Distribution and Characterization of *Borrelia burgdorferi* Isolates from *Ixodes scapularis* and Presence in Mammalian Hosts in Ontario, Canada

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ABSTRACT The blacklegged tick, *Ixodes scapularis* Say (Acari: Ixodidae), has a wide geographical distribution in Ontario, Canada, with a detected range extending at least as far north as the 50th parallel. Our data of 591 adult *I. scapularis* submissions collected from domestic animals (canines, felines, and equines) and humans during a 10-yr period (1993–2002) discloses a monthly questing activity in Ontario that peaks in May and October. The Lyme disease spirochete *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwalt & Brenner was detected in 12.9% of *I. scapularis* adults collected from domestic hosts with no history of out-of-province travel or exposure at a Lyme disease endemic area. Fifty-three isolates of *B. burgdorferi* were confirmed positive with polymerase chain reaction by targeting the *rrf* (5S)-*rrl* (23S) gene. Using DNA sequencing of the ribosomal species-specific *rrf* (5S)-*rrl* (23S) intergenic spacer region, all isolates belong to the pathogenic genospecies *B. burgdorferi* sensu stricto (s.s.). Nucleotide sequence analysis of a 218- to 220-bp amplicon fragment exhibits six cluster patterns and, collectively, these isolates branch into four phylogenetic cluster groups for both untraveled, mammalian hosts and those with travel to the northeastern United States (New Jersey and New York). Four of five geographic regions in Ontario had strain variants consisting of three different genomic cluster groups. Overall, our molecular characterization of *B. burgdorferi* s.s. shows genetic heterogeneity within Ontario and displays a connecting link to common strains from Lyme disease endemic areas in the northeastern United States. Moreover, our findings of *B. burgdorferi* in *I. scapularis* reveal that people and domestic animals may be exposed to Lyme disease vector ticks, which have wide-ranging distribution in eastern and central Canada.

KEY WORDS *Ixodes scapularis*, Lyme disease, *Borrelia burgdorferi*, distribution, Ontario

Lyme disease (Lyme borreliosis) is a bacterial, tick-borne zoonosis caused by the spirochete *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwalt & Brenner that is typically transmitted by certain ixodid ticks to a wide range of bird and mammalian hosts (Burgdorfer et al. 1982). In Canada, the first case of Lyme disease was diagnosed in a 13-yr-old girl from southwestern Ontario in 1977 based on her clinical manifestations (Bollegraaf 1988). Across eastern and central Canada, the blacklegged tick, *Ixodes scapularis* Say (northern populations previously considered as *Ixodes dammini* Spielman, Clifford, Piesman & Corwin [deer tick]) (Oliver et al. 1993, Keirans et al. 1996), is the principal vector of the Lyme disease spirochete. In far-western Canada, the western blacklegged tick, *Ixodes pacificus* Cooley & Kohls and perhaps *Ixodes angustus* Neumann are natural vectors in the transmission and enzootic cycling of *B. burgdorferi* (Banerjee et al. 1994). Both *I. scapularis* (e.g., Sanders and Oliver 1995) and *I. pacificus* (e.g., Clover and Lane 1995), which parasitize domestic animals (dogs, cats, horses, cattle, sheep, and goats) and humans (Durden and Keirans 1996), act as competent vectors of *B. burgdorferi*; however, nymphal *I. pacificus* are known to lose infection when they take a bloodmeal from western fence lizards, *Sceloporus occidentalis* Baird & Girard (Lane and Quistad 1998). In eastern Canada, Nuttall and Warburton (1911) first reported *I. scapularis* in 1904 on a human at Bracebridge, Ontario. Much later, all three motile developmental stages (larva, nymph, and adult) of *I. scapularis* were collected from white-tailed deer, *Odocoileus virginianus* (Zimmermann), at Long Point, Ontario, located on the north shore of Lake Erie (Watson and Anderson 1976). *B. burgdorferi* was first isolated in the province from *I. scapularis* (reported as *I. dammini*) col-

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lected at Long Point, an endemic area for Lyme disease (Barker et al. 1988. Lindsay et al. 1991).

Worldwide. the spirochete is known collectively as *B. burgdorferi* sensu lato (s.l.), and includes at least 11 genospecies or genomic groups. Across North America. three genospecies, namely. *B. burgdorferi* s.s.. *B. bissettii*. and *B. andersonii*, have been described. *B. burgdorferi* s.s.. which was initially isolated from blacklegged ticks from Shelter Island. New York. is the most dominant genospecies in the northern United States (Mathiesen et al. 1997). *B. bissettii* was first discovered in California as strain DN127 (Bissett and Hill 1987. Assous et al. 1993, Postic et al. 1998), and similar strains have been found in New York state (Anderson et al. 1988. Postic et al. 1998), Florida (Oliver et al. 1995), South Carolina (Lin et al. 2001). and northern Colorado (Schneider et al. 2000). *B. andersonii* was originally isolated from cottontail rabbits, *Sylvilagus floridanus* (J. A. Allen) and *Ixodes dentatus* Marx ticks collected in Dutchess County, New York (Anderson et al. 1988, 1989: Marconi et al. 1995), and later in Georgia (Oliver 1996, Lin et al. 2001) and Missouri (Mathiesen et al. 1997, Oliver et al. 1998). The *Borrelia* strain MI-8 from Florida (Oliver et al. 1995, Lin et al. 2004) is currently a partially described genospecies or genomic group.

