identified using the Bernoulli models (Figures 3.2, 3.3, 3.5). The significant temporal clusters for the rate of *I. scapularis* carriage are also comparable to the secondary temporal clusters identified in the spring with the Bernoulli model. These results suggest that Poisson models fit well with the expected temporal and spatial biology of *I. scapularis* in this region. We did not have any data on the travel history or

tick carriage for the dogs used in the denominator, and the analyses may have yielded different results if we had been able to exclude these dogs from the denominator.

Analyses of *I. scapularis* positive for *B. burgdorferi* carriage on companion dogs

Significant spatial and space-time high prevalence clusters of *B. burgdorferi*-positive *I. scapularis* carriage on dogs were identified with the Bernoulli model, but only when dogs with no history of travel georeferenced to the centroid of the owners' postal code were used for analyses. It should be noted that complete addresses were not requested from participants in this study; by using the centroid of the postal code, owner anonymity was protected. The location of this significant 425 km^2 spatial and space-time cluster is in the greater Napanee area, and adjacent to an area of increased Lyme disease risk described by Public Health Ontario (Public Health Ontario, 2016). The date of this space-time cluster occurred from September 22 to November 11, 2015, but no significant purely temporal clusters were identified. Although these results are based on a small number of cases, they are consistent with a *B. burgdorferi*-risk area identified by active surveillance (Clow et al., 2016).

Using the Poisson model, an increased rate of *B. burgdorferi*-positive *I. scapularis* carriage was detected, but the centroid of this significant space-time cluster was in Peterborough, Ontario. This finding is not in keeping with the current understanding for human and canine Lyme disease risk in Ontario (Public Health Ontario, 2016), and may represent a collection of adventitious ticks that arrived in 2014, survived the winter and molted into questing *B. burgdorferi*-positive adult ticks. This high-rate cluster of dogs carrying *B. burgdorferi*-positive *I. scapularis* should be interpreted with caution, and should not alarm the reader to an increased risk of human

Lyme disease in Peterborough, Ontario. As previously discussed, the Poisson method was only available for *I. scapularis* submissions from May 10 to August 8, 2015 and results are based on a small sample size of 3 dogs with no history of travel and 7 dogs when all dogs were included in the analyses. Further investigation involving data from multiple years from a larger study area will allow better interpretation of these findings.

A limitation to our study is that the home postal code or veterinary clinic may not reflect the actual location of tick exposure. In some analyses, the locations of spatial and spatio-temporal clusters were the same regardless of which data source was used for geolocation. Although the owner postal code was expected to provide a more precise indication of where the tick was acquired; these analyses did not necessarily result in clusters being identified in a more epidemiologically plausible location in space and space-time. The use of the permanent residence postal code is not necessarily indicative of where the animal was parasitized by a tick; when a submission was classified as 'no travel' we could not assume that the owner postal code was an appropriate georeference for the tick encounter. Collecting both the submitting veterinary location and the owner postal code provided us the opportunity to assess the potential implications of this type of misclassification. Using a spatial scan statistic for ticks geocoded to the veterinary clinic may be a useful proxy for the identification of *I. scapularis* risk areas for companion dogs in Ontario.

Almost 20% of participants reported travelling with their dog in the two weeks prior to tick removal. Globally there is an increase in the amount of travel for people and their pets, and animal movements are known to play a role in the changing distribution of tick species (Chomel, 2011; Kilpatrick and Randolph, 2012). With the exception of the

significant spatial and space-time cluster identified for *B. burgdorferi*-positive *I. scapularis* carriage, the inclusion of dogs with a history of travel (or missing information) had little influence on the size and shape of significant spatial, temporal and space-time clusters in this population of dogs. While we do not advocate including animals with a history of travel in spatial epidemiology studies, it is clear that surveillance systems without these data can still yield useful information. We recommend that future studies have a clearer definition for "travel 14 days prior to tick removal" by including a measure of distance from permanent residence, or travel outside of the residing county or census subdivision.

Our study identified significant clustering of *I. scapularis* carriage on domestic dogs using Bernoulli, space-time permutation and Poisson models, and each model has inherent advantages and limitations. Bernoulli models, while simple to operationalize, describe a proportional morbidity of *I. scapularis* carriage relative to other tick species. Caution is required when interpreting results from a Bernoulli model because an identified high-incidence cluster may be the result of an increase in *I. scapularis* carriage or may reflect a reduction in other tick species in that region. In addition, in areas where people have become accustomed to tick parasitism the ticks may not be submitted, and tick avoidance, personal protection, and animal tick control could be utilized and may influence the results (Ogden et al., 2015). Space-time permutation models are often used to detect areas where disease outbreaks are taking place (Kulldorff et al., 2005). Analyses with space-time permutation models can adjust for pre-existing purely spatial and/or purely temporal clustering, and do not require a measure of the population at risk (Malizia, 2013). However, space-time permutation models are reliant on the assumption

that changes in the population within the study area are homogeneous in space and consistent through time (Mack et al., 2012). Thus, knowledge of population dynamics is critical to avoid identification of a false positive or "false alarm" cluster. Poisson models are straight forward to interpret as they measure a rate of disease incidence, but measuring accurate and complete data for use as a denominator is challenging in domestic animal research (Stephen et al., 1998). However, we were able to obtain these data from some clinics, indicating that development of a system for tick surveillance using veterinary clinics is feasible.

The spatial scan statistic may be a valuable tool to monitor change, and to identify "hot spots" or unexpected events of tick attachment. Thus, in the future, our methods could be applied to other tick species. In the United States, the Lone Star tick, *Amblyomma americanum*, is forecasted to expand its geographical distribution to higher latitudes (Springer et al., 2015). Although no known *A. americanum* populations are established in Ontario, this tick has been recovered on passive surveillance; likely as an adventitious tick or acquired during travel to an *A. americanum* endemic area (Chap 2, Nelder et al., 2014). *Amblyomma americanum* is the primary vector for several human and animal pathogens (e.g., *Ehrlichia chaffeensis*, *Erhlichia ewingii*, and *Cytauxzoon felis*) (reviewed by, Goddard and Varela-Stokes, 2009), and its establishment in Ontario would be of considerable public, medical and veterinary health significance. The use of spatial scan statistics in sentinel veterinary hospitals, may allow for the early warning of clustering of medically important ticks in Ontario.

The goal of spatial epidemiology for tick-borne disease is to increase awareness and inform the public and animal health sectors regarding shifting patterns of disease

occurrence, and to aid in the development of successful prevention and control strategies (Eisen and Eisen, 2011). If the current trends of climate change persist, we expect the continued expansion of blacklegged ticks into new areas of Ontario where suitable habitat, hosts and environmental conditions exist for successful reproduction of *I. scapularis* (Ogden et al., 2008b; Clow et al., 2017). We have demonstrated that companion dogs in southeastern Ontario can be used as sentinels to detect significant clustering of *I. scapularis* and *B. burgdorferi*-positive *I. scapularis* carriage in one year and over a small geographical area using the spatial scan statistic. Compared to active surveillance, this method is relatively inexpensive and the inclusion of veterinary clinics and dogs may have a role in tick surveillance. Before integrating spatial scan statistics into surveillance programs, information about how these clusters change in time and space over a prolonged period of time will help validate these methods and better inform their usefulness in tick surveillance. The use of active surveillance in the same region and over multiple seasons will remain the gold standard for confirming established populations of *I. scapularis*, but spatial scan statistics may aid in early hypothesis generation and help to direct the investment of resources for time intensive surveillance strategies.

