

# *Ixodes scapularis* Ticks Collected by Passive Surveillance in Canada: Analysis of Geographic Distribution and Infection with Lyme Borreliosis Agent *Borrelia burgdorferi*

N. H. OGDEN,<sup>1,2,3</sup> L. TRUDEL,<sup>4</sup> H. ARTSOB,<sup>5</sup> I. K. BARKER,<sup>6</sup> G. BEAUCHAMP,<sup>1</sup> D. F. CHARRON,<sup>2</sup> M. A. DREBOT,<sup>5</sup> T. D. GALLOWAY,<sup>7</sup> R. O'HANDLEY,<sup>8</sup> R. A. THOMPSON,<sup>1</sup> AND L. R. LINDSAY<sup>5</sup>

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**ABSTRACT** Passive surveillance for the occurrence of the tick *Ixodes scapularis* Say (1821) and their infection with the Lyme borreliosis spirochaetes *Borrelia burgdorferi* s.l. has taken place in Canada since early 1990. Ticks have been submitted from members of the public, veterinarians, and medical practitioners to provincial, federal, and university laboratories for identification, and the data have been collated and *B. burgdorferi* detected at the National Microbiology Laboratory. The locations of collection of 2,319 submitted *I. scapularis* were mapped, and we investigated potential risk factors for *I. scapularis* occurrence (in Québec as a case study) by using regression analysis and spatial statistics. Ticks were submitted from all provinces east of Alberta, most from areas where resident *I. scapularis* populations are unknown. Most were adult ticks and were collected in spring and autumn. In southern Québec, risk factors for tick occurrence were lower latitude and remote-sensed indices for land cover with woodland. *B. burgdorferi* infection, identified by conventional and molecular methods, was detected in 12.5% of 1,816 ticks, including 10.1% of the 256 ticks that were collected from humans and tested. Our study suggests that *B. burgdorferi*-infected *I. scapularis* can be found over a wide geographic range in Canada, although most may be adventitious ticks carried from endemic areas in the United States and Canada by migrating birds. The risk of Lyme borreliosis in Canada may therefore be mostly low but more geographically widespread than previously suspected.

**KEY WORDS** *Ixodes scapularis*, Lyme borreliosis, *Borrelia burgdorferi*, spatial distribution, infection prevalence

*Ixodes scapularis* Say (1821) is the tick vector of several pathogens capable of causing zoonoses of public health importance, including Lyme borreliosis, anaplasmosis (formerly human granulocytic ehrlichiosis), and babesiosis (Thompson et al. 2001). Its geographic range extends from Texas in southern United States to eastern Canada (Dennis et al. 1998, Ogden et al. 2005). The highest densities of this tick occur in the northeastern and north central states of the United States that border Canada. The distribution of *I. scapularis* in Canada is thought to be limited to isolated populations on the northern shores of Lake Ontario and Lake Erie,

and the southeastern coast of Nova Scotia (Ogden et al. 2005). Increasing temperatures anticipated in southeastern Canada because of climate change are likely to increase the survival of *I. scapularis* and may permit the range of *I. scapularis* and the pathogens it transmits to expand northward (Ogden et al. 2006). Ixodid ticks have very limited capacity to move by themselves from the location they drop off their hosts (Falco and Fish 1991) and to move into new territory, the ticks must be carried on their hosts. Birds migrating northward in spring can carry *I. scapularis* long distances into Canada (Scott et al. 2001), presumably having acquired the ticks in northeastern and north central United States or in the few Canadian localities supporting endemic populations (Smith et al. 1996).

In this study, we have collated and analyzed data on *I. scapularis* ticks found in Canada by veterinarians, medical practitioners, wildlife specialists, and the general public, which were submitted to various organizations for identification and analysis for the agent of Lyme borreliosis, *Borrelia burgdorferi* s.l. Our objectives were to identify risk factors, including geographic range, for the occurrence of *I. scapularis* and *B. burgdorferi* in Canada to assist decision-making by health professionals in diagnosis and prevention and to obtain baseline information to help document pos-

<sup>1</sup> Groupe de Recherche en Épidémiologie des Zoonoses et Santé Publique, Université de Montréal, Québec, Canada J2S 7C6.

<sup>2</sup> Division of Enteric, Food and Waterborne Diseases, Public Health Agency of Canada, Ontario, Canada K1A 0L2.

<sup>3</sup> Corresponding author, e-mail: nicholas.ogden@umontreal.ca.

<sup>4</sup> Laboratoire de santé publique du Québec, Institut national de santé publique du Québec, Québec, Canada H9X 3R5.

<sup>5</sup> National Microbiology Laboratory, Zoonotic Diseases and Special Pathogens Section, Public Health Agency of Canada, Manitoba, Canada R3E 3R2.

<sup>6</sup> Canadian Cooperative Wildlife Health Centre, Department of Pathobiology, University of Guelph, Ontario, Canada N1G 2W1.

<sup>7</sup> Department of Entomology, University of Manitoba, Manitoba, Canada R3T 2N2.

<sup>8</sup> Department of Pathology and Microbiology, Atlantic Veterinary College, Prince Edward Island, Canada C1A 4P3.

sible future changes in the distribution of the tick in Canada.