In Eurasia, *Borrelia* species consisting of *B. afzelii* (Canica et al. 1993), *B. garinii* (Baranton et al. 1992). and *B. burgdorferi* s.s. (Baranton et al. 1992) have been cultured from *Ixodes ricinus* L. ticks and, likewise, the former two genospecies also have been cultured from *Ixodes persulcatus* Schulze in eastern Europe and Asia (Fukunaga et al. 2000). In the Mediterranean basin, *B. lusitaniae* (Le Fleche et al. 1997) and *B. valaisiana* also have been reported (Wang et al. 1997). In Japan, *B. japonica* has been isolated from *Ixodes ovatus* Neumann (Kawabata et al. 1993), *B. turdae* from *Ixodes turdus* Nakatsuji (Fukunaga et al. 1996), and *B. tanukii* from *Ixodes tanuki* Saito (Fukunaga et al. 1996). In mainland China, *B. sinica* has been isolated from *I. ovatus* (Masuzawa et al. 2001) and, specifically, in Taiwan *B. burgdorferi* s.s. has been discovered (Chao and Shih 2002). In Korea, *B. afzelii* has been isolated from *Ixodes nipponensis* Kitaoka & Saito and *Ixodes granulatus* Supino ticks (Kee et al. 1996).

Three genospecies were initially associated with Lyme disease in humans. namely, *B. burgdorferi* s.s. (Baranton et al. 1992), *B. afzelii* (Canica et al. 1993), and *B. garinii* (Baranton et al. 1992). More recently, two genospecies. *B. bissettii* (Picken et al. 1996, Strle et al. 1997) and *B. lusitaniae* (Collares-Pereira et al. 2004) have been found in Lyme disease patients in Europe.

The *B. burgdorferi* s.l. complex has wide genetic diversity. The presence of two tandem, duplicated copies of the *rrf* (5S) and *rrl* (23S) genes in *B. burgdorferi* s.l. is unique and, selectively, the species-specific intergenetic spacer region has been a valuable technique for studying genetic heterogeneity (Postic et al. 1994, Liveris et al. 1995). Recently, studies of North American isolates revealed considerable diversity (Lin et al. 2002). In Europe. different genospecies exhibited comparable clinical symptoms of Lyme disease patients (Assous et al. 1993). Significant regional variation of clinical symptoms in Eurasia has supported speculation that variation in disease presentation may be associated with variants of *B. burgdorferi* (Wienecke et al. 1994); perhaps. multiple strains and genospecies are present in Ontario. The aim of this study was to determine the geographic distribution of vector ticks in Ontario and to extrapolate the prevalence and genetic variation of *B. burgdorferi* in these ectoparasites.

Materials and Methods

Tick Collections. Blacklegged ticks were obtained primarily from veterinary clinics from five geographic areas in Ontario and were removed from domestic animals (canines, felines. and equines) and humans during a passive surveillance program (1993–2002). Before submitting ticks, veterinarians completed a tick–host information sheet indicating pertinent history of the hosts. including date of tick removal. attachment (loose or attached). geographic location, host travel. and Lyme disease vaccine status. A submission consisted of one or more ticks, which belonged to one tick species, removed from a single host. Whenever an established population of *I. scapularis* was evident in an area, we conducted active surveillance and collected immature ticks (larvae and nymphs) from live-trapped small mammals. In addition, adult ticks (males and females) were collected by flagging low-lying, woodland understory vegetation by using a 62- by 90-cm white, flannel-covered waterproof crib sheet (Dundee Mills. New York. NY). Ticks were identified to species and stage of development by using standard taxonomic nomenclature and keys (Clifford et al. 1961. Keirans and Clifford 1978. Durden and Keirans 1996). Live ticks were kept in ventilated polyethylene vials with tulle netting caps and then placed in ziplock plastic bags with moist paper towel. Ticks were sent promptly via overnight courier to the British Columbia Centre for Disease Control (BCCDC) for culturing and polymerase chain reaction (PCR) testing. Dead ticks were put directly into 70% ethanol for PCR testing: they were not tested if they were badly damaged or decayed. Live ticks were cultured. *I. scapularis* submissions were mapped by township by using latitude/longitude coordinates in a Geographic Information System (ArcGIS 9.0. Environmental Systems Research Institute Inc., Redlands, CA).

Source of *B. burgdorferi* Isolates from Ticks. *B. burgdorferi* isolates were obtained from blacklegged ticks by both active and passive surveillance. either by flagging or direct removal from domestic and wildlife mammals. For genetic and epidemiological comparison. 10 of the 53 isolates were cultured from *I. scapularis* adults removed from dogs, which had traveled to endemic areas in the northeastern United States (New Jersey and New York). Live blacklegged ticks were surface sterilized with 10% hydrogen peroxide for 10 min followed by 70% isopropyl alcohol and

Table 1. Prevalence of *B. burgdorferi* in *I. scapularis* adults removed from domestic hosts in Ontario with no history of out-of-province travel or exposure at an endemic area, 2000–2002

Yr	No. tested	Positive for *B. burgdorferi* (%)
2000	102	17 (16.7)
2001	98	12 (12.2)
2002	125	13 (10.4)
Total	325	42 (12.9)

washed three times with sterile distilled water. The midgut contents were placed in Barbour–Stoenner–Kelly (BSK) II medium, incubated at 34°C, and checked weekly by dark-field microscopy for live spirochetes for up to 30 d.