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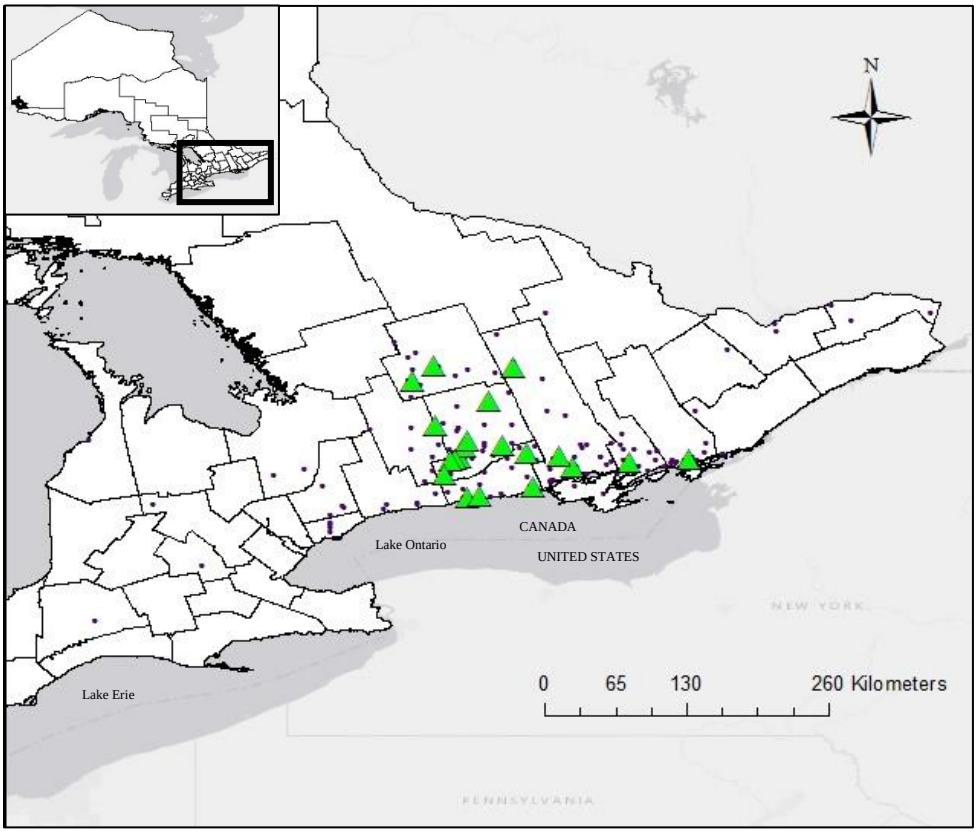

Figure 3.1. A map of the 20 veterinary hospitals (△) where ticks were submitted to the study from companion dogs in southeastern Ontario and the location of the geocoded centroid of each dog owner's postal code (•).

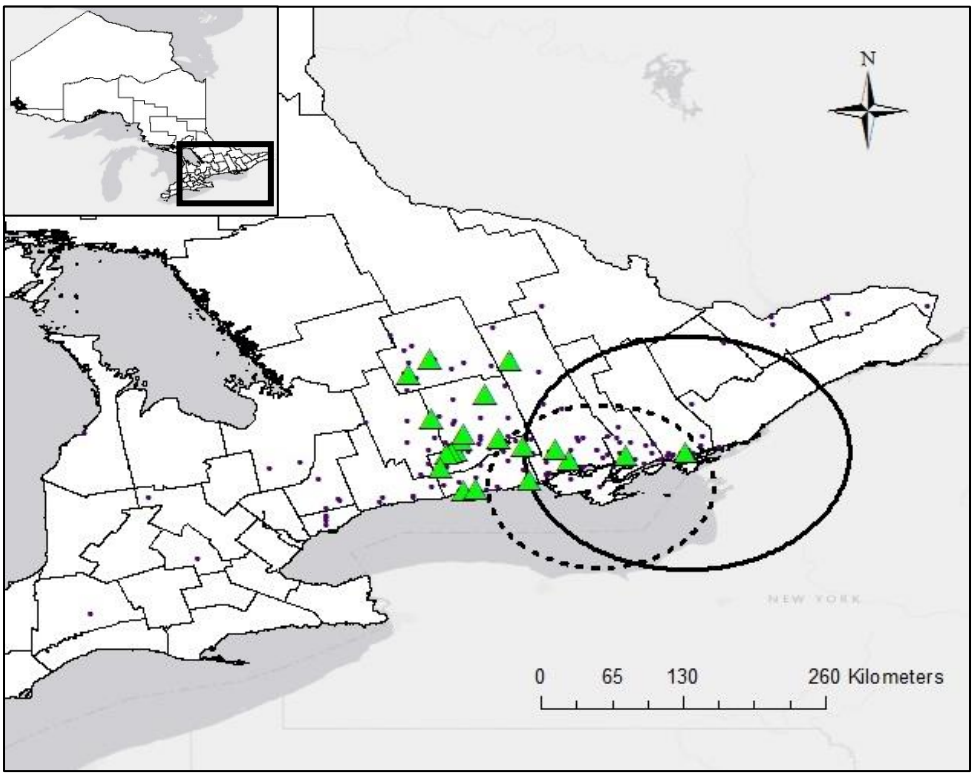

Figure 3.2. The most likely non-overlapping significant spatial clusters based on Bernoulli models of *Ixodes scapularis* carriage relative to the carriage of other tick species from all participating dogs in southeastern Ontario, from April to December 2015, using the submitting veterinary clinic (solid oval) or the centroid of the owner's postal code (hatched oval) for geographical reference.

