
Borrelia garinii in Seabird Ticks (*Ixodes uriae*), Atlantic Coast, North America

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Borrelia garinii is the most neurotropic of the genospecies of *B. burgdorferi* sensu lato that cause Lyme disease in Europe, where it is transmitted to avian and mammalian reservoir hosts and to humans by *Ixodes ricinus*. *B. garinii* is also maintained in an enzootic cycle in seabirds by *I. uriae*, a tick found at high latitudes in both the Northern and Southern Hemispheres. To determine whether *B. garinii* is present in seabird ticks on the Atlantic Coast of North America, we examined 261 *I. uriae* ticks by polyclonal antiborrelial fluorescent antibody. Ten of 61 ticks from Gull Island, Newfoundland, were positive for borreliae by this screen. Amplicons of DNA obtained by PCR that targeted the *B. garinii* rrs-rrla intergenic spacer were sequenced and matched to GenBank sequences for *B. garinii*. The potential for introduction of this agent into the North American Lyme disease enzootic is unknown.

In Europe, Lyme disease is caused by 3 genospecies of *Borrelia burgdorferi* (i.e., *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto), while in North America only *B. burgdorferi* sensu stricto, the genospecies present in *I. scapularis* ticks, has been implicated in human disease. *B. garinii*, the most neurotropic of these 3 genospecies, causes most neurologic Lyme disease in Europe, including cases of meningopolyneuritis and, rarely, encephalomyelitis (1–3). The presence of multiple pathogenic genospecies that cause Lyme disease in Europe complicates serodiagnostic testing (4).

In Eurasia, *B. garinii* is transmitted to avian and rodent hosts and to humans by *I. ricinus*, the sheep or forest tick,

and *I. persulcatus*, the taiga tick (5–9). *I. uriae*, the seabird tick, also maintains this agent in a “silent” enzootic cycle in seabirds at their nesting sites over a wide but discontinuous area (10–13). Although these 2 enzootic cycles are generally geographically and ecologically separate, interchange of *B. garinii* strains may occur at sites where both vectors coexist (14). The risk for seabird-associated strain types of *B. garinii* to cause Lyme disease, however, is not known (15).

Although *B. garinii* is present in seabird ticks in a nearly circumpolar distribution in both the Northern and Southern Hemispheres (12,13), including Alaska, the presence of *B. garinii* in *I. uriae* ticks at sites on the North Atlantic Coast has not previously been documented. We sought to determine whether *B. garinii* is present in ticks obtained from colonial seabird nesting sites on the Atlantic Coast of North America.

Methods

Seabird researchers working at several sites on the Atlantic Coast in the United States and Canada (Figure 1) submitted *I. uriae* ticks to our laboratory for analysis (16). Ticks were identified to species, stage, and degree of engorgement (17). A subset of ticks was dissected, and midguts were screened for spirochetes by fluorescent microscopy by using a polyclonal antiborrelial antibody (18).

DNA was extracted from *Borrelia*-positive ticks by using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). DNA amplification was performed in a designated room with genus-specific primers that include the partial sequence of rrs-rrla intergenic spacer region as described by Bunikis et al. (19) with use of negative con-

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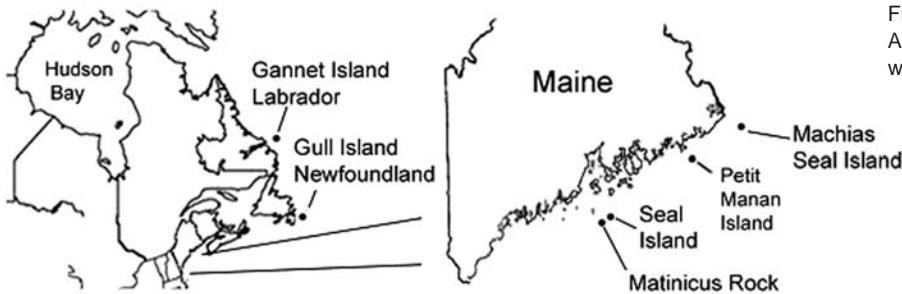


Figure 1. Locations in Maine (USA) and Atlantic Canada where *Ixodes uriae* ticks were collected.

trols. Amplification products were viewed on a 1% agarose gel containing 0.5 µg/mL ethidium bromide. At a second laboratory, ticks positive by fluorescent antibody screen were prepared as above for DNA extraction, and PCR was performed by using primers directed at the 16S ribosomal DNA. Sequences of amplicons obtained at both laboratories were confirmed to be *B. garinii* by comparison with known sequences in the GenBank database.