Culturing *B. burgdorferi* Isolates from Mouse Organs. Small mammals were euthanized using carbon dioxide and directly soaked in chlorine bleach solution for 10 min, transferred to distilled water for 1 min, placed in 70% isopropyl alcohol for 5 min, dipped in distilled water for 1 min, and laid on cotton batting to absorb excess aqueous liquid. Organs and tissues were dissected and placed promptly in BSK II medium with 6% rabbit serum and antibiotics (50 μg/ml rifampicin and 10 μg/ml kanamycin) as described previously (Barbour 1984), and then they were incubated at 34°C for 30 d. Cultures were checked weekly for live spirochetes by dark-field microscopy.

Prevalence of *B. burgdorferi* in Ticks. We specifically designated a 3-yr period (2000–2002) for determining *B. burgdorferi* prevalence in *I. scapularis*, including live and dead ticks (Table 1), because some pertinent data from previous years was previously documented (Banerjee et al. 1995, 1996, 2000). *I. scapularis* specimens were excluded from the prevalence part of this study if hosts had out-of-province travel or ticks were part of active surveillance conducted at established populations. "Prevalence" is the percentage of *I. scapularis* infected with *B. burgdorferi* by using PCR testing. "Endemic area for Lyme disease" means that *B. burgdorferi* is continuously cycling enzootically between reservoir-competent hosts and vector ticks in a given area.

Serology on Animal Hosts. During the same 3-yr period (2000–2002), suitable domestic animal hosts with *I. scapularis* infestations were tested for Lyme disease by using indirect immunofluorescence assay (IFA) and Western blot (MarDx Diagnostics, Inc., Carlsbad, CA). Hosts were excluded if they had either exposure at a Lyme disease endemic area, or they were administered antibiotics, the Lyme disease vaccine, or both between tick removal and the time that blood was drawn for serology, normally 4–6 wk after tick detachment, to minimize sampling bias and false positive results. Any hosts that disappeared or died were excluded.

Monoclonal Antibodies of *B. burgdorferi* Isolates. Spirochetal isolates were immunostained with monoclonal antibodies of *B. burgdorferi*, namely, species-specific H5332, which reacts with 31-kDa outer surface protein A (OspA); H107-IIF3, which is reactive with P39 (39 kDa); and *Borrelia* (genus)-specific H9724, which reacts with flagellin (41 kDa) (all provided by Alan G. Barbour, University of California, Irvine, CA).

PCR Amplification. DNA was extracted from pure or contaminated cultures using QIAGEN tissue kits (QIAGEN, Mississauga, Ontario, Canada). PCR was performed to amplify a portion of the variable spacer region between two conserved structures, the 3′ end of the 5S rRNA (*rrf*) and the 5′ end of the 23S rRNA (*rrl*), as described previously (Postic et al. 1994).

DNA Sequencing. The endonuclease *Mse*I was used to select the *rrf-rrl* intergenic spacer region and to characterize isolates as described previously (Postic et al. 1994). PCR products were purified using Microcon PCR centrifugal filter columns (Millipore, Billerica, MA) following the manufacturer's instructions. Dye-terminated fragments were produced using ABI Big-Dye terminator sequencing kits (Applied Biosystems, Foster City, CA) and purified by ammonium acetate-ethanol precipitation before analysis using an ABI Prism 310 DNA sequencer (Applied Biosystems). Generated DNA sequence data were analyzed and assembled using the SeqMan module within the Lasergene Sequence Analysis Software (DNASTAR, Madison, WI). A phylogenetic tree of the *Borrelia* sequences, including representative sequences of all *Borrelia* species downloaded from GenBank (National Center for Biotechnology Information, Bethesda, MD), was generated by Clustal X analysis by using the MegAlign Module of the Lasergene software.

Nucleotide Sequence Accession Numbers. Nucleotide sequences of the *rrf-rrl* spacer region from *B. burgdorferi* s.l. isolates have been deposited in the GenBank database (Table 2).

Results

Tick Submissions. During a 10-yr (1993–2002) passive surveillance program across Ontario, 591 *I. scapularis* submissions consisting of 625 adults (605 females and 20 males) were collected from domestic and human hosts with no recent history of out-of-province (Fig. 1). From these *I. scapularis*, 65 *B. burgdorferi*-positive ticks (14 live cultures; 51 PCR sequences from 51 dead ticks) were obtained. With the exception of one fully engorged female collected from the floor of a veterinary clinic, all ticks were removed directly from animal hosts (canines, felines, and equines) and humans; only adults were submitted from these hosts.

I. scapularis adults were recorded monthly, and the highest frequency occurred in October when 178 (30.1%) of 591 submissions were collected (Fig. 2). Bimodal questing activity of *I. scapularis* adults was apparent during the spring (late March to late June) and fall (early October to mid-December); however, a few adults were collected in July and August primarily from northern areas of the province, and a minimal number of winter submissions were collected in January and February along the north shore of Lake Erie at times when snow cover was absent. One *I. scapularis* adult was collected on 15 February 1998

Table 2. Isolates of *B. burgdorferi* cultured from faunal specimens collected in Ontario, 1993–2002