### Materials and Methods

**Collection of *I. scapularis* in Canada, 1990–2003.** Passive surveillance for *I. scapularis* began in Canada in the early 1990s driven by postal, telephone, and media solicitation from interested provincial and federal public health scientists. Ticks found by the public, usually as feeding stages attached to themselves or their pets, have been submitted to provincial and federal health organizations, and university researchers, in Canada directly by the public or via physicians and veterinarians, from all provinces east of Alberta. Those submitting ticks were asked to record the name of the location where the tick was found or submitted, the host species on which the tick was found, the date of collection, and whether the person or pet on which the tick was found had traveled out of Canada during the period the tick may have attached. In Québec, those submitting ticks also were asked to indicate whether the person or pet on which the tick was found had traveled outside of the census subdivision (CSD) in which they resided, even if they had not traveled outside the province.

The tick species were identified at university and provincial health laboratories or at the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC), where stage and state of engorgement of the ticks, and data collected with the submitted ticks were recorded. The state of engorgement of the ticks was coded as 0 (unfed, or no evidence of a bloodmeal), 1 (partially engorged), or 2 (appearance of full engorgement). Many ticks found by the public in Ontario are submitted to the Lyme Disease Association of Ontario, but data on ticks submitted to them during the period 1993–1999 were made available to us for this study.

Summary statistics of the numbers, sources, hosts, sex, stage, and collection dates of submitted *I. scapularis* were collated. Data on ticks submitted by members of the public who had a history of travel out of their province of residence during the likely engorgement period of feeding ticks were not included in our analyses.

Locations where the ticks were found were mapped in Arcview GIS version 3.2 (Environmental Systems Research Institute, Redlands, CA). The location for each tick was assigned to the centroid of the CSD where the tick was found because CSD was the finest common scale of resolution for the locations of some of the submitted ticks. The shape file of CSD boundaries used for mapping was obtained from the Statistics Canada Web site (<http://www.statcan.ca:8096/bsolc/english/bsolc?catno=92F0162X>).

**Detection of *B. burgdorferi* Infection in Submitted Ticks.** *I. scapularis* collected were tested for *B. burgdorferi* infection for surveillance purposes and to provide medical and veterinary practitioners with information to assist in deciding whether to treat potentially exposed individuals or their pets with antibiotics. Detection techniques undertaken in this

study changed over the course of the 13 yr of tick collections to keep pace with current technology. Before 1998, detection was by immunofluorescent antibody (IFA) staining of tick gut contents, culture in BSK-H medium followed by IFA staining of isolates with specific monoclonal antibodies to *B. burgdorferi* (Artsob et al. 1992), or a combination. From January 1998, one-step polymerase chain reaction (PCR), nested PCR, and then real-time PCR were used. Ticks were stored at  $-80^{\circ}\text{C}$  after identification and before DNA extraction unless they were submitted in 70% ethanol, in which case they were stored at  $4^{\circ}\text{C}$ . Here, we describe the DNA extraction and PCR amplification methodologies used in the detection of *B. burgdorferi* in the ticks. Reactions containing positive control DNA from known positive ticks and multiple water-only negative controls were included in all PCR runs.

**Extraction.** DNA was extracted from ticks using either DNAzol (Molecular Research Center, Cincinnati, OH) or QIAmp DNA mini kit or DNeasy 96 tissue kit (QIAGEN, Mississauga, Ontario, Canada). When QIAGEN kits were used, the protocol was optimized for digestion of tick exoskeleton and recovery of low-copy number DNA by incubating ticks in lysis buffer containing proteinase K for 90 min (mini kit) or overnight (tissue kit) at  $56^{\circ}\text{C}$ . DNA extraction efficiency was assessed using primers specific for the tick 16S rRNA gene (MM1 and MM2 primers; Table 1).

**Primers and Probes.** Primers for standard and real-time PCR were synthesized in-house at the NML DNA Core Facility using Beckman Oligo Synthesizers (Beckman Coulter Canada, Inc., Mississauga, Ontario, Canada). Dual-labeled TaqMan fluorogenic probes were synthesized by Applied Biosystems (Foster City, CA). The fluorescent dyes incorporated at the 5' and at the 3' ends of the probe were 6-carboxyfluorescein (FAM; reporter) and 6-carboxytetramethylrhodamine (TAMRA; quencher), respectively. Concentrated reaction mixture containing unlabeled primers and TaqMan MGB probe (FAM dye labeled) for real-time detection of the *Osp A* gene was provided by Applied Biosystems Assays-by-Design service for gene expression assays. Sequences for all primers and probes are provided in Table 1.

**Amplification of DNA by Standard PCR.** PCR reactions comprised  $5\ \mu\text{l}$  of DNA template and  $95\ \mu\text{l}$  of Mastermix, which contained 0.2 mM of each dNTP (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom),  $0.5\ \mu\text{M}$  forward and reverse primers, 5 U of AmpliTaq polymerase (Applied Biosystems), and 1.5 mM  $\text{MgCl}_2$  (Applied Biosystems 10 $\times$  buffer). Basic thermocycler conditions used were 4-min denaturation at  $94^{\circ}\text{C}$ , 35 cycles of 1-min denaturation at  $94^{\circ}\text{C}$ , 1-min annealing at  $\approx 55^{\circ}\text{C}$ , and 1-min extension at  $72^{\circ}\text{C}$ , followed by a final 10-min extension phase at  $72^{\circ}\text{C}$  (references for primer-specific annealing conditions are in Table 1). Amplification products were visualized by electrophoresis in ethidium bromide-stained 2% agarose gels.