Results

I. uriae ticks submitted from 6 seabird colony sites and several hosts in the United States and Canada were processed (Table). Through 2005, 880 *I. uriae* ticks recovered primarily from Atlantic puffin chicks or their nests, were submitted from Maine sites, and another 383 were submitted from sites on the Atlantic Coast of Canada. Over 200 ticks from Maine sites and 61 ticks from Canadian sites off the coast of Newfoundland and Labrador were screened for borreliae by fluorescent microscopy.

Spirochetes were detected only in ticks from Gull Island, Newfoundland (47° 15'N, 52° 46'W), where 9 of 22 adults and 1 of 39 nymphal ticks were positive. DNA was extracted from 2 of these ticks, and PCR showed a 1,900-base amplicon of the *rrs-rrl*s intergenic spacer region that matched with *B. garinii* on comparison with GenBank sequences. Two additional ticks were examined in the laboratory of Sam Telford (Tufts University School of Veterinary Medicine, Grafton, MA, USA) by means of

PCR targeting of 16S ribosomal DNA and again confirmed a match for *B. garinii* (GenBank bankit no. 800902 DG463373). Figure 2 shows the sequence from one of the ticks shown in an alignment with sequences from *B. burgdorferi* strain B31 and a representative *B. garinii* sample from GenBank.

Discussion

The finding of *B. garinii* in *I. uriae* ticks from Gull Island, Newfoundland, adds to the known distribution of this agent and increases the likelihood that this agent is present in other colonial seabird nesting sites on the Atlantic Coast of North America, as is the case in Europe. The recent emergence of *I. scapularis* in coastal Maine and some Maritime Canadian sites (20,21) brings these 2 enzootic cycles of different genospecies of *B. burgdorferi* into proximate areas, although their ecologic settings differ. The public health importance of this finding depends on the probability of the introduction of *B. garinii* into emergent *I. scapularis*-vectored *B. burgdorferi* s.s. cycles and its potential maintenance in this cycle. The public health effects also depend on the pathogenic potential for human disease caused by seabird-associated strains of *B. garinii* (15).

The remote geographic and, at some sites, isolated topographic location of colonial seabird colonies provide significant barriers to the introduction of *B. garinii* into other vector ticks and their reservoir hosts. *I. scapularis* is

Table. Submissions of *Ixodes uriae* from coastal Maine (USA) and Canada, 1996–2005

Site	Host species					On humans	Flag/drag sampling	Soil/litter sample
	Atlantic puffin (<i>Fratercula arctica</i>)	Murre (<i>Uria aalge</i>)/Razorbill (<i>Alca torda</i>)	Black guillemot (<i>Cepphus grille</i>)	Herring gull (<i>Larus argentatus</i>)	Common eider (<i>Somateria mollissima</i>)			
Machias Seal Island*	218					5	52	
Matinicus Rock*	258	23	25			210		
Petit Manan Island*	56		12					
Seal Island*	46							
Gannet Island†	111	88			8	72	31	
Gull Island‡	22			22		5	76	200

*Maine, USA.

†Labrador, Canada.

‡Newfoundland, Canada.

Bg AJ009753	TTGTTAATGGATGAAAGGAAGCCCTTAAAGCTTCGCTGTAGATGAGTCTGCGTCTTATT	209
Gull Island	TTGTTAATGATGAAAGGAAGCCCTTAAAGCTTCGCTGTAGATGAGTCTGCGTCTTATT	60
Bb X57404	TTGTTAATGATGAAAGGAAGCCCTTAAACANT-CGCTTGTAGATGAGTCTGCGTCTTATT	239

Bg AJ009753	AGCTAGTTGTTGGTGGTAAATGCTTACCAGGCGATGATAAGTAAACCGGCTGAGAGGGTG	269
Gull Island	AGCTAGTTGTTGGTGGTAAATGCTTACCAGGCGATGATAAGTAAACCGGCTGAGAGGGTG	120
Bb X57404	AGCTAGTTGTTGGTGGTAAATGCTTACCAGGCGATGATAAGTAAACCGGCTGAGAGGGTG	298