Isolate strain	Geographic location	Biological origin	Source	Cluster group	GenBank accession no.
		Southwestern Ontario			
96FT649	Long Point	*I. scapularis* (male)	Flagging	A	AY363421
96FT650	Long Point	*I. scapularis* (male)	Flagging	B	AY363420
96FT651	Long Point	*I. scapularis* (male)	Flagging	A	AY363419
96FT652	Long Point	*I. scapularis* (male)	Flagging	C	AY363418
96FT658	Long Point	*I. scapularis* (female)	Flagging	B	AY363417
99FT1072	Rondeau Park	*I. scapularis* (males)	Flagging	C	AY363403
99FT1078	Rondeau Park[a]	*I. scapularis* (females)	Flagging	C	AY363402
99FT1082	Rondeau Park	*I. scapularis* (females)	Flagging	C	AY363401
99FT1086	Rondeau Park	*I. scapularis* (females)	Flagging	C	AY363400
01FT1256	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363450
01FT1271	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363446
01FT1273	Turkey Point	*I. scapularis* (females)	Flagging	A	AY363445
01FT1329	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363443
01FT1330	Turkey Point	*I. scapularis* (males)	Flagging	C	AY363442
02FT1374	Turkey Point	*I. scapularis* (females)	Flagging	A	AY363440
02FT1375	Turkey Point	*I. scapularis* (females)	Flagging	A	AY363439
02FT1376	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363438
02FT1377	Turkey Point	*I. scapularis* (females)	Flagging	A	AY363437
02FT1378	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363436
02FT1379	Turkey Point	*I. scapularis* (females)	Flagging	A	AY363435
02FT1380	Turkey Point	*I. scapularis* (females)	Flagging	C	AY363434
02FT1409	Turkey Point	*I. scapularis* (larvae)	W.-f. mouse[b]	A	AY363432
02FT1430	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363431
02FT1431	Turkey Point	*I. scapularis* (females)	Flagging	B	AY363430
02FT1432	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363429
02FT1435	Turkey Point	*I. scapularis* (females)	Flagging	C	AY363428
02FT1438	Turkey Point	*I. scapularis* (males)	Flagging	A	AY363427
02FT1439	Turkey Point	*I. scapularis* (females)	Flagging	A	AY363426
ONT03FWT	Turkey Point	*P. leucopus* (male)	Ear lobe	A	AY363398
		Western Ontario			
97FT710	Mississauga	*I. scapularis* (female)	Dog	A	AY363416
00FT1212	Barrie	*I. scapularis* (female)	Dog	C	AY363447
02FT1447	Burlington[a]	*I. scapularis* (female)	Dog	D	AY363425
		Central Ontario			
98FT789	Toronto	*I. scapularis* (female)	Dog	A	AY363415
99FT1142	Cobourg	*I. scapularis* (female)	Dog	A	AY363399
01FT1349	Courtice	*I. scapularis* (female)	Dog	A	AY363441
		Eastern Ontario			
99FT1042	Kingston	*I. scapularis* (female)	Dog	A	AY363413
01FT1303	Kingston	*I. scapularis* (female)	Dog	B	AY363444
02FT1457	Ottawa[a]	*I. scapularis* (female)	Dog	D	AY363424
		Northern Ontario			
95FT402	Thunder Bay	*I. scapularis* (female)	Dog	B	AY363422
00FT1189	Terrace Bay	*I. scapularis* (female)	Dog	C	AY363448
02FT1390	Thunder Bay	*I. scapularis* (female)	Dog	C	AY363433
93FT1913	Kenora[a]	*I. scapularis* (female)	Dog	C	AY363423
99FT1041	Thunder Bay[a]	*I. scapularis* (female)	Dog	D	AY363414
		Out-of-province travel[c]			
99FT1045	Woodville[a]	*I. scapularis* (females)	Dog	D	AY363412
99FT1046	Woodville	*I. scapularis* (females)	Dog	D	AY363411
99FT1047	Woodville[a]	*I. scapularis* (females)	Dog	D	AY363410
99FT1048	Woodville	*I. scapularis* (females)	Dog	C	AY363409
99FT1049	Woodville[a]	*I. scapularis* (females)	Dog	D	AY363408
99FT1050	Woodville[a]	*I. scapularis* (males)	Dog	D	AY363407
99FT1051	Woodville	*I. scapularis* (males)	Dog	D	AY363406
99FT1052	Woodville	*I. scapularis* (males)	Dog	A	AY363405
99FT1053	Woodville	*I. scapularis* (males)	Dog	A	AY363404
01FT1218	Timmins	*I. scapularis* (females)	Dog	B	AY363449

[a] This isolate has a 220-bp amplicon fragment with a 2-bp insertion.
[b] W.-f. mouse, white-footed mouse, *P. leucopus*.
[c] Hosts traveled to northeastern United States.