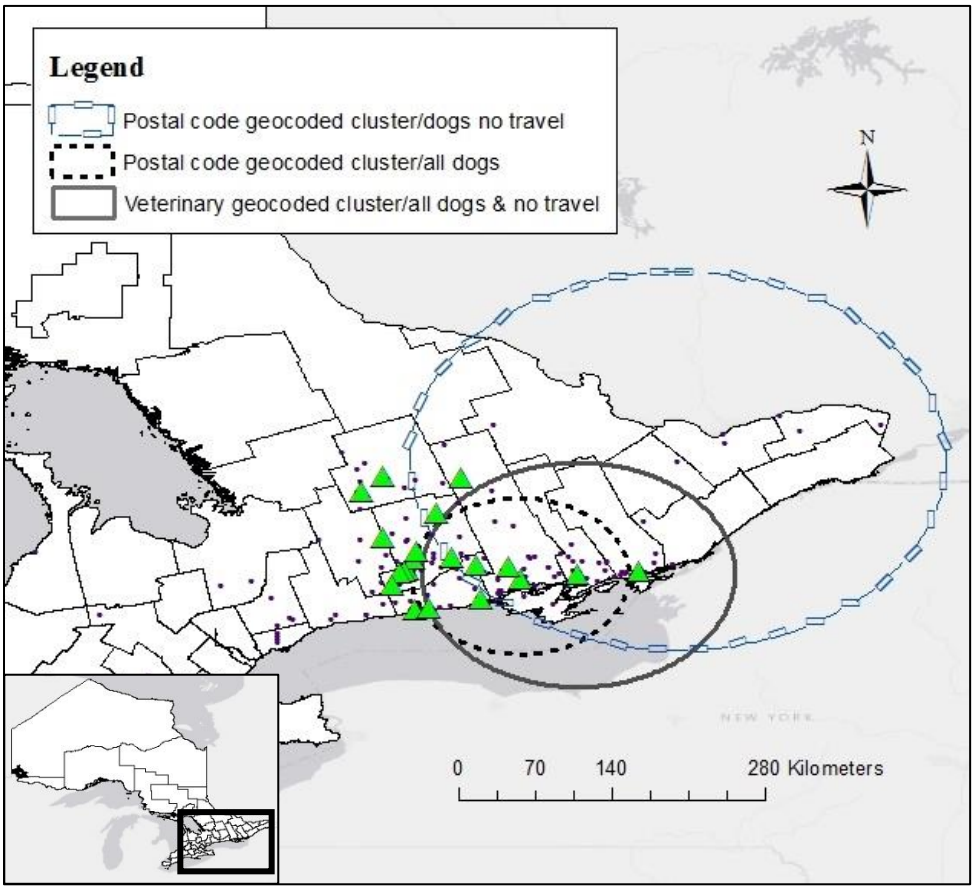

Figure 3.3. The locations of the non-overlapping significant space-time clusters based on Bernoulli models of *Ixodes scapularis* carriage relative to the carriage of other tick species from a population of dogs in southeastern Ontario using either the participating veterinary clinic or the centroid of the owner's postal code for geographical reference.

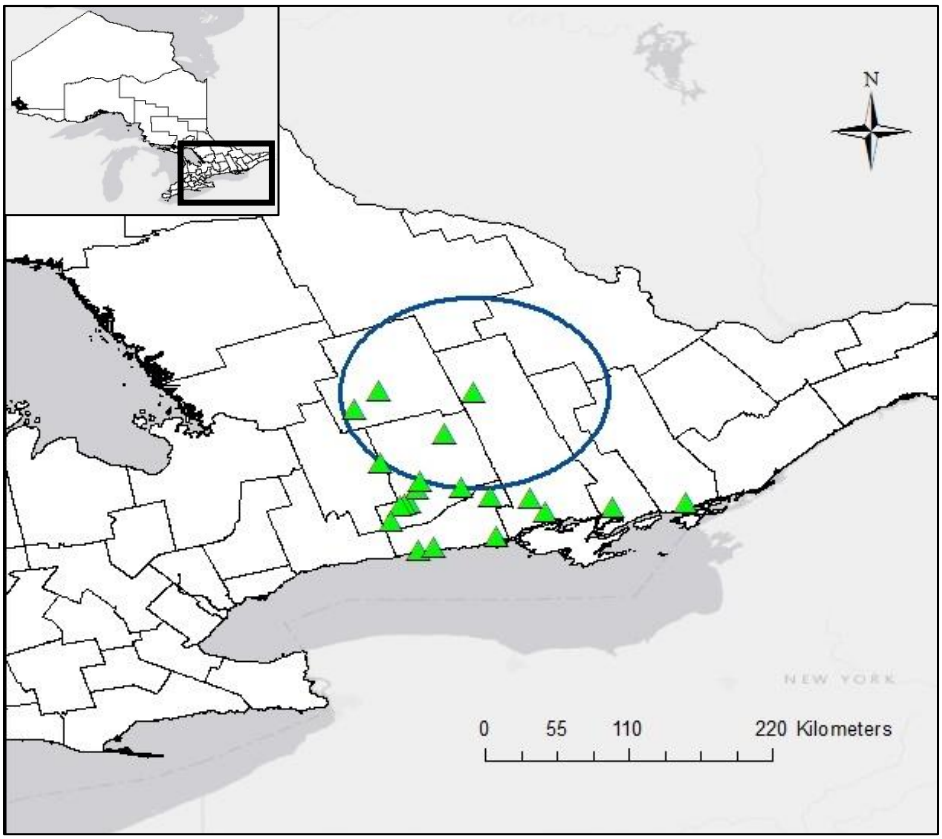

Figure 3.4. The location of the most likely significant clusters based on space-time permutation models, of *Ixodes scapularis* carriage from all dogs and those with no history of travel in the 14 days prior to tick removal (identical cluster size and location), in southeastern Ontario using the location of submitting veterinary clinic for geographical reference.

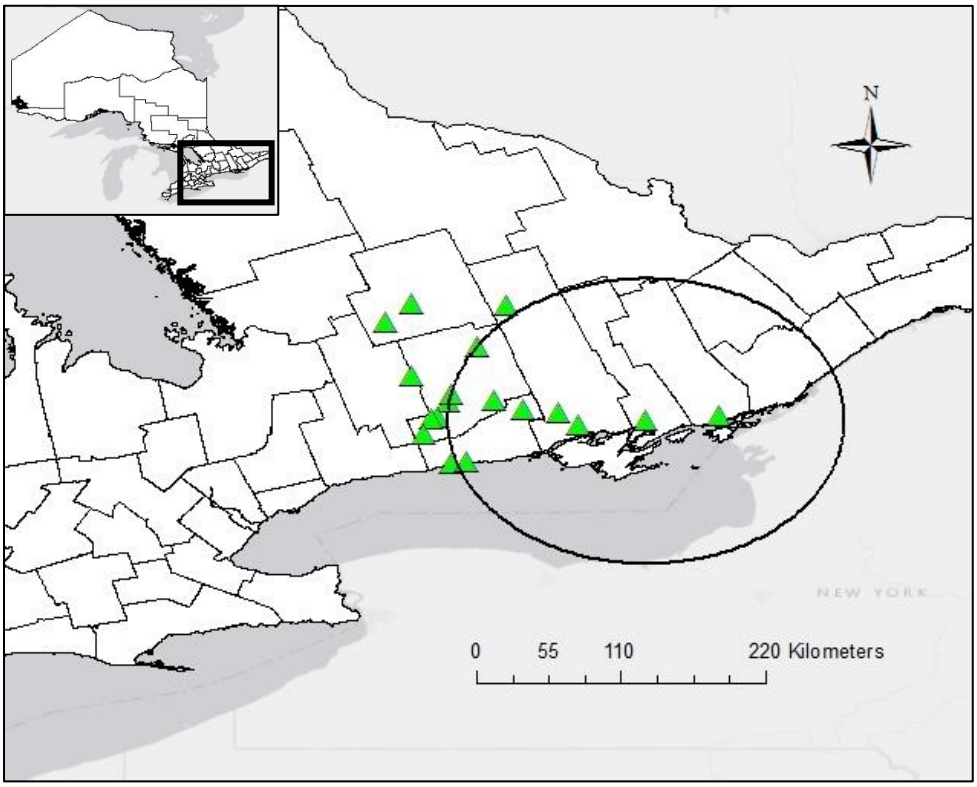

Figure 3.5. The location of the non-overlapping significant spatial (all dogs) and space-time clusters (all dogs and those with no history of travel), based on a Poisson model, of high rates of *Ixodes scapularis* carriage from a population of dogs in southeastern Ontario from which ticks were collected from 18 veterinary clinics between May 10 and August 8, 2015.

