The search for *Ixodes dammini* and *Borrelia burgdorferi* in Nova Scotia

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CR BELL, HB SPECHT, BA COOMBS. The search for *Ixodes dammini* and *Borrelia burgdorferi* in Nova Scotia. *Can J Infect Dis* 1992;3(5):224-230. Twenty-four *Ixodes dammini* ticks (23 adults and one nymph) have been recovered in Nova Scotia since 1984. There has not been a systematic search for larvae and none has been identified. The recovery of the nymph from a road-killed yellow throat bird, *Geothypis trichas*, in late May 1990 supports the contention that migrating birds are bringing deer ticks into the province every spring. In March and April 1991, four adult deer ticks were identified, suggesting that these ticks had overwintered. These deer tick specimens indicate that it is possible that *I dammini* is becoming established in Nova Scotia, if it is not already established. There has been no evidence for the existence of *Borrelia burgdorferi* in the province. The spirochete was not cultured from 650 *Dermacentor variabilis* ticks, nor were antibodies detected in a small sample of feral rodents using an indirect fluorescent antibody test. A survey of 137 dog sera samples, analyzed by enzyme-linked immunosorbent assay, also proved negative. There has been no confirmed indigenous case of Lyme disease in Nova Scotia to date.

Key Words: *Borrelia burgdorferi*, *Dermacentor variabilis*, *Ixodes dammini*, Lyme disease, Nova Scotia

La recherche de *Ixodes dammini* et de *Borrelia burgdorferi* en Nouvelle-Écosse

RÉSUMÉ: On a trouvé 24 tiques *Ixodes dammini* (23 adultes et une nymphe) en Nouvelle-Écosse depuis 1984. Les larves n'ont pas été systématiquement recherchées et aucune n'a été identifiées. La découverte d'une nymphe chez une fauvette masquée (*Geothypis trichas*) tuée sur la route, à la fin mai 1990 appuie l'hypothèse selon laquelle les oiseaux migrateurs apportent la tique du wapiti dans la province chaque année. En mars et avril 1991, quatre tiques du wapiti adultes ont été identifiées, suggérant que ces tiques avaient hiverné et que *I dammini* est en voie de s'établir en Nouvelle-Écosse – si ce n'est déjà fait. Rien ne semble indiquer la présence de *Borrelia burgdorferi* dans la province. Aucun spirochète n'a été mis en culture à partir de 650 *Dermacentor variabilis* (tique du chien) et aucun anticorps n'a été décelé par immunofluorescence indirecte dans un petit échantillon de rongeurs sauvages. L'analyse de 137 sérums de chien effectuée par la méthode enzymo-immunologique a donné elle aussi des résultats négatifs. Jusqu'à ce jour, aucun cas indigène de borréliose de Lyme n'a été confirmé en Nouvelle-Écosse.

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Figure 1) Distribution of *Dermacentor variabilis* and the location of the 1988 field sites in southwestern Nova Scotia. (Solid circles represent 1985 data)

TICK-BORNE LYME DISEASE WAS RECOGNIZED AND CHARACTERIZED in North America in the early 1970s (1,2). There have now been thousands of cases reported throughout the contiguous United States which have contributed to the present awareness of the etiology and epidemiology of the disease in the United States (3,4). Information on the disease in Canada is not as complete.

The first case of Lyme disease in Canada was diagnosed in 1977 (5), and at a symposium on Lyme disease in Canada conducted at the University of Guelph in January 1991 (6), it was revealed that 65 indigenous cases of the disease had been reported to the Laboratory Centre for Disease Control in the previous seven years (personal communication). Fifty-four of these cases were limited to Ontario, with only one case occurring in the Maritimes, in New Brunswick (7). However, there has been some concern expressed by health professionals about the potential for the disease in Nova Scotia largely because of the proximity to disease foci in the state of Maine (8) and the possibility that migrating birds could be introducing infected *Ixodes*

dammini ticks into the province each spring (9). Certainly because of the very large numbers of *Dermacentor variabilis* ticks in Nova Scotia (10), introduced at the turn of the century, there is palpable concern among the general public. These factors provided the impetus in 1984 for this continuing search for the Lyme disease agent, *Borrelia burgdorferi*, and the vector, *I dammini*, in Nova Scotia.

MATERIALS AND METHODS

1988 Collection of *D variabilis*: In the summer of 1988 ticks were collected from seven field sites within the area endemic for *D variabilis* in south western Nova Scotia. Adults were collected using a 0.5 m² flag dragged over vegetation along roadsides and clearings near woods. The boundaries of the area sampled were established from the studies of Garvie et al (10) and Dodds et al (11). Data for the distribution in 1985 were obtained by flagging of vegetation, as described, at random sites, together with submissions from the public (Figure 1).