**Amplification of DNA by TaqMan PCR Assays.** Reaction mixtures of primers targeting the flagellin gene

Table 1. Primer and probe sequences used to assess DNA extraction and to detect *B. burgdorferi*

Primer name	Sequence	Reference
MM1 <sup>a</sup>	actgtggtcggatgtgcct	Designed in-house
MM2 <sup>a</sup>	gcacatcccatgccatctcgaaagcg	Designed in-house
1F	gcattaacgctgctaate	Wise and Weaver (1991)
2F	ttgcaggctgcattccaa	Wise and Weaver (1991)
OspA2	gttttgtaatttcaactgctgacc	Persing et al. (1990)
OspA4	ctgcagcttggaaatcaggcacttc	Persing et al. (1990)
fla outer1	aagtagaaaaagtcttagtaagaatgaagga	Johnson et al. (1992)
fla outer 2	aattgcatactcagctactattcttagat	Johnson et al. (1992)
fla inner 1	cacatattcagatgcagacagaggttcta	Johnson et al. (1992)
fla inner 2	gaaggtgctgtagcaggctgctgctgt	Johnson et al. (1992)
OspA 1b (outer)	gtagcagcctgcagcaga	Persing et al. (1990)
OspA 4b (outer)	gatactagtggtttgccatc	Persing et al. (1990)
OspA 2b (inner)	gcgtttcagtagattgcctg	Persing et al. (1990)
OspA 3b (inner)	tcaagtgtggtttgacatag	Persing et al. (1990)
5' F	ttgtgacagagtgtatgataatggaa	Zeidner et al. (2001)
5' R	actcctccggaagccacaa	Zeidner et al. (2001)
5' probe	tgctaaaaactgaggagattgt	Zeidner et al. (2001)
FlaF1A	agcaaataggcttccaa	Schwaiger et al. (2001)
FlaR1	gcaatcattgccattgcaga	Schwaiger et al. (2001)
Fla probe	tgctacaacctcatctgtctatgtagcatctttatttg	Schwaiger et al. (2001)
OspA F	cttggaaatcaggeactcaacttt	Designed in-house
OspA R	tgagtctgattgttactgtaattgtgt	Designed in-house
OspA probe	cacaaggtcttagttttttac	Designed in-house

<sup>a</sup> These primers target the 16S rRNA gene of ticks of the genus *Ixodes* and were used in control on DNA extractions.

were prepared in 2x×TaqMan Universal Mastermix (Applied Biosystems) to contain 300–600 nM primers and 200 nM probe. Primers directed at *B. burgdorferi* 23S rRNA genes also were diluted in 2× Universal Mastermix with a concentration adjusted to 700 nM for primers and 150 nM for probes. Detection of a sequence of the Osp A gene was performed using Assays-by-Design 20× mixture of primers and probe (Applied Biosystems) also diluted in the aforementioned buffer to yield 900 nM primers and 250 nM probe. Amplification was carried out on either an ABI Prism 7700 sequence detection system or ABI 7900HT by using 96-well optical plates. After incubation at 50°C for 2 min (to activate the enzyme AmpErase) and 10 min at 95°C (to denature AmpErase and activate AmpliTaq Gold Polymerase), the samples were subjected to amplification (95°C for 15 s, 60°C for 1 min, 40 cycles). After amplification and real-time data acquisition, analysis was performed using the Sequence Detection System software (Applied Biosystems).

**Evolution of Diagnostic Algorithm.** Before 1998, all ticks were tested by IFA staining of ticks gut contents, isolation in BSK-H medium (assay 1 in statistical analyses), or a combination. From January 1998, *B. burgdorferi* was detected by IFA, culture, and PCR with 1 F and 2 F primers targeting the flagellin gene (Wise and Weaver 1991) and OspA2 and OspA4 primers targeting the Osp A gene (Persing et al. 1990a) (assay 2 in statistical analyses).

From January 1999 to October 2003, a nested PCR reaction for the flagellin gene was used (Johnson et al. 1992) for ticks submitted from humans to increase sensitivity. In this PCR, the first round of amplification used external primers fla outer 1 and 2, and 2 μl of the first stage product was added to 98 μl of Mastermix containing internal primers fla inner 1 and 2 and re-amplified (assay 3 in statistical analyses). Because of

inconsistent results with the flagellin screening primers 1 F and 2 F, they were replaced with internal nested primers fla inner 1 and 2 in October 2001 to screen extracts from ticks off nonhuman hosts. Again, any positive samples were confirmed with OspA2 and OspA4 (assay 4 in statistical analyses).

In October 2003, a nested PCR reaction targeting the Osp A gene (Persing et al. 1990b) was introduced for confirmation of nested flagellin positive samples (assay 5 in statistical analyses).

Real-time PCR using a dual-labeled probe was introduced in November 2003. This test was designed for use when >20 samples were being processed. DNA extracts were screened with primers targeting the 5' end of the flagellin gene (Zeidner et al. 2001), and confirmation of positives was performed with primers FlaF1A and FlaR1 targeting the 3' end of the flagellin gene (Schwaiger et al. 2001) (assay 6 in statistical analyses).

**Statistical and Spatial Analyses.** *Environmental Risk Factors for I. scapularis Occurrence and Spatial Analysis.* Only data from Québec were used in these analyses to minimize misclassification errors: data from this province permitted selection of ticks that more certainly attached to people or animals in their CSD of residence. No resident *I. scapularis* populations are known in the province, and no multiple submissions from one location have occurred, which could signal the occurrence of a resident population. Furthermore, in the different provinces, tick surveillance began in different years, methods and routes of tick and data collection differed, and wide variations in the geographic size of CSDs in some provinces affected spatial analyses. All of these could contribute to misclassification errors.