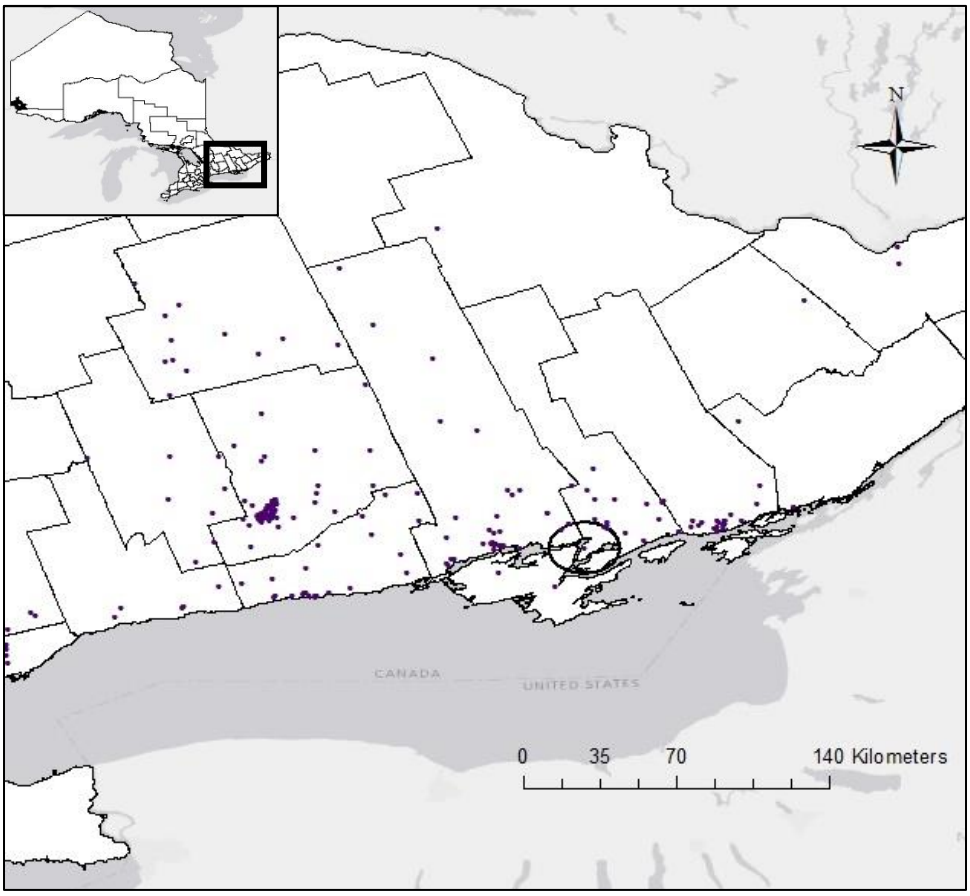

Figure 3.6. The location of most likely non-overlapping significant spatial and space-time postal code geocoded clusters, based on a Bernoulli model, for *Ixodes scapularis* positive for *Borrelia burgdorferi* carriage relative to carriage of *I. scapularis* negative for *B. burgdorferi* on dogs in southeastern Ontario with no history of travel in the two weeks prior to tick removal.

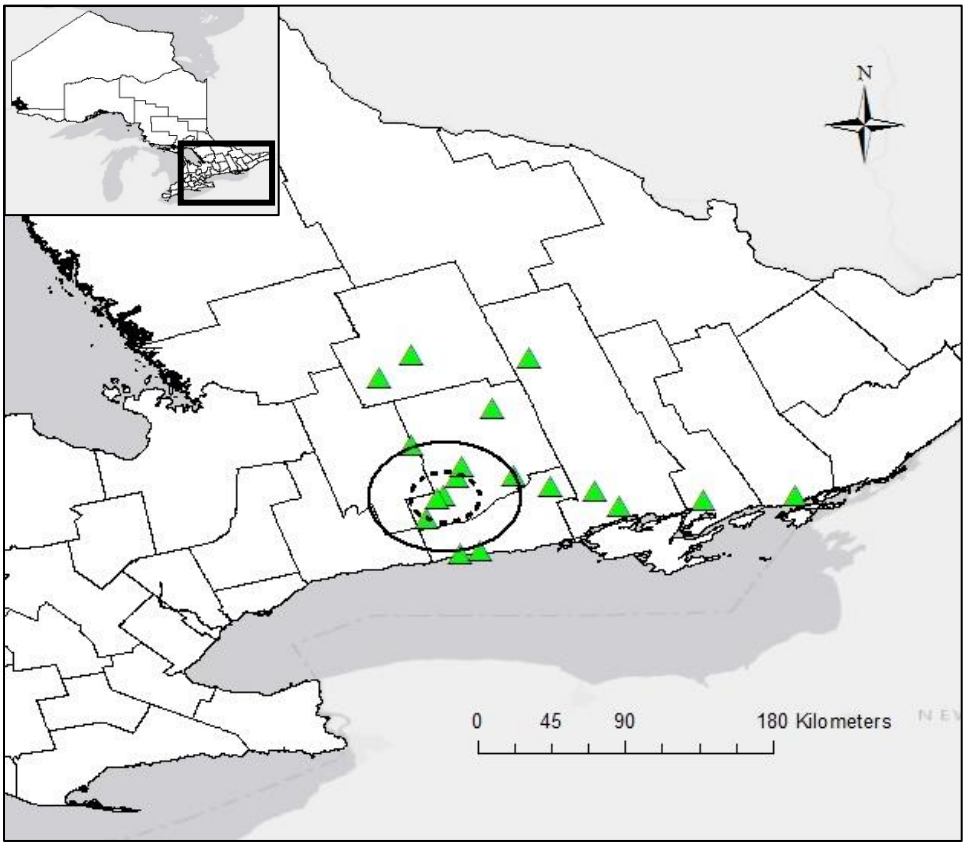

Figure 3.7. The most likely non-overlapping significant space-time clusters, based on Poisson models, of high rates of *Ixodes scapularis* positive for *Borrelia burgdorferi* carriage submitted from all dogs (solid oval) and those with no history of travel in the two weeks prior to tick removal (hatched oval) geocoded to the location of the submitting veterinary clinic.

Table 3.1. Summary of data on ticks removed from dogs submitted from April to December 2015 in southeastern Ontario used to perform scan statistics with Bernoulli models to identify spatial, temporal, and space-time clusters of 1) *Ixodes scapularis* carriage compared to carriage of other tick species and 2) carriage of *Borrelia burgdorferi*-positive *I. scapularis* relative to *B. burgdorferi*-negative *I. scapularis*.

	Dogs with no history of travel	Dogs with a history of travel*	Dogs with unknown travel history	All dogs
Ixodes scapularis	330	82	51	463
B. burgdorferi positive	*16*	*15*	*4*	*35*
B. burgdorferi negative	*314*	*67*	*47*	*428*
Other tick species	50	25	2	77
Total dogs	**380**	**107**	**53**	**540**

*History of travel in the 14 days prior to tick removal was reported by the dog owner on a questionnaire

Table 3.2. Results of spatial and space-time scans using Bernoulli and space-time permutation models to identify clusters of *Ixodes scapularis* carriage relative to carriage of other tick species on pet dogs. Ticks were collected from pet dogs during April to December, 2015 in the study area of southeastern Ontario, and georeferenced to both the 20 participating veterinary clinics and the centroid of the 221 unique owner postal codes*.