1988 Collection of small mammals: Live trapping for small mammals was performed at the seven sites from

TABLE 1
***Ixodes dammini* recovered in Nova Scotia**

Location	Stage/sex	Host	Date
Aylesford	Adult/female	Cat	July 84
Halifax	Adult/female	On lawn	Nov 84
Little River	Adult/female	Dog	Nov 84
Amherst	—	Human	Nov 88
Waterville	Adult/female	Dog	May 89
Canard	Adult/female	Human	Oct 89
Black River	Adult/female	Dog	Oct 89
Centreville	Adult/female	Cat	Nov 89
White Rock	Adult/female	Cat	Nov 89
Petite Rivière	Adult/female	Cat	Nov 89
Plympton	Adult/female	Dog	Nov 89
Central Grove	Adult/female	Cat	Nov 89
Bear River	Adult/female	Dog	Nov 89
Antigonish	Adult/female	Human	June 90
Windsor	Nymph	Yellow throat	June 90
Antigonish	Adult/male	Human	July 90
Second Peninsula	Adult/female	Dog	Oct 90
Church Point	Adult/female	Human	Nov 90
Port Mouton	Adult/female	Cat	Nov 90
Yarmouth	Adult/female	Veterinary sample	Fall 90
Weymouth Falls	Adult/female	Dog	March 91
Sandford	Adult/female	Human	March 91
Gullivers Cove	Adult/male	Cat	April 91
Margaretsville	Adult/female	Human	April 91

mid May to August. The sites were: Granite village, Queens County; Lower Shag Harbour, Shelburne County; Ellenwood Provincial Park, Yarmouth County; Meteghan Station, Digby County; South Milford, Annapolis County; Lequille, Annapolis County; and West Paradise, Annapolis County.

Approximately 100 to 130 traps (Sherman 9 inch live traps; HB Sherman, Florida) were set each night at each site for four nights to give an average of 450 trap nights per site and 4960 trap-nights for all sites for the entire season (West Paradise was sampled for three weeks).

Ticks and mammals were returned to Acadia University each week for identification and processing. Ticks were identified with the keys of Kierans and Litwak (12), Kierans and Clifford (13) and Sonenshine (14).

1988 Cultivation of *B burgdorferi* from ticks and small mammals and seroconversion: Adult ticks from each site were pooled into sets of five by sex. After surface sterilization with 70% volume/volume ethanol, 10% volume/volume hydrogen peroxide and rinsing with sterile water, the ticks were homogenized in a sterile mortar and pestle with 2.0 mL phosphate buffered saline (PBS, pH 7.4).

Mammals were anesthetized, exsanguinated by cardiac puncture and the spleen and kidney excised and homogenized in a Potter Elvehjem tissue grinder in 2.0 mL PBS. Blood samples were allowed to clot by incubation

at 37°C for 20 mins; sera were then separated by centrifugation and stored at -70°C.

Samples (50 µL) of tick and mammal organ homogenate were inoculated, in duplicate, into 8 mL Barbour-Stoenner-Kelly (BSK II) medium with antibiotics (15) and incubated for up to three months at 33°C. Positive controls were run by inoculation with *B burgdorferi* ATCC 35210. The presence of spirochetes in the medium was determined after at least two months incubation by epifluorescence microscopy with acridine orange (16,17).

Antibodies to *B burgdorferi* in the *Peromyscus leucopus* specimens were determined using the indirect fluorescent antibody (IFA) test described by Wilkinson (18), with slight modifications by Artsob (personal communication) using fluorescein isothiocyanate-conjugated rabbit antimouse polyclonal sera (Cedarlane Laboratories Ltd).

1990 Canine seroprevalence testing: Antibodies to *B burgdorferi* in domestic dogs were tested in sera collected in 1990 with the assistance of three veterinary clinics in the province. These were: Bayview Animal Clinic, Digby (Dr N Pothier); TriCounty Veterinary Services, Arcadia, near Yarmouth (Dr T O'Brien); and Cape Breton Veterinary Services, Sydney (Dr B Buick). The location of these clinics is shown in Figure 2. The veterinarians were asked to bleed up to 50 dogs from each clinic. The dogs had to be over six months old and to have always lived in Nova Scotia. If the dogs had visited an area endemic for Lyme disease the owners were asked to indicate this on an accompanying questionnaire. Sampling began at the beginning of May 1990 and continued until the beginning of November, when the vials were collected. Each dog was bled once only, and all sera were kept frozen after separation.