To investigate potential risk factors for *I. scapularis* occurrence, the number of *I. scapularis* ticks per CSD

was the outcome variable in regression analyses in SAS (version 8.2, SAS Institute, Cary, NC). A negative binomial regression model was used (dictated by regression diagnostics) in which the natural logarithm of the human population per CSD in 2001 ("Ln population") was included as an offset to account for the almost linear relationship of this variable with the numbers of tick submissions. Mean, maximum, minimum, and standard deviation of normalized difference vegetation index (NDVI) for each CSD and CSD latitude and longitude were included as explanatory variables. Data on CSD populations and locations were obtained from the Statistics Canada Web site (<http://www.statcan.ca/bsolc/english/bsolc?catno=95F0495X2001002>). NDVI data were obtained from four cloud-free orthorectified Landsat ETM+ images downloaded from the Geogratis Web site of Environment Canada (<http://geogratis.cgdi.gc.ca/>). These images covered much of southern Québec province, including Montérégie (row 14, tracks 28 and 29), Estrie (row 13, track 29), and Outaouais (row 16, track 29) regions. These images were all captured in June 2001, except the image for Outaouais, which was captured in late August 1999. The data were extracted from the images using ENVI (version 4, Research Systems, Inc., Boulder, CO) and then imported into Arcview GIS, in which the Spatial Analyst extension was used to compute mean, maximum, minimum, and standard deviation of NDVI values for 605 of the 1,476 polygons (excluding areas covering water bodies) outlined by the Québec CSD shape file. The image identification (ID) number was a factorized explanatory variable in the regression model. Correlation coefficients among mean, maximum, minimum, and standard deviation of NDVI values were low ( $<0.4$ ), so these could be included together in multivariable models without significant confounding. The most parsimonious model was obtained by backwards and forwards substitution and elimination of the explanatory variables. The level of statistical significance was  $P < 0.05$ .

To test the hypothesis that undiscovered reproducing *I. scapularis* populations exist in southern Québec, we investigated the potential for spatial clustering of submitted ticks, evidence of which may signal the locations of tick populations. The null hypothesis was that any observed clustering could be explained by the spatial dispersion of environmental risk factors for *I. scapularis* identified in the most parsimonious regression model described above. The potential for clustering in the raw data (locations of ticks reported) was investigated using Moran's *I* method (Moran 1950) and then Oden's *I*pop method (Oden 1995) to account for the human population in each CSD, in ClusterSeer (Terraseer, Crystal Lake, IL). We then investigated to what degree any observed clustering was explained by the factors in the multivariable regression model. To do so, we compared the degree of spatial clustering among residuals of similar magnitude and direction, obtained in the intercept-only regression (although with Ln population included as an offset), with the degree of clustering among similar

residuals obtained in the regression model that included all significant explanatory variables. For both models, Cuzick and Edwards test (Cuzick and Edwards 1990) in ClusterSeer was used to detect significant clustering of "cases" (CSDs for which regression model residuals were greater than 2 SDs above the predicted), among "controls" (CSDs with residuals less than 2 SDs above the predicted). The level of statistical significance was  $P < 0.05$ .

*Factors Influencing B. burgdorferi Infection Prevalence in Submitted Ticks.* The PCR result was the binary outcome in a logistic regression model in STATA/SE 8.0 for Windows (Stata Corporation, College Station, TX) in which the province of origin of the tick (coded 1–7), the sex and stage of the tick, the year the tick was collected, the cohort to which the tick belonged (coded 1–14), the state of engorgement of the tick, the test/algorithm used to determine the test result, and the host of origin were the explanatory variables. Adult ticks collected from September to December and from January to August of the subsequent year were considered to belong to the same cohort (Ogden et al. 2004). The test or algorithm of test results used to determine whether a sample was positive or negative were coded from 1 to 6. Detection by culture and IFA was coded 1, the most recent real-time PCR was coded 6, and the various one-step and nested PCRs used were coded 2–5, the latter being the last standard PCR before the adoption of real-time PCR. The hosts were coded as 1 for humans, 2 for dogs, 3 for cats, and 4 for ticks collected from wildlife. The level of statistical significance was  $P < 0.05$ .

## Results

**Summary Statistics and Geographical Distribution of Submitted *I. scapularis*.** Several tick species were submitted, but in this study we focused on *I. scapularis*. In total, 2,054 *I. scapularis* were submitted from January 1990 to December 2003. A further 265 were submitted to the Lyme Disease Association of Ontario during the period 1993–1999. Together, they comprised 2,233 adult females, 63 adult males, 22 nymphs, and one larva. The median number of ticks per submission was 1 and the maximum was 120 (a year's collection by one individual located within a resident *I. scapularis* population in Nova Scotia). In 37 cases, submissions contained more than one tick (multiple submissions), which were mostly all adult females, but males and females were submitted together in seven cases, all but two being from locations close to known resident populations in Ontario and Nova Scotia. Most ticks (1,725) were found on household pets (dogs or cats), although 323 were found on humans. These data are summarized in Table 2.

The numbers of ticks submitted from Ontario, Québec, and the Atlantic provinces (in years where data from both PHAC and the Lyme Disease Association of Ontario are available) increased year by year (Fig. 1). The annual numbers submitted from Manitoba increased until 2000 but declined in more recent years. Ticks were submitted in all months of the year, but

**Table 2. Numbers, stages, and sources of submitted ticks**

	Province								Total
	SK	MB	ON	QC	NB	NS	PEI	NF	
All ticks	5	355	302	1088	156	217	157	39	2,319
Stage									
Adult females	3	308	286	1081	151	208	157	39	2,233
Adult males	2	38	7	6	3	7	0	0	63
Nymphs	0	9	8	1	2	2	0	0	22
Larvae	0	0	1	0	0	0	0	0	1
Host/source									
Dogs	2	239	25	680	89	162	102	28	1,327
Cats	3	55	0	251	20	17	46	6	398
Humans	0	51	33	155	44	30	7	3	323
Other sources, including unknown sources	0	10	244	2	3	8	2	2	271
Submissions of multiple ticks									
No. of submissions	0	2	5	18	0	8	4	0	
Median ticks (range) per submissions		2 (2)	3 (2-6)	2 (2-6)		2 (2-120)	2 (2)		

MB, Manitoba; NB, New Brunswick; NF, Newfoundland; NS, Nova Scotia; ON, Ontario; PEI, Prince Edward Island; QC, Quebec; SK, Saskatchewan.