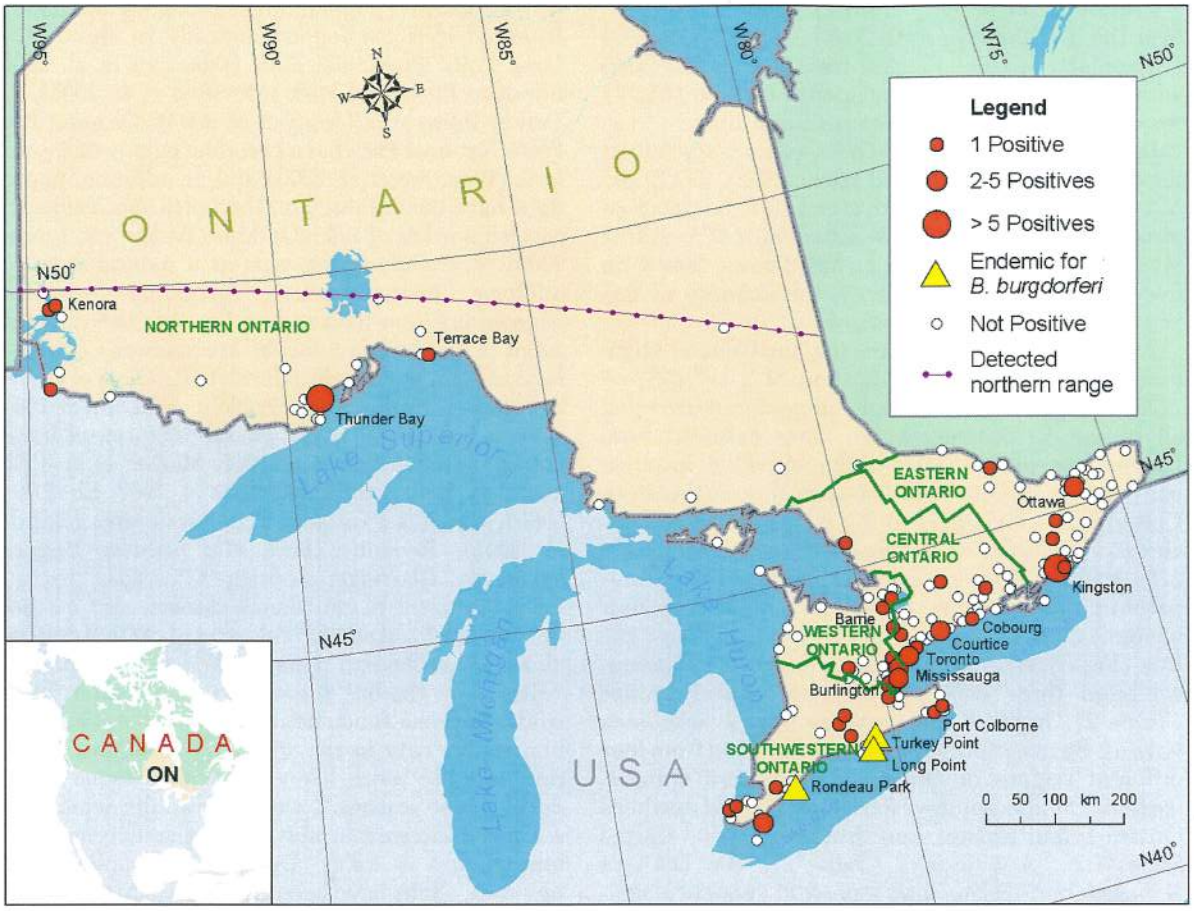

Fig. 1. Distribution of *I. scapularis* tested for *B. burgdorferi* collected from Ontario hosts with no out-out-province travel and the northern detected range, 1993–2002.

at Point Pelee National Park when the ambient air temperature was 6°C. We report the first known occurrence of *I. scapularis*, an attached, partially engorged female, collected from a horse on 3 December 2001 at Wainfleet, Ontario.

In 1993, 26 veterinary clinics initially participated, which steadily increased to 193 clinics by 2002. The number of *I. scapularis* submissions started with seven in 1993 and rose continually to 129 in 2002, by heightening awareness and strengthening participation with veterinary clinics. A wide geographic distribution of *I. scapularis* extended across the province from Pelee Island (41° 47′ N, 82° 40′ W) in the south, to as far north as Timmins (48° 28′ N, 81° 21′ W) in northeastern Ontario, and Minaki (49° 59′ N, 94° 40′ W) in northwestern Ontario. Notably, one fully engorged female from Terrace Bay (48° 47′ N, 87° 09′ W) on the north shore of Lake Superior laid a full batch of eggs, which developed into viable larvae. Based on the 591 submissions, the dotted line on the map provides the detected northern geographic range of *I. scapularis* in Ontario, which extends to the 50th parallel (Fig. 1). Only *I. scapularis* adults were collected from domestic and human hosts; larval and nymphal developmental stages were obtained from small mammal wildlife hosts collected from focal areas with established populations of *I. scapularis*.

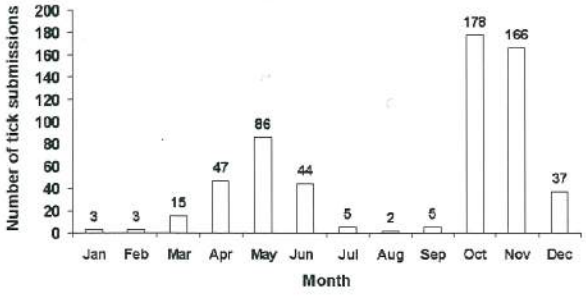

Fig. 2. *I. scapularis* submissions in Ontario, with no out-of-province travel, by month, 1993–2002.

***B. burgdorferi* Isolates.** Fifty-three *B. burgdorferi* s.s. isolates (43 Ontario-based and 10 out-of-province) were cultured and selected from faunal specimens for genetic comparison; specifically, they were obtained from live *I. scapularis* and a white-footed mouse, *Peromyscus leucopus* (Rafinesque), collected in Ontario (Table 2). The majority of isolates were from southwestern Ontario, especially three areas with breeding colonies of *I. scapularis*. All isolates were reactive to monoclonal antibodies of *B. burgdorferi*, namely, OspA, P39, and flagellin.

Prevalence of *B. burgdorferi* in Ticks. Specifically, over the 3-yr period (2000–2002), 42 (12.9%) of 325 *I. scapularis* adults collected from untraveled hosts within the five geographic regions of Ontario (Fig. 1) were positive for *B. burgdorferi* (Table 1).

Serology of Animal Hosts. Of the canines and felines tested during the 3-yr period (2000–2002), 24 (100%) of 24 symptomatic hosts with attached *B. burgdorferi*-positive *I. scapularis* were reactive with IFA and/or Western blot serology for Lyme disease; based on five-band criteria for positivity, the majority of IgG Western blot tests were positive.