	Cluster type	History of travel[a]	Population in cluster	Latitude (°N)	Longitude (°W)	Radius (km)	Date of 2015 cluster	Observed	Expected	Observed/ expected	Relative risk	*P*-value
Veterinary clinic	Spatial	No travel	96	44.3	76.4	77.17		91	83.37	1.09	1.13	0.109
		All dogs	228	44.3	76.4	106.54		213	195.49	1.09	1.17	<0.001
	Space-time	No travel	118	44.2	76.9	102.50	Sept 22 – Dec 30	118	102.47	1.15	1.24	<0.001
		All dogs	171	44.2	76.9	102.50	Sept 22 – Dec 30	171	146.62	1.17	1.26	<0.001
	Space-time permutation	No travel		45.0	77.9	72.39	Jun 2 – Sept 24	23	7.06	3.26		<0.001
		All dogs		45.0	77.9	72.39	Jun 2 – Sept 25	30	10.57	2.84		<0.001
Postal code centroid	Spatial	No travel	122	44.4	76.3	110.99		114	105.95	1.08	1.12	0.595
		All dogs	229	44.0	77.2	74.35		214	196.35	1.09	1.17	0.003
	Space-time	No travel	119	45.2	76.1	172.76	Sept 22 – Dec 30	119	103.34	1.15	1.24	0.001
		All dogs	169	44.2	77.4	71.25	Sept 22 – Dec 30	169	144.90	1.17	1.26	0.001
	Space-time permutation	No travel		44.2	77.4	1.09	Dec 11 – Dec 19	3	0.045	66.00		0.067
		All dogs		44.4	81.4	221.9	Jun 2 – Sept 25	14	3.33	4.21		0.113

* If the owner postal code was not provided, the centroid of the veterinary clinic postal code was used (18 postal codes for all dogs and 4 postal codes for dogs with no history of travel)

[a] Separate scans were performed for all dogs and those with no history of travel in the 14 days prior to tick removal

Table 3.3. Results of temporal scans using Bernoulli models to identify primary and secondary clusters of *Ixodes scapularis* carriage on pet dogs collected from April to December, 2015 in the study area of southeastern Ontario.

	History of travel[a]	Date of 2015 cluster	Population in cluster	Observed	Expected	Observed/ expected	Relative risk	*P*-value
Temporal clusters of *I. scapularis* carriage	No travel	Sept 22 – Dec 7	199	199	172.82	1.15	1.38	< 0.001
		Apr 4 – May 27	81	74	58.62	1.26	1.60	< 0.001
	All dogs	Sept 22 – Dec 30	299	298	256.36	1.16	1.46	< 0.001
		Apr 15 – Jun 5	149	126	102.01	1.24	1.99	< 0.001

[a] Separate scans were performed for all dogs and those with no history of travel in the 14 days prior to tick removal

Table 3.4. Results of spatial, temporal and space-time scans based on Poisson models to identify high-rates of canine *Ixodes scapularis* carriage in the study area of southeastern Ontario from May 10 to August 8, 2015*.

Cluster type	Travel history[a]	Population	Cases	Latitude (°N)	Longitude (°W)	Radius (km)	Date of 2015 cluster	Observed	Expected	Observed/ expected	Relative risk	*P*-value
Spatial	No travel	665	80	44.2	76.9	108.28		52	38.66	1.34	1.99	0.063
	All dogs	665	110	44.2	76.9	108.28		74	53.16	1.39	2.20	0.003
Temporal	No travel		80				May 10 to Jun 13	63	34.46	1.83	4.90	0.001
	All dogs		110				May 10 to Jun 13	86	47.38	1.82	4.74	0.001
Space-time	No travel	665	80	44.2	76.9	108.28	May 10 to May 30	35	10.36	3.38	5.23	0.001
	All dogs	665	110	44.2	76.9	108.28	May 10 to Jun 13	62	23.07	2.69	4.87	0.001

* The population denominator is comprised of the total number of canine veterinary appointments per week from the 18 participating veterinary clinics

[a] Separate scans were performed on all dogs and those with no history of travel in the 14 days prior to tick removal

Table 3.5. Results of spatial and space-time scans using Bernoulli and space-time permutation models to identify clusters of *Ixodes scapularis* positive for *Borrelia burgdorferi* carriage relative to carriage of *B. burgdorferi*-negative *I. scapularis* on pet dogs. Ticks were collected from pet dogs during April to December, 2015 in the study area of southeastern Ontario, and georeferenced to both the 20 participating veterinary clinics and the centroid of the 221 unique owner postal codes*.

	Cluster type	History of travel[a]	No. Cases	No. Controls	Latitude (°N)	Longitude (°W)	Radius (km)	Date of 2015 cluster	Observed	Expected	Observed/ expected	Relative risk	*P*-value
Veterinary clinic	Spatial	No travel	16	314	44.3	76.4	40.05		7	2.76	2.53	3.73	0.200
		All dogs	35	428	44.3	76.4	40.05		11	6.58	1.67	1.98	0.694
	Space-time	No travel	16	314	44.3	76.4	40.05	Sept 22 to Oct 16	5	0.58	8.59	12.05	0.157
		All dogs	35	428	44.2	76.9	0	Sept 22 to Oct 20	6	0.83	7.22	8.50	0.120
	Space-time permutation	No travel	16		44.2	77.4	32.20	Oct 19 to Oct 27	2	0.25	8.00		0.468
		All dogs	35		44.1	78.5	15.18	Jun 1 to Jun 16	3	0.43	7.00		0.522
Postal code centroid	Spatial	No travel	16	314	44.2	77.0	11.63		4	0.39	10.31	13.42	0.041
		All dogs	35	428	44.2	77.0	0		4	0.53	7.56	8.41	0.189
	Space-time	No travel	16	314	44.2	77.0	11.63	Sept 22 to Nov 11	4	0.19	20.63	27.17	0.024
		All dogs	35	428	44.2	77.0	11.63	Sept 22 to Oct 19	4	0.30	13.23	14.81	0.241
	Space-time permutation	No travel	16		44.3	77.7	12.81	Oct 19 to Oct 27	2	0.25	8.00		0.704
		All dogs	35		44.4	78.4	8.47	Jun 9 to Jun 16	2	0.11	17.50		0.874

* If the owner postal code was not provided, the centroid of the veterinary clinic postal code was used (18 postal codes for all dogs and 4 postal codes for dogs with no history of travel).

[a] Separate scans were performed for all dogs and those with no history of travel in the 14 days prior to tick removal

Table 3.6. Results of temporal scans using Bernoulli models to identify clusters of *Borrelia burgdorferi*-positive *Ixodes scapularis* on pet dogs collected from April to December, 2015 in the study area of southeastern Ontario.

	History of travel[a]	Date of 2015 Cluster	Population in cluster	Observed	Expected	Observed/ expected	Relative risk	*P*-value
Carriage of *B. burgdorferi* positive I. scapularis	No travel	Aug 21 to Sept 22	2	2	0.10	20.63	23.43	0.153
	All dogs	Oct 16 to Oct 20	37	9	2.80	3.22	3.99	0.179

[a] Separate scans were performed for all dogs and those with no history of travel in the 14 days prior to tick removal.

Table 3.7. Results of spatial, temporal and space-time scans based on Poisson models for high-rates of carriage of *Borrelia burgdorferi*-positive *Ixodes scapularis* on dogs in the study area of southeastern Ontario from May 10 to August 8, 2015*.