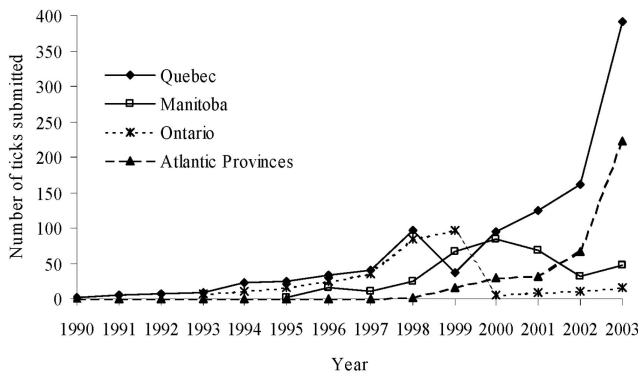
most adults were submitted in one of two discrete periods: April to July (38.2% of adult ticks) peaking in May or October to December (57.9% of adult ticks) peaking in October (Fig. 2). Nymphs were submitted from May to August with most being submitted in June and July (Fig. 2). The one larva was submitted from Ontario in September.

Ticks were submitted from all provinces from Saskatchewan in the west to Newfoundland in the east (Fig. 3). Almost all were submitted from CSDs within 200 miles of the U.S. border, corresponding with the regions of Canada with the highest human populations. Of the total 2,319 ticks submitted, 40 (excluding the 120 in one submission from Nova Scotia) were submitted from CSDs in which a resident *I. scapularis* population is known to occur (Fig. 3).

**Risk Factor and Spatial Analysis.** The Ln population was a highly significant explanatory variable in bivariable negative binomial regression analyses (coefficient = 1.043, SE = 0.053,  $P < 0.001$ ). In models in which the Ln population was an offset, average NDVI for the CSD was significantly associated with an increased number of ticks submitted from that CSD

(coefficient = 1.862, SE = 0.482,  $P < 0.001$ ), whereas the numbers of ticks were significantly less the further north was the CSD (coefficient = -1.604, SE = 0.181,  $P < 0.001$ ). Minimum, maximum, and standard deviation of the NDVI values in each CSD, and the ID number of the LandsatTM image from which NDVI data were calculated and were not significant ( $P > 0.1$  for all).

There was significant spatial clustering of the number of ticks submitted per CSD (Moran's I = 0.065,  $P < 0.05$ ) after accounting for human population (Ipopt statistic = 1.341,  $P < 0.001$ ). There was similar spatial clustering of the residuals in the model in which Ln population was an offset (Cuzick and Edwards statistical distance test statistic = 9.352,  $P = 0.001$ , Fig. 4a), but when mean NDVI values and latitude were included in the model, the clusters became indistinct and there was no significant spatial structure to the data (distance test statistic = 3.101,  $P > 0.1$ ; Fig. 4b). These findings supported the null hypothesis that clustering could be explained by the spatial disposition of environmental risk factors rather than providing evidence for discrete *I. scapularis* populations.



**Fig. 1.** Number of *I. scapularis* submitted from Québec, Manitoba, Ontario, and the combined Atlantic provinces (New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland and Labrador). The numbers of ticks submitted in Ontario from 1993 to 1999 include those submitted to the Lyme Disease Association of Ontario. The number of ticks submitted in the Atlantic provinces in 2003 excludes the 120 ticks in one submission from a person living close to the resident population in Nova Scotia.

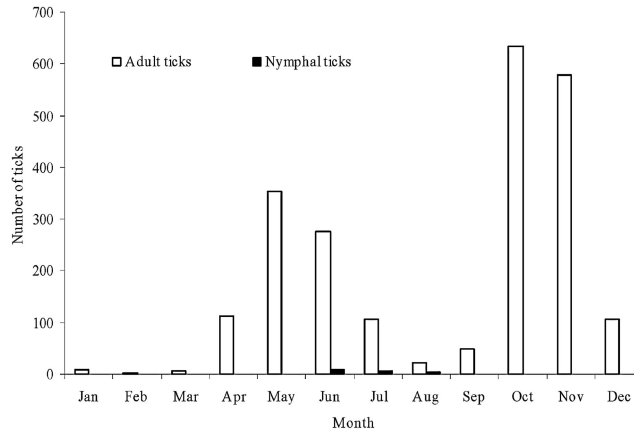


Fig. 2. Number of adult and nymphal *I. scapularis* ticks submitted from all provinces, by month of submission.