PCR Amplification, Sequencing, and Genetic Alignment Analysis. PCR amplification of a *rrf* (5S)-*rrl* (23S) intergenic spacer amplicon gene revealed that all of the 53 isolates, which were cultured from *I. scapularis* collected from both untraveled, mammalian hosts and those with travel to the northeastern United States, belonged to the widespread genospecies *B. burgdorferi* s.s. Further DNA sequencing of a 218- to 220-bp amplicon fragment showed six cluster patterns and, combined, they branched into four genomic cluster groups as noted on the phylogenetic tree (Fig. 3); four of five geographic areas in Ontario exhibited three different genomic cluster groups (Table 2). In total, nine isolates from *I. scapularis* have a 2-bp insertion: five isolates originated from four different regions of Ontario (i.e., eastern Ontario, western Ontario, southwestern Ontario, and northern Ontario); four isolates were from the coastal United States (i.e., New Jersey) (Table 2). The DNA of *B. burgdorferi* isolates had a percent sequence similarity ranging from 98.3 to 100%. Specifically, for the 43 isolates, which were cultured from *I. scapularis* adults attached to hosts with no history of out-of-province travel, the nucleotide percentage of sequence likewise ranged from 98.3 to 100%. The phylogenetic analysis comparing the isolates in this study to the *B. burgdorferi* s.l. isolates from Asia, Europe, and North America revealed a sequence divergence of 17.5% for nucleotide substitution (Fig. 3). An isolate (ONT03FWT) cultured from the ear lobe of a feral *P. leucopus*, genetically matched one (02FT1409) cultured from the corresponding, attached *I. scapularis* larvae.

Discussion

Human Lyme disease cases are reported annually in Ontario. Some of these cases have no history of out-of-province travel or exposure at an endemic area for Lyme disease. This study shows there is genetic diversity of locally contracted *B. burgdorferi* strains that are widely distributed across Ontario. Given adequate immune response time, the domestic hosts that were bitten by *B. burgdorferi*-infected *I. scapularis* were serologically positive and had clinical symptoms of Lyme disease. Our findings provide validity for locally contracted cases of Lyme disease in Ontario.

With increased knowledge and participation from veterinarians, a steady increase in the number of *I. scapularis* submissions was seen over the 10-yr period. Using active surveillance, we studied four areas with established populations of *I. scapularis* and found *B. burgdorferi* cycling enzootically in three areas: Long Point Provincial Park (Banerjee et al. 2000), Rondeau Provincial Park (Morshed et al. 2003), and Turkey Point area (Scott et al. 2004). Of note, Point Pelee National Park has a breeding colony of *I. scapularis* (Banerjee et al. 2000) and, in addition, populations have been sighted on the north shore along the eastern portion of Lake Ontario (Barker and Lindsay 2000). *I. scapularis* also acts as a natural vector of etiologic microorganisms, including *Anaplasma phagocytophilum* Bakken and Dumler, the rickettsial agent of human granulocytic anaplasmosis (formerly human granulocytic ehrlichiosis) (Pancholi et al. 1995, des Vignes and Fish 1997, Bakken and Dumler 2001); *Babesia microti* (Franca), causal organism of human babesiosis (Piesman et al. 1986, Mather et al. 1990); and deer tick virus (Telford et al. 1997, Ebel et al. 1999), which is a variant of Powassan virus (Kuno et al. 2001). Recently, *Bartonella henselae* Regnery, Anderson, Clarridge, Rodriguez-Barradas, Jones & Carr, the agent of cat scratch disease, also has been detected in *I. scapularis* (Eskow et al. 2001, Adelson et al. 2004) and human patients (Podsiadly et al. 2003).

Based on the 591 *I. scapularis* submissions in this study, our data fundamentally show that adults have bimodal activity in the spring and fall, and Ontario residents are more likely to acquire Lyme disease during these seasons. *I. scapularis* adults demonstrate a marked increase in host-seeking activity when the temperature is ≥4°C (Duffy and Campbell 1994); however, adults have been collected in Maryland during the coldest months when the temperature was as low as −2°C (Carroll and Kramer 2003). Collections of *I. scapularis* in the early spring show that this tick species overwinters in Ontario and, logically, the snow cover acts as an insulating layer against frigid ambient air temperatures, which can dip to −44°C at Kenora in northern Ontario. Our findings show the northern detected geographic range of *I. scapularis* in Ontario extends, at least, to the 50th parallel. Although *I. scapularis* had been reported previously in northern regions of the province (Banerjee et al. 1995, 1996), our ability to monitor the distribution of ticks in far-northern Ontario was limited by the availability of veterinary clinics, which are currently all located south of the 50th latitude.

I. scapularis nymphs have been suggested as the principal vector for Lyme disease. Given the fact that *I. scapularis* nymphs were not submitted by veterinarians and the public during this decade-long study, our new findings show that *I. scapularis* adults are the main mode of *B. burgdorferi* transmission. Apparently, minute size was not a recognition factor because larvae and nymphs of other common tick species were observed and collected and submitted from dogs and cats. In contrast, we collected *I. scapularis* nymphs from wildlife mammalian hosts during active surveillance in tick-endemic areas. Because *I. scapularis* adults have an additional bloodmeal, they have a greater opportunity of acquiring spirochetes from infected hosts. Our tick–host–pathogen study provides