Cluster type	Travel history[a]	Cases	Latitude (ºN)	Longitude (ºW)	Radius (km)	Date of 2015 cluster	Observed	Expected	Observed/ expected	Relative risk	*P*-value
Spatial	No travel	3	44.3	78.4	15.18		3	0.65	4.62	infinity	0.083
	All dogs	7	44.0	78.2	48.52		6	2.55	2.35	10.44	0.157
Temporal	No travel	3				May 31 to Jun 13	3	0.50	5.97	infinity	0.025
	All dogs	7				May 10 to Jun 20	7	3.75	1.96	infinity	0.053
Space-time	No travel	3	44.3	78.4	15.18	May 31 to Jun 13	3	0.12	25.84	infinity	0.011
	All dogs	7	44.3	78.4	33.25	May 31 to Jun 20	5	0.51	9.79	31.78	0.025

* The population denominator is comprised of the total number of canine veterinary appointments per week over 13 weeks from the 18 participating veterinary clinics.

[a] Separate scans were performed on all dogs and for those with no history of travel in the 14 days prior to tick removal.

CHAPTER 4: SUMMARY DISCUSSION & CONCLUSIONS

This study provides an up-to-date summary of Ixodidae tick species that parasitize companion dogs, and the prevalence of tick-borne pathogens of interest, in an emerging area for Lyme disease risk in southeastern Ontario. Using a case-case study design, we determined canine risk factors for *Ixodes scapularis* carriage relative to carriage of other tick species, as well as risk factors for the carriage of *Borrelia burgdorferi*-positive *I. scapularis* relative to negative ticks. Spatial, temporal and space-time clusters of *I. scapularis* and *B. burgdorferi*-positive *I. scapularis* were also identified within this approximately 11, 000 km^2 area of Ontario.

4.1 IMPLICATIONS OF RESEARCH FOR COMPANION DOG HEALTH

While seven species of ticks were recovered from parasitized dogs, 85.6% of these dogs were parasitized with *I. scapularis* (Chapter 2). All *I. scapularis* ticks collected from dogs were adults; only 2.2% of dogs were carrying nymphal or larval ticks of other species. Immature tick stages are not commonly recovered in companion animal tick studies and are likely overlooked due to their small size (Földvári and Farkas, 2005; Burroughs et al., 2016).

In keeping with recent human tick surveillance in Ontario (Nelder et al., 2014), 98.7% of dogs in our study were parasitized by four species of ticks: *I. scapularis*, *Dermacentor variabilis, Ixodes cookei* and *Amblyomma americanum*. In Ontario, *A. americanum* is considered an adventive or introduced tick (Lindquist et al., 2016), but its collection in this study and in other passive surveillance programs in the province is

notable (Nelder et al., 2014). The range of *A. americanum* is expanding northwards in the United States, and with forecasted climate change, establishment of this medically important tick in Ontario is plausible (Ogden et al., 2006b; Springer et al., 2015). In Ontario, *Rhipicephalus sanguineus* is also not known to reproduce in the wild, and although there are limited data regarding this species in the province, it has been reported to establish indoors (e.g., dog kennels and homes) (Lindquist et al., 2016). Worldwide, *Rhipicephalus sanguineus* is considered the most ubiquitous tick parasitizing dogs (Dantas-Torres, 2010), but in this study only 0.55% of dogs were parasitized with *R. sanguineus*; however, one puppy imported from Texas was heavily infested. This finding highlights the importance of ectoparasite prevention to limit the importation of exotic and rare parasites on traveling animals (Keirans and Durden, 2001).

This study identified some client confusion regarding the use of ectoparasite prevention (e.g., misidentifying a flea prevention product as an acaricide); an accurate assessment of the influence of tick prevention on tick species carriage would have required product confirmation by the veterinary clinic. Future research regarding the efficacy of tick prevention might be better addressed in a randomized control trial or a cohort study, where tick prevention use can be more accurately defined both by the nature of the chemical and the timing of its application.

Risk factors for *I. scapularis* carriage relative to all other tick species were identified using a case-case study design (Chapter 2). Other tick species, including *D. variabilis* and *I. cookei*, are potential vectors for zoonotic pathogens (Ebel, 2010; Wood et al., 2016), yet *I. scapularis* is currently the most medically important tick in Ontario

(Nelder et al., 2016). Advantages of a case-case design were that all dogs were identified through the same surveillance system, and all participants had an equal incentive (i.e., finding a tick on their dog) to contribute to the study (McCarthy and Giesecke, 1999; Pogreba-Brown et al., 2014). This resulted in an exceptional (92.6%) questionnaire response rate. The odds of *I. scapularis* carriage were significantly greater along the north shore of Lake Ontario, a known high risk area (Public Health Ontario, 2016), relative to the most northern region of the study area (approximately 60 km away). The spatial distribution of the *I. scapularis* collected from dogs were further explored in Chapter 3. The odds of *I. scapularis* carriage relative to other tick species were greatest in the spring and fall relative to the summer, which fits well with the known seasonal biology of adult *I. scapularis* in Ontario (Lindsay et al., 1999). Furthermore, in this population of dogs parasitized by ticks, female dogs and dogs weighing over 30kg had a significantly greater odds of *I. scapularis* carriage relative to male dogs and medium/small sized dogs, respectively. The only lifestyle habit that was significantly associated with a reduced risk of *I. scapularis* carriage was visiting a farm within the week prior to tick removal, which is plausible since the habitat preference for *I. scapularis* is forested areas (Lindsay et al., 1998; Ogden et al., 2006a). Previous studies using a case-control study design have found a significant increase in tick infestation for younger, male, sexually intact dogs, as well as gundogs, terriers and pastoral breeds (Raghavan et al., 2007; Smith et al., 2011). In contrast, dogs with short hair and toy breeds were at a significantly reduced risk for tick infestation (Raghavan et al., 2007;

Smith et al., 2011). Our results illustrate that the epidemiology of tick parasitism can vary between tick species, and that care should be taken in generalizing results.

The prevalence of *B. burgdorferi* and *Anaplasma phagocytophilum* in this study (Chapter 2) are similar to the prevalence of these pathogens found in other passive surveillance studies in Ontario and elsewhere in Canada (Dibernardo et al., 2014; Nelder et al., 2014). The small sample size of dogs carrying *B. burgdorferi*-positive *I. scapularis* did not allow sufficient statistical power to investigate multivariable models specific to *B. burgdorferi* carriage, but univariable analyses provided some important factors for veterinarians to consider when making recommendations for tick bite and Lyme disease prevention for dogs. For the analyses, the collection of travel information was important due to the occurrence of focal areas of *B. burgdorferi*-positive ticks in Ontario (Public Health Ontario, 2016). Despite not differentiating between travel to a high-risk versus low-risk Lyme disease area, dogs with a history of travel in the 14 days prior to tick removal had a significantly increased odds of *B. burgdorferi*-positive tick carriage relative to dogs that did not travel. This finding may be unique to this region of Ontario because of its proximity to known areas for Lyme disease risk. Nonetheless, a tick prevention product effective for *I. scapularis* should be recommended for dogs that travel in southeastern Ontario. Relative to older dogs, puppies under 1 year of age were at increased odds of carriage of *B. burgdorferi*-positive *I. scapularis*. In addition, dogs weighing less than 10 kg had increased odds of *B. burgdorferi*-positive *I. scapularis* carriage relative to medium and large dogs. These results support the importance of tick prevention in young dogs and those under 10 kg, who may be presumed to have a “low