**Prevalence of *B. burgdorferi* Infection.** Of 1,816 ticks collected from humans and animals in Canada with no history of travel out of the country, 228 (12.5%) were considered positive for *B. burgdorferi* infection (Table 3). None of the explanatory variables tested were significantly associated with variations in

prevalence when investigated individually ( $P > 0.05$  for all) or in a multivariable model ( $\chi^2 = 35.83$ ,  $df = 32$  [after removal of confounding variables],  $P > 0.1$ ). Of the 256 ticks collected from humans and tested (after assessment of DNA extraction efficiency), 26 (10.1%) were positive for *B. burgdorferi*. Sixteen of

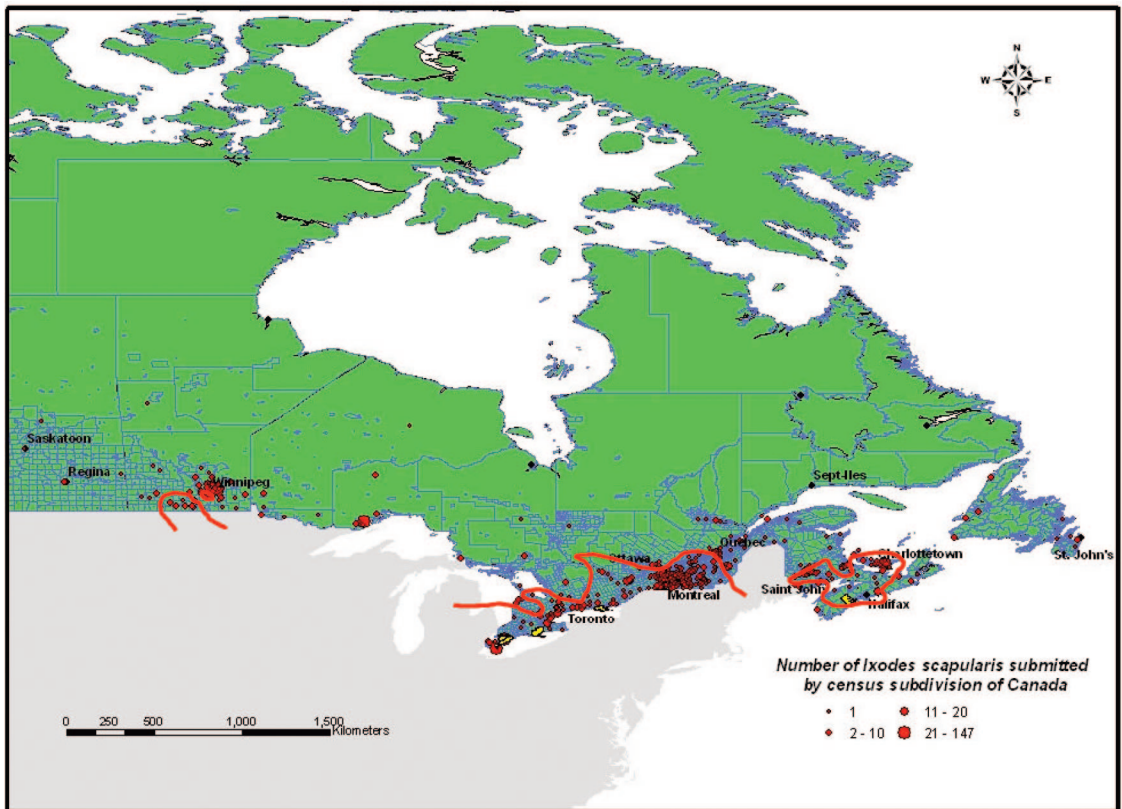
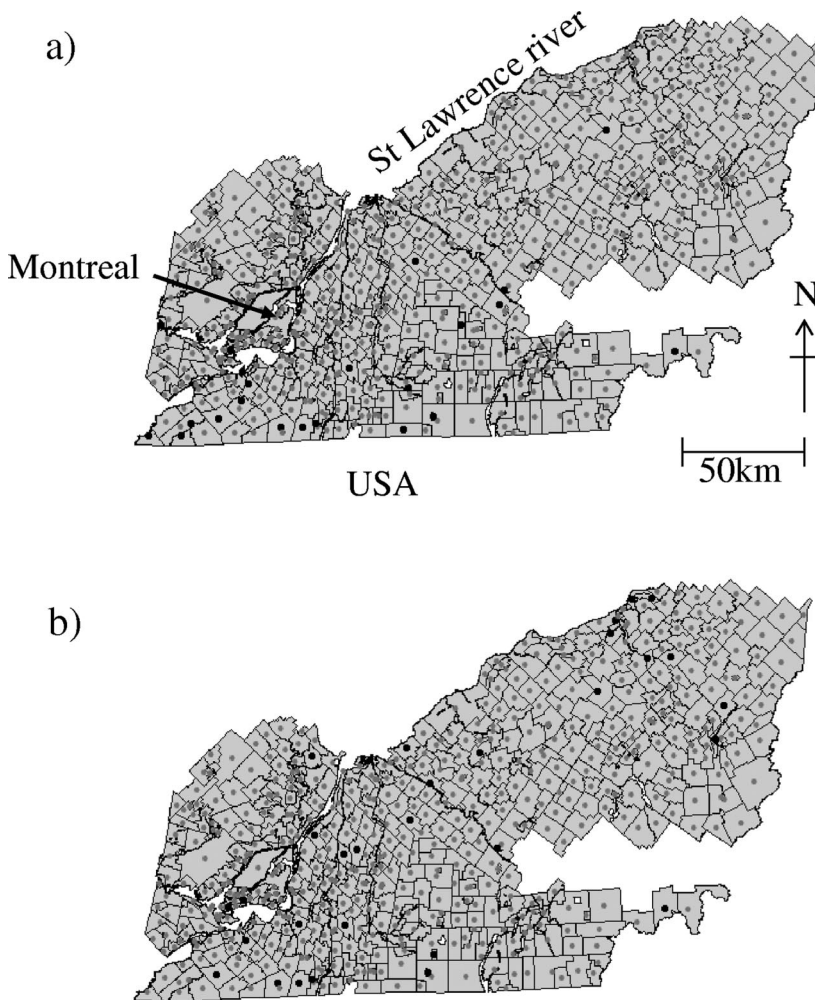


Fig. 3. Distribution of *I. scapularis* submitted from January 1990 to December 2003 (red circles that are centered on the centroid of the census district from which they were submitted). Only data from individuals that had no history of recent travel out of the census district are shown. Census districts in which resident *I. scapularis* populations are known to occur are highlighted in yellow. The boundaries of all census districts are shown as gray lines. These data include those obtained from the Lyme Disease Association of Ontario for 1993–1999. The red line shows approximate temperature limits for *I. scapularis* establishment, deduced in Ogden et al. 2005a.