This phase of the project was performed in collaboration with the Maine Medical Center in Portland, Maine, which supplied all blood sampling equipment and arranged for analysis at the Veterinary Diagnostic Laboratory of Tufts University. The sera were shipped frozen to Boston and analyzed for seroconversion by enzyme-linked immunosorbent assay (19).

1984 to 1991 Collection of *I dammini*: All *I dammini* specimens were submitted by the general public. Initial identification was through the keys mentioned above (11-14). Independent confirmation was obtained by sending the specimen to at least one of the following authorities: JE Kierans, Georgia Southern University; EE Lundquist, Agriculture Canada, Ottawa; WD McEnroe, University of Massachusetts; or EH Lacombe, Maine Medical Center.

In 1990 live trapping was conducted in Digby County from May 12 to June 21 in an attempt to recover *I dammini* from small mammals. Attention was focused on Digby County because many of the deer ticks identified had been submitted from this part of the province. Traps (Ugglan multiple live traps; Hillerstorp, Sweden)

Figure 2) Location of *Ixodes dammini* recovered in Nova Scotia and the three veterinary clinics participating in the canine serosurvey

were set for a total of 1017 trap-nights in numerous localities. These included Barton, Belliveau Cove, Brighton, Centerville, Concession, Jeremy's Mill, Major's Point, Medway Lake, Plympton, Riverdale, St Mary's Bay and Weymouth.

RESULTS

Occurrence of *B burgdorferi* with *D variabilis*: A total of 650 ticks (405 male, 245 female) were collected from the 1988 sampling programme. Examination of BSK II media did not reveal spirochetes in any of the tubes, although there was heavy growth of rods and cocci. Tubes with filamentous structures suggestive of spirochetes were shipped to RA Wirtz of the Walter Reed Army Medical Center in Washington DC. Indirect fluorescent antibody testing with both species-specific monoclonal H5332 and genus-specific H9724 confirmed that they all were negative. Control tubes produced positive growth of *B burgdorferi*.

Occurrence of *B burgdorferi* and seroconversion in small mammals: The 1988 live trapping captured 87 animals consisting of one *Blarina brevicauda*, one *Napaeozapus insignis*, two *Tamiasciurus hudsonicus*, three *Tamias striatus*, 36 *Peromyscus leucopus* and 44

Clethrionomys gapperi. Inoculation of spleen and kidney homogenates in BSK II for spirochetes all proved negative. Indirect fluorescent antibody testing performed on 20 of the *P leucopus* serum samples all proved negative (titres less than 1:8). The remaining 16 specimens could not be tested because of inadequate blood recovery.

The 1990 trapping program captured 19 mice representing two species (18 *Zapus hudsonius* and one *P leucopus*) and 27 shrews representing two species, all of which died upon capture. No *I dammini* were found on any of these animals.

Seroprevalence in dogs: A total of 137 serum samples were collected from the three veterinary clinics (50 from Digby, 46 from Arcadia and 41 from Sydney). Analyses at Tufts University revealed that all were negative – in fact, well below the critical optical density of 0.170, which represents a value three standard deviations above that of sera from dogs from nonendemic areas.

Occurrence of *I dammini*: Since 1984 a total of 23 adult and one nymphal *I dammini* have been recovered from Nova Scotia (Table 1), all through submissions from the general public. The distribution of these ticks did not follow any obvious pattern (Figure 2). Prior to 1991 the majority of adult deer ticks were submitted in

the fall, the earliest submission occurring in June 1991 has been exceptional in that four adult ticks were recovered in March and April.

The one nymph was recovered from a recently migrated yellow throat bird, *Geothypis trichas* (Linnaeus), which was killed after colliding with the windshield of a car in Windsor.

DISCUSSION

The distribution of *D variabilis* in Canada is in southern Ontario, southern Manitoba, southeastern Saskatchewan and southwestern Nova Scotia (5,20). There was initial concern that this tick could act as a vector for Lyme disease because specimens found in the field were infected with *B burgdorferi* (21,22). Evidence has since accumulated which suggests that *D variabilis* may not be a competent vector for the disease largely because of the tick's inability for transtadial transmission (23,24). Thus the infected *D variabilis* found by Anderson *et al* (21) and Barker *et al* (22) presumably reflect ticks which have recently fed on hosts infected with the spirochete but will not maintain the bacterium long enough to represent a major transmission route to humans.

The data presented here on the zero infection of 650 *D variabilis* specimens from Nova Scotia is in keeping with this idea. It is also in keeping with more recent findings from Ontario where *D variabilis* ticks sampled outside the endemic area of Long Point have never shown infection with spirochetes (personal communication), and also from Manitoba, where a large survey of *D variabilis* produced negative results (25,26).

