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The prevalence of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) and the Agent of Human Granulocytic Ehrlichiosis (Rickettsiaceae: Ehrlichieae) in *Ixodes scapularis* (Acari:Ixodidae) Collected During 1998 and 1999 from Minnesota.

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ABSTRACT We tested 103 adult *Ixodes scapularis* Say from 12 counties in Minnesota for the presence of *Borrelia burgdorferi* and the causative agent of human granulocytic ehrlichiosis (HGE), using polymerase chain reaction (PCR). A total of 17 ticks (16.5%) was positive for *B. burgdorferi* using nested PCR for the flagellin gene, or both PCR for the *osp*A gene and nested PCR for the flagellin gene. A total of four ticks (3.8%) was positive for the agent of HGE using nested PCR for 16S rDNA. Counties in Minnesota with established and recently reported populations of *I. scapularis* both had ticks infected with *B. burgdorferi*. The agent of HGE was only detected in counties with established *I. scapularis* populations.

KEY WORDS *Borrelia burgdorferi*, human granulocytic ehrlichiosis, *Ixodes scapularis*, Minnesota

IXODES SCAPULARIS SAY is the primary vector for the etiological agents of Lyme disease and human granulocytic ehrlichiosis (HGE) (McQuiston et al. 1999, Piesman et al. 1999). Knowledge of *I. scapularis* distribution and the prevalence of pathogens in those ticks is important for developing strategies to prevent the transmission of these two human disease-causing agents (Dennis et al. 1998, Stafford et al. 1998).

A statewide survey of *I. scapularis* ticks collected in Minnesota in 1985 and 1986 showed that 10% were infected with *Borrelia* sp., using a genus-specific immunoassay (Drew et al. 1988). Twenty-two percent of *I. scapularis* collected from deer harvested in St. Croix State Park in east central Minnesota in 1989 were infected with *B. burgdorferi* (Gill et al. 1993). However, there have been no recent statewide surveys of *B. burgdorferi* occurrence in Minnesota ticks.

The HGE agent was first reported as a human pathogen from cases in Minnesota and Wisconsin (Bakken et al. 1994). Eight percent of ticks collected in Wisconsin in 1982 and 1991 were infected with the HGE agent (Pancholi et al. 1995). However, current data on the prevalence of the agent of HGE in *I. scapularis* from Minnesota are lacking.

Indications of a wider distribution of *I. scapularis* in Minnesota than was previously reported (Sanders and Guilfoile 2000) led us to investigate the prevalence of *B. burgdorferi* and the agent of HGE in *I. scapularis* ticks collected from 12 different counties in Minnesota.

Materials and Methods

Tick Collection. Of the 103 adult *I scapularis* ticks tested, 85 (83%) were collected during the Fall of 1998 and 1999 by grouse hunters from their hunting dogs. Of the remainder of the ticks tested, one tick was collected from a human, two were collected from horses, three were collected from cats, and 12 were collected from deer (Sanders and Guilfoile 2000). Once identified, specimens were stored in sterile microcentrifuge tubes at –70°C before DNA isolation.

DNA Isolation. DNA was isolated from most of the ticks by using the Qiagen DNeasy kit for animal tissues (QIAGEN, Chatsworth, CA), with minor modifications of the protocol. Female ticks were cut in half longitudinally; one-half was analyzed immediately, and the other half was stored at −80°C for further study, if necessary. Male ticks were cut into several pieces, all of which were used in the DNA isolation procedure. Tick fragments were placed in a sterile 1.5-ml microcentrifuge tube containing 180 μl PBS and homogenized using a sterile, disposable microtube pestle. Proteinase K (20 μl of a 20 mg/ml solution) was added and the sample was vortex mixed. The lysate was then incubated overnight in a 55°C water bath with periodic mixing. From this point, the manufacturer's procedure was followed.

DNA STAT-60 was also used to isolate DNA from 38 female *I. scapularis* (Tel-Test, Friendswood, TX) as

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described previously (Mauel et al. 1999). Purified DNA samples were stored at −20°C.