**Fig. 4.** Location of the centroids of “test” (black dots) and “control” (gray dots) CSDs in southern Québec. In both figures, test CSDs were defined as those for which residuals were 2 SDs or more above the value predicted by a regression model, and control CSDs were less than 2 SDs above the regression line. (a) Residuals were obtained from the intercept-only regression model which included the natural log of the human population of the CSD as an offset. (b) Residuals were obtained from the multivariable model in which mean NDVI and latitude were explanatory variables. There was significant spatial clustering in a but not in b.

these positive ticks were unfed, but seven were partially engorged.

### Discussion

This study demonstrated a broad geographic distribution of *I. scapularis* ticks in Canada, which is in marked contrast to the small number of endemic populations known to exist here. Most ticks were found on pets, particularly dogs, but almost 14% were found on humans. Some of these were infected with *B. burgdorferi*. The number of ticks submitted in this passive surveillance study may only represent a small fraction of *I. scapularis* that are attaching to pets and possibly humans in Canada each year. These ticks may, therefore, represent an existing and probably underestimated challenge to veterinary and public health in

Canada. Passive surveillance in endemic regions in the United States have yielded considerable numbers of nymphal *I. scapularis* (Johnson et al. 2004), but few nymphs were submitted even from known endemic areas in this study, which may suggest a lower awareness of ticks in Canada, and a correspondingly lower likelihood of detecting immature ticks. In Québec, however, the tick species most frequently submitted in passive surveillance was *Ixodes cookei* (Packard), 65% of which were immature ticks (L.T., unpublished). Although the risk for contracting Lyme borreliosis would be much lower in areas where *I. scapularis* has not been shown to be endemic, these findings reinforce the need for clinician or veterinarian awareness of the possible occurrence of Lyme borreliosis in patients/pets who do not have a history of travel to known tick-endemic areas.

**Table 3. Prevalence of *B. burgdorferi* infection in submitted ticks by province, year of collection, test algorithm, tick sex and stage, host, and state of engorgement**

Factor	No. positive (%)	95% CI
<b>Province</b>		
Quebec	128/984 (13.0)	11.0–15.3
Manitoba	34/349 (9.7)	6.8–13.3
New Brunswick	24/151 (15.9)	10.5–22.7
Newfoundland	4/21 (19.0)	5.4–41.9
Nova Scotia	13/86 (15.1)	8.3–24.5
Ontario	5/45 (11.1)	3.7–24.0
Prince Edward Island	20/180 (11.1)	6.9–16.6
<b>Yr</b>		
1990–1996	8/69 (11.6)	8.2–26.7
1997	7/55 (12.7)	5.3–24.5
1998	18/144 (12.5)	7.6–19.0
1999	14/138 (10.1)	5.7–16.4
2000	34/216 (15.7)	11.1–21.3
2001	21/258 (8.1)	5.1–12.2
2002	39/283 (13.8)	9.7–18.0
2003	87/653 (13.3)	10.8–16.2
<b>Test algorithm<sup>a</sup></b>		
1	15/124 (12.1)	6.9–19.2
2	67/509 (13.2)	10.3–16.4
3	21/186 (11.3)	8.0–18.0
4	74/611 (12.1)	9.6–15.0
5	15/129 (11.6)	6.7–18.4
6	35/253 (13.8)	9.8–18.7
<b>Tick sex and stage<sup>a</sup></b>		
Adult female	220/1693 (13.0)	11.4–14.7
Adult male	5/63 (7.9)	2.6–17.6
Immature ticks	0/19 (0)	0–17.6
<b>Host<sup>a</sup></b>		
Human	26/256 (10.1)	5.1–12.2
Dog	140/1147 (12.2)	10.4–14.2
Cat	58/392 (14.8)	11.4–18.7
Wildlife	4/16 (25.0)	7.3–52.4
<b>State of engorgement<sup>a</sup></b>		
Unfed	20/216 (9.2)	5.7–13.9
Partially engorged	142/1086 (13.1)	11.1–15.2
Fully engorged	56/409 (13.7)	10.1–16.9
Total tested	228/1816 (12.5)	11.1–14.2

<sup>a</sup> Some data were missing. Test results from these ticks were not included in statistical analyses.

The number of ticks submitted from Québec and the Atlantic provinces, and possibly Ontario, increased during the study. This trend may have been associated with increased public awareness of *I. scapularis* and the pathogens it transmits. However, projected climate change may improve the survival of *I. scapularis* in Canada and northern states of the United States (Ogden et al. 2006), and the study coincided with a period in time that may have shown the first evidence of global warming (Root et al. 2003).