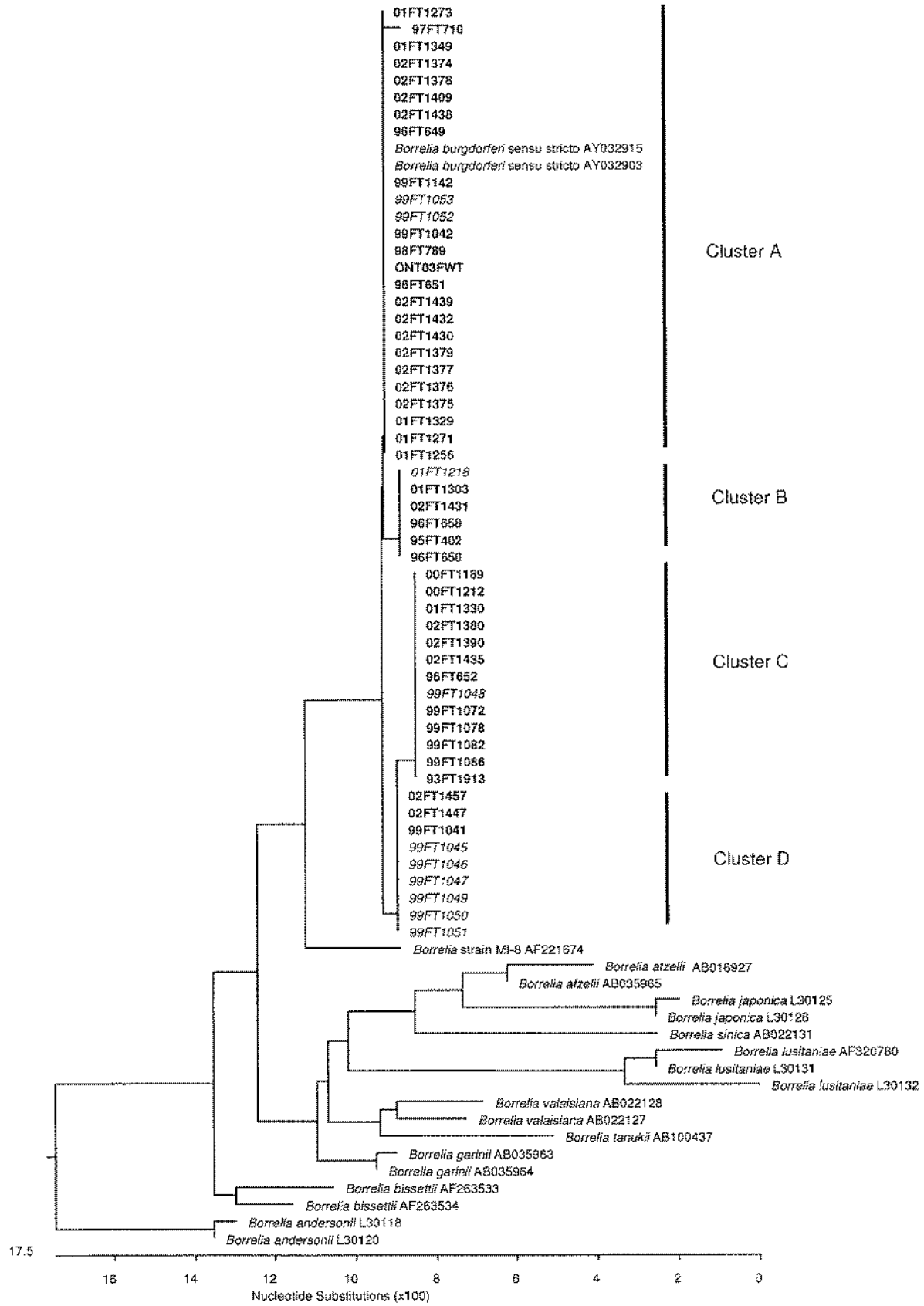

Fig. 3. Phylogenetic tree of *B. burgdorferi* isolates collected in Ontario determined by DNA sequencing of *rrf-rrl* interspacer amplicons. The 43 Ontario-based isolates are in bold, standard print.

notable evidence that *I. scapularis* adults are the primary vector for *B. burgdorferi* infection rather than nymphs in nonendemic areas in Ontario.

Migratory songbirds provide wide geographic distribution for Lyme disease vector ticks primarily during yearly spring migration. Immature *I. scapularis* are transported northward in Canada as Neotropical and short-distance migrants fly annually to nesting and breeding grounds in boreal and subarctic regions of the Northern Forest Avifaunal Biome, which stretches from Yukon to Newfoundland and Labrador. Ultimately, some passerine species act as competent reservoirs of *B. burgdorferi* and can infect both larval and nymphal *I. scapularis* (Anderson and Magnarelli 1984; Anderson et al. 1986, 1990; Weisbrod and Johnson 1989; Stafford et al. 1995; Richter et al. 2000). During late May to early June, heightened questing activity of *I. scapularis* nymphs in the northern United States corresponds with the gleaning activity and departure of spring migrants to Canada (Anderson and Magnarelli 1984). Within a week of attachment, these immature *I. scapularis* may be dispersed adventitiously from northern Alberta to Nova Scotia, and some of these ticks are infected with *B. burgdorferi* (Morshed et al. 1999, 2005; Scott et al. 2001). Blacklegged ticks may be transported further north than the 50th parallel in Ontario because Scott et al. (2001) reported *I. scapularis* on passerine migrants as far north as the town of Slave Lake (55° 17′ N, 114° 46′ W) in northern Alberta. Morshed et al. (2005) reported *B. burgdorferi*-positive *I. scapularis* collected from songbirds at Delta Marsh, Manitoba (50° 11′ N, 98° 12′ W) and Long Point, Ontario (42° 31′ N, 80° 10′ W), which substantiates the wide dispersal of infected ticks during spring migration in central and eastern Canada. *I. scapularis* acquired in the southern United States, where *B. burgdorferi* isolates have greater heterogeneity (Oliver 1996; Lin et al. 2001, 2002), seem to drop off before reaching Canada. Consequently, we have only encountered *B. burgdorferi* s.s., which has most likely originated from Lyme disease endemic areas in the northern United States. These passerine migratory patterns provide further evidence for the expanding range of *I. scapularis* and *B. burgdorferi* and, pragmatically, the potential establishment of new endemic Lyme areas.