Tick 16s rDNA PCR. Tick lysates were initially screened by PCR for tick 16S rDNA to verify that samples contained amplifiable DNA. Primers 16S +1 and 16S − 2 were used in the reaction mixture, and reactions were performed in either a Perkin-Elmer model 480 or 2400 thermocycler as previously described (Black and Piesman 1994). Reactions were considered positive if they generated a 335-bp DNA fragment.

***Borrelia burgdorferi* PCR.** Tick lysates with amplifiable DNA (based on PCR of tick 16S rDNA sequences) were next tested for the presence of *B. burgdorferi.* PCR for the *osp*A gene, using the OSPA149 and OSPA319 primers, was performed as described previously (Rys 1993), except that isopsoralen was not used, dUTP was substituted for dTTP, Advan*Taq* DNA Polymerase (Clontech, Valencia, CA) was used instead of *Taq* polymerase, and the final reaction volume was 25 μl. Reactions were considered positive if they generated a 195 bp DNA fragment.

All ticks lysates with amplifiable DNA were also tested using nested PCR to detect the flagellin gene of *B. burgdorferi* as described previously (Johnson et al. 1992), except that dUTP was used in place of dTTP, Advan*Taq* Polymerase was used in place of *Taq* polymerase (Clontech, Valencia, CA), the final reaction volumes were 25 μl, and 1 μl of a 50-fold dilution of PCR products from the first reaction was used in the second reaction. Reactions were considered positive if they generated a 390-bp DNA fragment.

HGE PCR. Nested PCR for HGE 16S rDNA was used to test the samples for the presence of the causative agent of human granulocytic ehrlichiosis as described previously (Massung et al. 1998) except that dUTP was used in place of dTTP, Advan*Taq* Polymerase (Clontech, Valencia, CA) was used instead of *Taq* DNA polymerase, and the final reaction volumes were 25 μl. Reactions were considered positive if they generated a 546 bp DNA fragment.

Detection of PCR Products, Sequencing of PCR Products. All PCR DNA fragments were analyzed after electrophoresis in a 2% agarose/0.5× TBE gel stained with Gelstar stain (FMC Bioproducts, Rockland, ME) to enhance the visualization of DNA bands. Before DNA sequencing, PCR products were purified with Centri-Spin 40 columns (Princeton Separations, Adelphia, NJ) as directed by the manufacturer. The HGE PCR products were sequenced using the ge2 primer (Massung et al. 1998); the *B. burgdorferi* flagellin PCR products were sequenced using the inner one *fla* primer (Johnson et al. 1992), using a Big Dye 3.0 cycle sequencing kit on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA).

Contamination Control. To prevent contamination of the PCR reactions with DNA from previous reactions, DNA purification was done in a laminar flow hood in one room, PCR set-up was in a laminar flow hood in a second room, and PCR reactions and sample analysis were done in a third room. Separate sets of dedicated pipettors were used for sample set-up and sample analysis, and filtered tips were always used. dUTP was used in all reactions to allow for the elimination of DNA contamination using Uracil-N-glycosylase.

Negative and positive control reactions were used in each set of PCR reactions. Negative controls used water rather than tick lysate and positive controls were either purified pathogen DNA or tick lysates from previous positive reactions.

Table 1. Number of *I. scapularis* ticks collected in Minnesota in 1998 and 1999, infected with *B. burgdorferi* and the agent of HGE from counties with established and recently reported populations

	No. ticks tested	No. positive for B. burgdorferi (%)	No. positive for the HGE agent (%)
	Counties with established populations[a]		
	34	7 (21)	4 (12)
	Counties with recently reported tick populations[b]		
	69	10 (14)	0 (0)
Total	103	17 (16.5)	4 (3.9)

[a] Counties in Minnesota with established *I. scapularis* populations that were tested and had positives for HGE or *B. burgdorferi* were Aitkin, Kanabec & Pine.

[b] Counties in Minnesota with recently reported *I. scapularis* populations that were tested and had positives for *B. burgdorferi* were Beltrami, Cass, Hubbard & Itasca (Drew et al. 1988, Sanders and Guilfoile 2000).