Attempts have been made to identify established populations in Ontario (Barker et al. 1992), Manitoba, and the Atlantic provinces. Multiple or repeated submissions of *I. scapularis* from the same locations have in many cases been followed up by intensive field studies (L.R.L., unpublished), but to date the number of locations known to contain reproducing populations of *I. scapularis* in Canada remains at seven (Fig. 3; Ogden et al. 2005). The criteria for identification of a reproducing population in a given location are the presence of all three stages of the tick (identified on their hosts or collected while questing) in at least two consecutive years, although to date all suspect popu-

lations identified in one year have yielded ticks in the following year. The geographic distribution of the submitted ticks was very much wider than that of identified reproducing populations (Fig. 3).

Many of the ticks were submitted from locations too cold for self-sustaining *I. scapularis* populations (i.e., too cold to support the whole life cycle, Ogden et al. 2005) but not too cold to prevent successful molting of one stage to the next (Lindsay et al. 1996). We infer that most of the submitted ticks were “adventitious ticks” derived from immature *I. scapularis* carried by migrating birds into and through Canada from tick populations resident in northeastern and north central states of the United States, and in southern Canada. Migratory bird-borne *I. scapularis* could explain the wide geographic occurrence of many of the submitted ticks for three reasons: 1) the timing of northward migration of ground feeding passerines is coincident with the seasonal activity of host-seeking immature *I. scapularis* (particularly nymphs) in the United States and in southern Canada (Smith et al. 1996, Lindsay et al. 1999b); 2) large numbers of migratory birds pass through southeastern and south central Canada each year (Smith et al. 1996); and 3) the direction and speed of spring migratory birds are sufficient to carry *I. scapularis* long distances into Canada (Klich et al. 1996, Scott et al. 2001).

The spatial distribution of *I. scapularis* in southern Québec may be consistent with this hypothesis. The geographic range for *I. scapularis* (adventitious or resident), in Québec and elsewhere in Canada, elucidated in this study is in part a reflection of the density of the human population finding and submitting the ticks. Accounting for this, tick submissions in the selected area of southern Québec were more numerous from CSDs that had higher mean NDVI values, an indication that the CSDs may have more land cover by woodland, as found in another study (Dister et al. 1997). This is consistent with woodland being a habitat suitable for survival of engorged nymphs during their development into adult ticks (Lindsay et al. 1998), and refueling stops for *I. scapularis*-carrying migrating passerine species that must already have made landfall in woodland habitats suitable for *I. scapularis* (Morris et al. 2003). Tick submissions were less numerous the farther north the CSDs were within the selected area of southern Québec (within which temperature conditions may be suitable for *I. scapularis* establishment; Ogden et al. 2005a). This could be consistent with 1) declining densities of migratory ground-feeding passerines with increasing latitude as the birds find breeding sites and 2) a declining likelihood that ticks remain attached to the birds the further the birds travel from any potential source tick populations in the United States. There was no spatial clustering that may signify dissemination of ticks from discrete resident, reproducing populations, over and above that of the environmental risk factors such as habitat. However, the occurrence of such populations cannot be ruled out by passive surveillance data alone. At present, we have no reason to suspect that the distribution of submitted ticks in southern Québec is in any way dif-

ferent from other regions of southeastern Canada where *I. scapularis* populations are currently unknown.

The prevalence of *B. burgdorferi* infection in the adult ticks was comparable with that observed in questing adult *I. scapularis* collected in Minnesota (16%; Layfield and Guilfoile 2002), higher than that observed in some studies in Michigan (6%; Walker et al. 1998) and Wisconsin (4.2%; Caporale et al. 2005), but lower than that observed in questing adults in selected areas of northeastern states (New Jersey, 43% [Varde et al. 1998] and 50% [Schulze et al. 2005]). Some migratory bird species may clear existing infections in immature ticks that feed on them (Rand et al. 1998), thereby reducing the overall prevalence of infection, but further studies are required to explain the observed prevalence and to understand the diversity and origins of *B. burgdorferi* infection in the ticks.

The seasonality in tick collections was similar to that of *I. scapularis* activity in northeastern and north central states of the United States, and in southern Canada (Yuval and Spielman 1990, Lindsay et al. 1999a). The risk of infection from adventitious ticks in Canada is therefore likely to be the early spring and autumn activity periods of adult ticks. Where reproducing *I. scapularis* populations exist, the spring-early summer activity period of nymphal ticks provides an additional high-risk period for human infection (Thompson et al. 2001).

This study indicates that there is a widespread risk of contracting Lyme borreliosis in Canada, extending eastward from Saskatchewan to Newfoundland. In addition, *I. scapularis* has been found in Alberta (Scott et al. 2001), and *B. burgdorferi*-infected *Ixodes pacificus* Cooley & Kohls have been demonstrated in British Columbia (Banerjee 1995). In most regions, the risk of *B. burgdorferi* infection in humans may be low given that the risk is posed by adult ticks derived from migratory bird-borne immatures, rather than by nymphal ticks in reproducing tick populations (Falco et al. 1996). Nevertheless, humans and pets in many parts of Canada may be bitten by infected adult ticks, some of which may feed long enough to transmit *B. burgdorferi* (as shown by their state of engorgement; Schwan and Piesman 2002).

Bird-borne adventitious ticks could act to seed reproducing tick populations and endemic cycles of *B. burgdorferi* as climate change renders temperatures in Canada and northernmost United States more suitable for *I. scapularis* survival. Risk factors for tick attachment in regions where *I. scapularis* populations are not established are season (spring and autumn), woodland habitats, and proximity to the U.S. border. Health professionals and veterinarians in Canada should be aware of the current risk of Lyme borreliosis as well as the possibility of an increased risk because of the potential establishment of more reproducing populations of *I. scapularis* in the coming decades.

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