risk" (e.g., assumed to not frequent tick habitat) for *I. scapularis* and *B. burgdorferi* exposure. A larger sample size would allow for a multivariable model to investigate potential issues of confounding between the breed, history of travel, weight and age of the dog and carriage of *B. burgdorferi*-positive ticks. The increased odds of *B. burgdorferi*-positive tick carriage in younger and smaller dogs may also reflect a difference in the ability of the PCR test to identify *B. burgdorferi* in ticks removed from older and heavier dogs, who may have been previously vaccinated with a Lyme vaccine, or previously exposed to *B. burgdorferi*. Research has shown that *B. burgdorferi* can be eliminated from vector ticks when they feed on outer surface protein A (OspA) and OspC immunized hosts (Fikrig et al., 1992; Lafleur et al., 2009). Information on the history of Lyme vaccination and historical results of *B. burgdorferi* testing were not collected on our questionnaire. Although this information was collected retrospectively, it is incomplete and has not been investigated. Further investigation into the influence of Lyme vaccine on the prevalence of *B. burgdorferi* found on PCR testing of *I. scapularis* removed from companion dogs is warranted.

4.2 IMPLICATIONS OF RESEARCH FOR TICK AND TICK-BORNE DISEASE SURVEILLANCE

Spatial analyses using Bernoulli, Poisson and space-time permutation models identified significant clustering in space, time and space-time for the carriage of *I. scapularis* and *I. scapularis* positive for *B. burgdorferi* in companion dogs from southeastern Ontario (Chapter 3). Most significant high-incident clusters identified in our study were within the risk area noted by Public Health Ontario, and are congruent with

the significant clusters identified by active tick surveillance in Ontario (Public Health Ontario, 2016; Clow et al., 2016). The use of a space-time permutation model identified a space-time cluster in Bancroft, Ontario, an area that is not currently a "hot spot" for *I. scapularis*. The space-time permutation model can be biased by changes in the background population (i.e., population shift bias) (Kulldorff and Hjalmars, 1999; Kulldorff et al., 2005), and further investigation is warranted. Our results support the usefulness of companion dogs as sentinels for the identification of epidemiologically plausible clusters of *I. scapularis* surveillance in Ontario. The accuracy of the spatial scan statistic for spatial analyses, concerning tick parasitism, needs to be assessed over time, but it may require less effort and financial resources relative to active surveillance.

Domestic animals share the same environment as their owners, and they are an excellent source for ticks (Bouchard et al., 2015). However, passive tick surveillance programs have sometimes excluded specimens from companion animals due to the overwhelming numbers of ticks (Nelder et al., 2014). As a result the objectives of passive tick surveillance in some jurisdictions are not amenable to the inclusion of ticks from all host species.

The use of veterinary practices for spatial analyses could provide a finer scale for geographic referencing compared to county or public health unit location, while maintaining de-identified data to assure owner privacy. Several considerations are warranted before establishing an operationally useful system for tick surveillance in sentinel veterinary clinics. Firstly, veterinary hospitals occasionally operate in a harried environment, and even though best efforts to complete research projects are put forward,

at times there is not enough staff support to gather all the data required. As such, all ticks recovered may not be submitted; for accurate surveillance and spatial analyses, the distinction between "no ticks recovered" and "no ticks submitted" is important. In this research project, we aimed to minimize the time and input required by veterinary staff for quality data collection, but substantial time was spent collecting missing information. However, our findings demonstrate that minimal data are required for successful spatial analyses to monitor changes in tick species and tick-borne pathogen prevalence (e.g., clinic and owner home postal code, host species, travel history). Secondly, animal health record management systems vary between veterinary clinics; the collection of a denominator for analyses (e.g., number of canine vaccines, appointments, or transactions) could be generated automatically by specifically programming the individual veterinary software to collect this information. Lastly, some veterinary clinics offer to send ticks away to be identified and PCR tested at a private reference laboratory. An effort to account for, or incorporate, this additional tick testing in sentinel surveillance should be considered.

4.3 LIMITATIONS

Our study involved dogs that frequented a veterinary hospital and therefore by design excluded dogs that do not access veterinary care. However, this was the only practical and economical way to enrol companion dogs into the study, and this sampling method has been used in other investigations (Burroughs et al., 2016). Veterinary clinics were chosen by convenience, and while a random selection of veterinary clinics would have been preferable, this was not feasible due to the limited number of veterinary

practices in the three study areas of interest. The human population density is greatest along Lake Ontario. Thus, in an effort to obtain comparable sample sizes between the three study regions, the proportion of total veterinary clinics participating varied: 18.1%, 40.0% and 85.7% for the Lakeshore, Middle and Northern regions, respectively (Chapter 2). Increasing the scale of the study across Ontario would allow for a better understanding of the regional risks for tick exposure, however special attention to study design is required to ensure the collection of high quality data.

A limitation of the case-case study design is that risk factors for tick carriage may be identical between the different tick species, and we may have failed to identify other significant factors that are the same between comparison groups (McCarthy and Giesecke, 1999; Pogreba-Brown et al., 2014). A drawback of the two-page questionnaire was that a large number of risk factors were examined which may have increased the odds of a Type I statistical error (Dohoo et al., 2009). The questionnaire also contained some open-ended questions (e.g., reason for veterinary visit, location of travel, how often do you check your dog for ticks) which were difficult to code for analyses (Thrusfield, 2007). In addition, very few risk factors examined were significant. Consequently, we recommend the use of a short questionnaire with few open ended questions for surveillance purposes.

4.4 FUTURE CONSIDERATIONS

Thirty-five dogs on this study were carrying at least one *B. burgdorferi*-positive *I. scapularis,* and following up with these animals may provide some clinical evidence for canine Lyme disease in Ontario. This study may serve as the basis for hypothesis

generation and sample size calculations for future studies on the incidence of canine Lyme disease in Ontario. In this study, ticks were only collected from April to December 2015. Future studies could consider tick collection throughout the winter. Few ticks are likely to be recovered in the winter months, yet climate change is expected to extend the seasonal occurrence of *I. scapularis* in Ontario (Ogden et al., 2006b; Ogden and Lindsay, 2016). Investigating how the significant spatial, temporal and space-time clusters identified for *I. scapularis* and *B. burgdorferi*-positive tick carriage compare to human Lyme disease incidence data, and establishing how these clusters change over time are essential components to evaluating the use of companion animals in sentinel tick surveillance.

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APPENDIX A

UNIVERSITY of GUELPH

April 6, 2015

Dear Participant:

Thank you for your interest in this study investigating ticks on pet dogs in Ontario. I am a Masters student in the Department of Pathobiology at the University of Guelph, and my study is being funded by a grant from the Ontario Veterinary College Pet Trust Fund.

In the past decade there has been a great deal of media attention and a heightened awareness about Lyme disease and black-legged ticks in Southern Ontario. The goal of our study is to document which species of ticks are attaching to pets, and determine if the exposure to ticks varies with location, time of the year, and the lifestyle of dogs. To answer our research question we need help from pet owners who have never seen a tick as well as people who have removed tick(s) from their dog. By taking 5 or 10 minutes to fill out a short questionnaire you can help us determine the risk factors associated with tick carriage in dogs.