The recovery of 24 *I dammini* from various locations around the province is more intriguing and relevant to potential Lyme disease. This collection of 24 deer ticks represents the highest recovery in Canada, excluding Long Point (27). Larvae have not been observed in the province (see addendum) so these specimens cannot be considered members of an indigenous population. A statistically valid search for larvae has never been conducted and is needed, in light of the number of adult specimens recovered.

The deer ticks have been submitted from most regions of the province with the exception of Cape Breton Island (see addendum). Such a dispersion is probably the result of ticks dropping off birds as they alight on their northward migration. Certainly the infestation of birds in the New England states has been well documented (9,28,29), and the recovery of the nymph on a Yellow throat in the first week of June 1990 supports this contention. The 19 adult *I dammini* collected in years prior to 1991 could then represent nymphs introduced on birds in the same years. It was believed that such populations posed little threat to the establishment of an indigenous population because these ticks would probably perish during the winter.

The recovery of four adult *I dammini* (the three

females were all engorged) in early spring 1991 is an interesting development. It is likely that these ticks had overwintered; the spring bird migration had not yet begun, and there are no records of adult *I dammini* infesting birds (30). Of course, even these overwintered ticks may have originated from nymphs introduced by birds the previous summer; it nonetheless raises the possibility of a spring breeding season in addition to a fall season (31). The provision of two breeding seasons increases the chance of successful mating which is recognized as the most serious impediment to the completion of the three-host life cycle of the deer tick.

I dammini have been recorded in the state of Maine as far north as Machias (32), with indigenous populations documented further south (8,33,34). Some of these more isolated deer tick populations, such as the one on Monhegan Island, may have been introduced by birds (personal communication). Generally, the distribution of deer ticks in the state follows a 32 km strip next to the coast, which could reflect the moderating influence of the ocean on temperature. Nova Scotia has a similar oceanic climate; indeed, there are pockets such as the Annapolis Valley where the climate provides a warm summer (35), which could be amenable to the establishment of *I dammini*. Certainly the province appears no more inhospitable to *I dammini* than the northern states of Maine or Wisconsin with their established foci (36,37).

If *I dammini* is moving into Nova Scotia, there is no evidence that *B burgdorferi* is present. In addition to the negative results reported here on the cultivation of the spirochete from small mammals, seroconversion in small mammals, and seroconversion in dogs, there has been no confirmed case of Lyme disease in the human population (personal communication). Magnarelli *et al* (38,39) found high titres of *B burgdorferi* in 20.5% of the rodent population in endemic areas of New England, and Anderson *et al* (40) were able to culture spirochetes from 75% of the small mammal population in Connecticut. Obviously Nova Scotia does not compare to these high incidence areas.

The canine serosurvey represents more persuasive evidence on the low incidence in Nova Scotia. The data presented here do not suffer from the quoted problems of cross-reactivity and false positives (41) as it is exceptional for its lack of reactivity. Lindenmayer *et al* (19) after a comparison of the enzyme-linked immunosorbent assay used in the present study reported a maximum specificity of 93.5% (18). A false positive percentage of 6.5% did not apply to these data. The 41 samples taken from Sydney were designed as negative controls because of the lack of ticks *D variabilis* and *I dammini* on Cape Breton Island. The questionnaire completed by the owners confirmed that this was the case. In contrast, the dogs sampled in Digby and Yarmouth Counties experienced a considerable tick load. The exceptionally low level of reactivity in all three

populations suggests that Lyme disease is not yet present in the province's dogs.

The complete lack of evidence of *B burgdorferi* in Nova Scotia is reassuring news for the general public. Perhaps it is not too surprising considering the limited number of the classic vector, *I dammini*, and the confirmation that the abundant tick, *D variabilis*, is not a competent vector. The situation deserves careful monitoring in the years ahead. The deer ticks found in Nova Scotia over the past seven years could represent small pockets of established populations or may indicate adventitious populations attempting to establish themselves. If the latter, then the millions of birds migrating through the province every year and bringing nymphs with them constitute a formidable 'attempt' each spring which should not be ignored. Weisbrod and Johnson (42) demonstrated that approximately 1% of

the birds in the central flyway were infested with *I dammini*, and of these deer ticks about 20% were infected with *B burgdorferi*.

Maine is now considered an emerging area for Lyme disease, with 26 documented cases (14 acquired in the state) and indigenous populations of *I dammini* in the south of the state (32). The vector is crucial to the epidemiology of the disease (43), and in order to predict if Nova Scotia could become an emerging area for Lyme disease in Atlantic Canada, further clarification of the status of *I dammini* in the province is required.

ADDENDUM

A gravid *I dammini* adult was discovered on Cape Breton Island in the summer of 1991. The eggs ultimately hatched into larvae. Full details have been submitted to *Canada Communicable Disease Report*.

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