Results

Ixodes scapularis that had been collected as part of a previous study of tick distribution (Sanders and Guilfoile 2000) were analyzed for the presence of tick-borne pathogens. The ticks were adults (55 females and 48 males) and most were collected from dogs by grouse hunters during the Fall of 1998 and 1999. All the ticks that tested positive for either pathogen were collected from dogs.

Analysis of Two DNA Isolation Methods. A previous report indicated that DNA-STAT 60 was more effective at isolating DNA from ticks for detection of HGE DNA, compared with a previous version of the Qiagen DNeasy kit (Mauel et al. 1999). Therefore, we tested the amplification of tick 16S rDNA from a total of 38 female *I. scapularis* using both the Qiagen DNeasy method and DNA-STAT 60. Using the DNeasy method, 27 of 38 females (71%) were PCR positive for tick 16S rDNA. Using DNA-STAT-60, 22 of the 38 females (59%) were PCR positive for tick 16S rDNA. We therefore used the DNeasy method for the balance of the DNA preparations.

PCR Amplification of Tick 16S rDNA. Initially, the tick lysates were tested for the presence of amplifiable DNA using PCR with primers specific for the tick 16S rDNA gene. A total of 103 of the 148 ticks tested positive for 16S rDNA (70%); ticks with amplifiable DNA were collected in 12 counties in Minnesota (Table 1).

Prevalence of *B. burgdorferi* and the Agent of HGE. The 103 ticks with amplifiable DNA were then further tested for the presence of *B. burgdorferi* and the agent of HGE by using PCR. Seventeen ticks tested positive for *B. burgdorferi* (16.5%) (Table 1). Nine of the infected ticks were males and eight were females. Four-

teen of the ticks tested positive by both *osp*A and nested flagellin PCR, and three tested positive by nested flagellin PCR only. Products from three different flagellin PCR reactions were sequenced and all of them matched the *B. burgdorferi* flagellin gene sequences in Genbank.

Four ticks (3.8%) tested positive for the presence of the causative agent of HGE (three females and one male) (Table 1). Products from two HGE PCR reactions were sequenced and both matched the HGE 16S rDNA gene sequences in Genbank. A single tick was co-infected with both *B. burgdorferi* and the causative agent of HGE. *Borrelia burgdorferi* was detected in counties with historically endemic and recently established populations of *I. scapularis* (Table 1). The agent of HGE was found only in counties with historically endemic populations of *I. scapularis* (Table 1).

Discussion

Preventing the transmission of Lyme disease and human granulocytic ehrlichiosis requires knowledge of the geographic range of the vector, *I. scapularis*, and the occurrence of pathogen carriage by those ticks. In this report, we determined that blacklegged ticks from seven counties in Minnesota carried *B. burgdorferi*, the agent of HGE, or both pathogens.

The prevalence of *B. burgdorferi* in *I scapularis* in our study (16.5%) was similar to that reported from ticks collected in Minnesota in 1985 and 1986 (10%) (Drew et al. 1988). The overall prevalence of the agent of HGE in our study (3.8%) was somewhat lower than that reported from ticks collected in Wisconsin in 1982 and 1991 (7.9%) (Pancholi et al. 1995). However, the prevalence of the agent of HGE in counties with historically reported populations of *I. scapularis* was somewhat higher (12%) than reported previously (Table 1) (Pancholi et al. 1995).

Blacklegged ticks from historically endemic sites as well as ones from recently identified areas (Drew et al. 1988, Sanders and Guilfoile 2000) carried *B. burgdorferi.* This suggests that, at least in Minnesota, the environmental and biological factors that allow for the growth and expansion of *I. scapularis* populations also help maintain this pathogen in ticks. Physicians who may treat patients with tick-borne diseases and others at risk for contracting tick-borne diseases should be aware of the potential for expansion of the range of *I. scapularis* and the likelihood of a concomitant expansion in the range of tick-borne diseases.

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