If you find a tick on your dog, we can send it to the National Microbiology Laboratory in Winnipeg for tick identification and pathogen testing. It will take a few weeks for the results to come in, and your veterinarian will contact you when he or she receives the information.

In some veterinary clinics, the blood test for heartworm disease also tests for various tick borne diseases (e.g., the SNAP® *4Dx*® Plus test from Idexx). If your clinic uses one of these tests, we would like your permission to look at your dog's medical record to document the result of the most recent tests. We would also like to see if your dog has ever been treated for Lyme disease. Your signature is required on the survey to access this information. You may complete the survey and decline access to your dog's medical record.

You will not get any personal benefit from participating in this study, but your participation may help us understand the risk factors associated with tick carriage in dogs. There are no foreseeable risks of harm in completing this survey. This study will also provide information regarding the tick species and tick-borne diseases that pets encounter in Ontario.

Participation in our study is voluntary and your answers will in no way affect your relationship with your veterinarian. You do not have to answer any questions you are uncomfortable answering. All personal information will be removed before the research

results are published. You may withdraw from our study at any time without penalty by contacting Christine James. By completing and returning the questionnaire, you indicate your voluntary consent. The faculty advisors and graduate student will be the only individuals who can access the information you provide.

If you consent to take part in this project, your name can be entered into a draw for a chance to win an iPad mini worth approximately $300.00. We hope to recruit 900 people, making the chance of winning the prize 1 in 900 – but this number is subject to change.

Once the study is completed, our results will be shared with your veterinary clinic. Please feel free to contact me, if you have any questions about this project.

Thank you for helping us determine the risks of tick attachment in Ontario pets.

Sincerely,

Christine James

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Please address any questions about ethical concerns and your rights as a research participant to: S. Auld, Director, Research Ethics reb@uoguelph.ca 519-824-4120 X56606

APPENDIX B

UNIVERSITY of GUELPH

University of Guelph 2015 Research Project:
A study of the risks of ticks on pet dogs in Ontario.

DATE

Day: _____ Month: _____ Year: _____

This questionnaire is designed for one dog. If you have more than one dog, please answer the questions with respect to the dog who had a tick removed today. If your dog did not have a tick today, please answer the questions with respect to your dog with the name that comes first in the alphabet.

ABOUT YOUR DOG

Dog's name:	Breed:	Weight: lb □ kg □
	Dog's Age: months □ years □	Male □ Female □ Neutered or Spayed Yes □ No □
Dog's Hair Length: Short □ Medium □ Long □ Other □ _____	Colour *(Please check all that apply)* : Black □ Yellow □ Brown □ White □ Grey □ Other □ _____	
Reason for veterinary appointment today:		

LIFESTYLE

When your dog goes for a walk is he or she on a leash? *Please check one*	a) Always - More than 80% of the time walked on a leash	□	
	b) Sometimes - 20% to 80% of the time walked on a leash	□	
	c) Rarely – Less than 20% of the time walked on the leash	□	
In an average week, how far does your dog walk? *Please check one*	a) Less than 1 km per week	□	Other □ *(Please specify)*
	b) From 1 to 5 km per week	□	
	c) More than 5 km per week	□	_____

In the last 7 days, where has your dog visited or spent time? *Please check all that apply*

Inside my house □	Dog park □	Country Road □	Forest □	Other □ *(Please specify)*
In my yard □	Public Park □	Sidewalk □	Field □	
In a boarding kennel □	Provincial Park □	Beach □	Farm □	_____

On an average day, how many hours does your dog spend outside during the following seasons:

	Less than 1 hour	1-3 hours	More than 3 hours
Spring (Mar, Apr and May)	□	□	□
Summer (Jun, Jul, and Aug)	□	□	□
Fall (Sept, Oct, and Nov)	□	□	□

Do you go camping with your dog? YES □ NO □

In the past 14 days have you travelled with your dog? YES □ NO □

If you answered "yes" to travel with your pet in the past 14 days please specify where you traveled:

Name of City/Provincial or National Park etc._____ Province/State_____

TICK EXPOSURE

Have you ever found a tick in your house?	YES □	NO □	I don't know □
Would you be able to recognize a tick on your pet?	YES □	NO □	I don't know □
Have you removed a tick from yourself or a family member while in Ontario?	YES □	NO □	I don't know □
Has the dog for this questionnaire ever had a tick?	YES □	NO □	
Have you found a tick on this dog in the past 14 days?	YES □	NO □	

Over →

If a tick was found on your dog today, please answer the following questions:

Number of ticks removed today: _____

Do you know where your dog picked up the tick(s)? Yes □ No □ *(If yes please specify)* _____

Where on your dog's body were the ticks removed today: *Please check all that apply.*

Head □	Chest □	Feet □	Left Side □
Ears □	Abdomen □	Front Legs □	Right Side □
Neck □	Back □	Back Legs □	Tail □

TICK PROTECTION

Do you avoid taking your dog to certain places if ticks are present?	YES □	Occasionally □	NO □
Do you regularly check your dog for ticks?	YES □	Occasionally □	NO □
If "yes", how often do you check your dog for ticks? *(Please specify)* _____			
Do you use tick prevention products for your dog?	YES □	Occasionally □	NO □

If you do use a tick prevention product for your dog, please answer the following questions:

Where do you get your tick prevention product for your dog?

Veterinarian □	Hardware Store □	On-line product □
Pet Store □	Grocery Store □	Other □ *(Please specify)* _____

What type of tick prevention product do you use for your dog?

Preventative Collar □	Oral tablet □	Other □ *(Please specify)*
Topical Medication □	Spray □	_____

If you are using a Veterinary tick prevention product which product are you currently using:

Revolution® (topical) □	Preventic® Tick collar □	Bravecto™ (chewable tablet) □
K9 Advantix II® (topical) □	Seresto® Flea/Tick collar □	NexGard™ (chewable tablet) □
Other □ *(Please specify)* _____		

When was the last time this product was administered or applied? Date if known: _____

Within the last 30 days □ More than one month ago □ More than six months ago □

ABOUT YOU

Last Name:	Gender: Male □ Female □	Your Age: 18-25 □ 26-35 □ 36-45 □ 46-55 □ 55-65 □ 65+ years □
City:	Is your primary residence in a: Village □ Town □ Rural area □ Farm □	
Postal Code:	Suburb □ City □ Other □ _____	
Number of Dogs in the home: _____	Number of Cats in the home: _____	Number of Children in the home under 12 years of age: _____

Medical Record Access: In some veterinary clinics, the blood test for heartworm disease also tests for various tick borne diseases (e.g., the SNAP® *4Dx®* Plus test from Idexx). If your clinic uses one of these tests, we would like your permission to look at your dog's medical record to document the result of the most recent tests. We are also interested to see if your dog has ever been treated for Lyme disease. **I will allow access to my dog's medical record: YES □ NO □**

Name (please print): _____ **Signature:** _____

iPad Mini Draw: If you are interested, your name will be entered into a draw for a chance to win a new iPad mini.

Prize will be drawn in December 2015. ***Enter my name into the draw for a chance to win an iPad mini: yes □ no □***

Please enter your contact information. You will only be contacted if you win the draw.

Telephone number: _____

or E-mail address: _____

Please insert your completed questionnaire into the available envelope.

Thank you for taking the time to fill out our questionnaire about your dog!

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