Our data on DNA sequencing of the *rrf-rrl* intergenic spacer revealed four genetic clusters of *B. burgdorferi* s.s. in Ontario collected by both active and passive surveillance. Isolates with a 2-bp insertion submitted from five distinctly separate locations (i.e., Kenora, Thunder Bay, Burlington, Ottawa, and Rondeau Park) closely resemble isolates collected from four *I. scapularis* attached to a dog from Woodville, which had traveled to New Jersey. Not only do these strain variants demonstrate heterogeneity within different geographic regions of Ontario, they also show genetic diversity geographically across the province. The close similarity of the Florida MI-8 strain to Ontario isolates suggests that it mutated and was carried northward during the past 10,000 yr by Neotropical migrants to glaciated areas including northern United States and eastern Canada. Our evidence also shows DNA genetic similarities with *B. burgdorferi* strains from the northeastern United States (New Jersey, New York) and, likewise, divulges an epidemiological link to these endemic areas.

Isolates of the genospecies *B. burgdorferi* s.s., prevalent in North America, belong to a group with proven human pathogenicity. During our study, two *B. burgdorferi*-positive *I. scapularis* were collected from humans; however, tissues from these ticks could not be cultured as they were submitted dead. One dog with clinical symptoms and sequential positive serology, which was initially bitten by a culture-positive *I. scapularis*, was studied for 5 yr and was treated with antimicrobials for chronic Lyme disease. Based on positive serology and clinical symptoms of the 24 dogs, *B. burgdorferi* s.s. strains were able to survive and effectively colonize their canine hosts. With the wide distribution of *B. burgdorferi*-positive *I. scapularis* in Ontario, patients can contract Lyme disease and related tick-transmitted diseases without having frequented an endemic area. Left undiagnosed and untreated, Lyme disease can produce protean clinical manifestations (cardiac, cutaneous, musculoskeletal, neurologic, neuropsychiatric, neurocognitive, and urologic), and become a persistent illness with debilitating symptoms in mammalian hosts, including humans (Preac-Mursic et al. 1989, Häupl et al. 1993), dogs (Straubinger et al. 1997), and cats (Gibson et al. 1995). During the current study, some dogs and cats became very symptomatic after *B. burgdorferi*-positive *I. scapularis* were removed, and follow-up testing on these hosts was positive for Lyme disease by using IFA and/or Western blot serology. Gross clinical manifestations, including fever, anorexia, arthritis, stiffness, lameness. depression, encephalopathy, and aggressive disposition, have been reported in dogs (Evans et al. 1995) and cats (Gibson et al. 1995). Complete, fatal heart block has been documented in canines (Levy and Duray 1988).

When immature *I. scapularis* are dispersed across Ontario, several wildlife animals can act as natural hosts. Deer mice, *Peromyscus maniculatus* (Wagner), have a wide distribution throughout Ontario and are reservoir-competent hosts (Rand et al. 1993). Similarly, *P. leucopus* is common in southern Ontario, and maintains *B. burgdorferi* infection as a natural reservoir (Bosler et al. 1983, Anderson et al. 1985, Donahue et al. 1987). We cultured *B. burgdorferi* from a white-footed mouse collected from an endemic area on the north shore of Lake Erie. In contrast, white-tailed deer are not a competent reservoir host of *B. burgdorferi* (Telford et al. 1988); however, they do act as amplifying hosts of *I. scapularis*, especially adults (Durden and Keirans 1996). Rand et al. (2004) reported that when white-tailed deer were completely eliminated from Monhegan Island, an initially Lyme endemic island off the coast of Maine, *I. scapularis* were substantially reduced but not extirpated. Understandably, migratory songbirds continued to introduce immature *I. scapularis* there. With songbirds acting as dispersing agents, endemic areas in the northern United States

supply a source of *I. scapularis* for eastern Canada and, similarly, endemic areas in the upper Midwest provide cross-border sustenance for northern Ontario and central Canada. Moreover, Lyme disease endemic areas on the north shore of Lake Erie may act as epicenters of *B. burgdorferi*-infected *I. scapularis* for local and more northern regions. Additional vigilance is needed to screen for several zoonotic diseases because Ontario has the vertebrate biodiversity that is required to support *I. scapularis*, which can harbor and transmit multiple tick-borne pathogens.

In conclusion, we provide evidence that *I. scapularis* has a wide distribution in Ontario extending at least as far north as the 50th parallel. *B. burgdorferi* was present in ≈12.9% of *I. scapularis* adults removed from domestic hosts with no history of out-of-province travel, which indicates the potential of contracting Lyme disease and other tick-borne diseases in nonendemic areas. Not only do our genetic analyses show genetic variation of *B. burgdorferi* s.s. isolates collected in Ontario, they also reveal a connecting link with common strains in the northeastern United States. Based on the wide distribution of *B. burgdorferi*-positive *I. scapularis* in Ontario, medical and veterinary professionals need to be aware of the risk for Lyme disease in eastern and central Canada.

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