Bg AJ009753	AACGGTCCACACTGGAAGCTGAGATAACGGTCCAGACTCTACCGGAGGCGACAGCTAAGAAAT	329
Gull Island	AACGGTCCACACTGGAAGCTGAGATAACGGTCCAGACTCTACCGGAGGCGACAGCTAAGAAAT	180
Bb X57404	AACGGTCCACACTGGAAGCTGAGATAACGGTCCAGACTCTACCGGAGGCGACAGCTAAGAAAT	356

Bg AJ009753	CITCCGCAATGGGCGAAGCCCTGACGAGCGACACTGCCTGATGAAAGAGGTCGAAAGA	389
Gull Island	CITCCGCAATGGGCGAAGCCCTGACGAGCGACACTGCCTGATGAAAGAGGTCGAAAGA	240
Bb X57404	CITCCGCAATGGGCGAAGCCCTGACGAGCGACACTGCCTGATGAAAGAGGTCGAAAGA	415

Bg AJ009753	TTGTAAATTCITTTTATAAATGAGGAATAAAC-TTGTAGGAATGACAAAGTGTATGACG	448
Gull Island	TTGTAAATTCITTTTATAAATGAGGAATAAAC-TTGTAGGAATGACAAAGTGTATGACG	299
Bb X57404	TTGTAAATTCITTTTATAAATGAGGAATAAGCCITTTGTAGGAATGACAAAGTGTATGACG	475

Bg AJ009753	TTAATTTATGATAAAGCCCGGCTAATTACGTCGCAAGCAGCCGCGTAATACGTAAAGGGG	508
Gull Island	TTAATTTATGATAAAGCCCGGCTAATTACGTCGCAAGCAGCCGCGTAATACGTAAAGGGG	359
Bb X57404	TTAATTTATGATAAAGCCCGGCTAATTACGTCGCAAGCAGCCGCGTAATACGTAAAGGGG	535

Bg AJ009753	CGAGCGTGTGTCGGGATTAATTGGGCGTAAAGGGTGAGTAAAGCGGATATATAAGCTATGTC	568
Gull Island	CGAGCGTGTGTCGGGATTAATTGGGCGTAAAGGGTGAGTAAAGCGGATATATAAGCTATGTC	419
Bb X57404	CGAGCGTGTGTCGGGATTAATTGGGCGTAAAGGGTGAGTAAAGCGGATATATAAGCTATGTC	593

Figure 2. Alignments of 16S RNA sequences from GenBank: Bg AJ009753, *Borrelia garinii*; Bb X57404, *B. burgdorferi* strain B31, Gull Island, Newfoundland, Canada.

dispersed to remote coastal islands of the North American Atlantic Coast during bird migration (22,23), but its establishment at these sites requires the presence of deer (24–26). With rare exception, deer are absent from sites with large seabird colonies, which are usually limited to offshore islands. Dispersal of infected *I. uriae* to proximate coastal areas by seabird hosts is unlikely because most species of seabirds parasitized by *I. uriae* are highly philopatric and forage at sea. One exception might be gulls, which may move between coastal and island sites. Passerine birds, which may forage near seabird colonies, provide another potential mechanism for dispersal of *B. garinii*, either through movement of infected *I. uriae* ticks or by serving as reservoir hosts of this agent. However, the frequency of parasitism of passerine birds by *I. uriae* ticks is unknown.

If *B. garinii* was introduced into *I. scapularis* ticks, its maintenance in this cycle would depend on the vector competence of *I. scapularis* for *B. garinii*, the reservoir competence of available hosts, and perhaps the population genetics and strain diversity of *B. garinii* (14,27). Although *I. scapularis* is vector-competent for transmission of *B. garinii* to rodents, its efficiency of transmission appears lower than for *B. burgdorferi* s.s. (28). In addition, the vector competence of *I. scapularis* for seabird-associated strains of *B. garinii* has not been tested. The presence of similar ribotypes of *B. garinii* in *I. ricinus* ticks on a European island suggests that interchange of different *B. garinii* strains in different ecologic cycles may occur (14).

To determine the public health importance of *B. garinii* in seabird colonies along the North Atlantic coast, additional studies on the issues of dispersal, vector competence, and reservoir host competence are needed. All strain

types of *B. garinii* may not be pathogenic for humans, and future studies should also address the potential for seabird-associated strains to cause disease.

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Dr Smith is an infectious disease physician and codirector of the Vector-borne Disease Laboratory at the Maine Medical Center Research Institute. His research interests include the ecology of Lyme disease emergence and the strain diversity of *B. burgdorferi*.

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