Comparison of Tick Feeding Success and Vector Competence for *Borrelia burgdorferi* Among Immature *Ixodes scapularis* (Ixodida: Ixodidae) of Both Southern and Northern Clades

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ABSTRACT Northern and southern *Ixodes scapularis* Say populations differ greatly in density, host utilization, and especially questing behavior of the immatures. Haplotypes of *I. scapularis* in North America can be divided into two major clades—the All American Clade (haplotypes A through J) and the Southern Clade (M through O). This genetic variation may affect feeding success and vector competence. This study compared feeding success of larval *I. scapularis* measured by time-to-drop-off and subsequent transmissibility success of *Borrelia burgdorferi* to mice using ticks from Mississippi, Connecticut (both F haplotype), and Louisiana (haplotype O). Northern ticks (CT) fed to repletion much faster than MS and LA ticks: overall, 73.6% of CT ticks had dropped off mice at Day 3 compared to only 1.7% and 6.6% of ticks dropped off for MS and LA ticks at that same time point. As for vector competence, 4 of the 4 mice in each case (MS or CT) that had been fed on by infected nymphs tested positive for *B. burgdorferi*. In a second experiment, 5 of the 6 mice tested positive for *B. burgdorferi* after exposure to infected LA ticks as compared with 3 of the 4 mice exposed to infected CT ticks. These data demonstrate that there is no difference in northern and southern populations of *I. scapularis* in their ability to transmit *B. burgdorferi*, but the ability of the northern populations to feed rapidly on rodents exceeds that of southern populations.

KEY WORDS Ixodes scapularis, Immatures, Lyme disease, Feeding success, Vector competence

Introduction

Lyme borreliosis (LB), caused by one or more "genospecies" of *Borrelia burgdorferi*, is a systemic tick-borne illness displaying a variety of clinical manifestations that occurs over much of the world in temperate zones (Gray et al. 2002). In North America, *Borrelia burgdorferi sensu stricto* is the etiologic agent of LB, which is transmitted by *Ixodes scapularis* Say ticks in the northeastern and midwestern United States, while *Ixodes pacificus* Cooley and Kohls, is the vector along the Pacific Coast. Immature ticks acquire the infection in nature while feeding as larvae and nymphs on infected reservoir vertebrate hosts such as small mammals or birds. In the northeastern and midwestern United States, the cycle of tick transmission of *B. burgdorferi sensu stricto* is driven by a focus on *Peromyscus* mice, chipmunks, shrews, and to some extent birds, but in the southern United States, little is known about the reservoirs, other than that lizards may be involved (Apperson et al. 1993, Levin et al. 1996, Durden et al. 2002). Confounding the issue is controversy (often extreme) about whether and to what extent "true" Lyme borreliosis occurs in the southern states (Auwaerter et al. 2011, Goddard et al. 2012, Clark et al. 2013), thus highlighting the need for ecological and epidemiological research on LB in that region.

Tick–host surveys are important in determining host–vector–pathogen relationships. However, host surveys by themselves do not provide specific information about relative success of tick development. The host species' influence on tick development and molting success is largely unexplored beyond a few studies found in the scientific literature (Bishopp and Hixson 1936, Trager 1939, Hixson 1940, Sonenshine and Atwood 1967, Amin 1969, Koch and Hair 1975, Moraru et al. 2012). Theoretically, when ticks take larger bloodmeals (and quicker) on one host as opposed to another, this means greater host–parasite synchronization, and that the particular host is well-suited for that tick species (Koch and Hair 1975).

I. scapularis populations from northern and southern parts of their range differ greatly in population density, host utilization, and, particularly, questing behavior of the immatures (Piesman 2002, Goddard and Goddard 2008, Goddard and Goddard 2010). The division

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between "All American" and "Southern" lineages was first established by Norris (Norris et al. 1996), but Qiu further refined the classification, stating that *I. scapularis* haplotypes in North America can be divided into two major clades—the All American Clade (haplotypes A through J), and the Southern Clade (M through O; Qiu et al. 2002). A recent analysis utilizing single-nucleotide polymorphisms supports this view, showing that *I. scapularis* ticks collected from Mississippi and Georgia display greater genetic variation than those from New Jersey or Virginia (Van Zee et al. 2013). This genetic variation may affect tick feeding success and vector competence among southern *I. scapularis*. Previous studies have compared the vector competence of *I. scapularis* collected from the northern and southern populations (Piesman and Sinksky 1988, Sanders and Oliver 1995), but these studies were performed before it was possible to characterize the genetic background of the populations used in the experiment. The present study compares feeding success of larval *I. scapularis* measured by time-to-drop-off and subsequent transmissibility success of *B. burgdorferi* to mice using ticks from Mississippi (F haplotype), Connecticut (F haplotype), and Louisiana (O haplotype).

Materials and Methods

Mice and Ticks. Mice used in these experiments were CD-1 females, 4–5 wk of age, purchased from Charles River Laboratories (Wilmington, MA). Mice were handled according to approved protocols on file with the Centers for Disease Control and Prevention, Division of Vector Borne Diseases Animal Care and Use Committee Protocol numbers 12-003 and 09-002. *I. scapularis* colonies were derived from three states: Connecticut (CT), Mississippi (MS), and Louisiana (LA). The CT colony originated from female *I. scapularis* collected in Bridgeport, CT, in 2009 and maintained as previously described (Piesman 1993). MS ticks were collected as adults from vegetation during March 2011 in Marshall County, MS, and fed on rabbits per previous protocols (Piesman 1991). LA ticks were derived from a colony maintained at the Tulane Primate Center, Covington, LA (but originally collected nearby).

Molecular Genetics. The genetic background of each tick colony was established by sequencing a 433 bp DNA fragment of the mitochondrial 16S rDNA gene (Van Zee et al. 2013). This fragment is enough to allow classification by haplotype as previously described (Qiu et al. 2002). Both the CT and MS ticks were classified as haplotype "F," the most common haplotype of the "Northern Clade" or "All American Clade" (Norris et al. 1996), whereas the LA ticks were haplotype "O," a haplotype restricted to southern states in its distribution.

Detection of *B. burgdorferi*. Nucleic acids were isolated from ticks using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) and a Mini-Beadbeater (Biospec, Bartlesville, OK; Hojgaard et al. 2014). To test for the presence of *B. burgdorferi*, a multiplex TaqMan PCR reaction was performed, targeting both *B. burgdorferi* and *I. scapularis*. As a control for both the DNA purification and the PCR reaction, a set of primers and probe against the *actin* gene of *I. scapularis* was used (Hojgaard et al. 2014). For detection of *B. burgdorferi* DNA, previously described primers and probes for the flagellar filament cap gene (*fliD*) were used (Dolan et al. 2011). The multiplex PCR reactions were performed using iQ Multiplex Powermix (Bio-Rad), with primers in a final concentration of 300 nM, and probes in a final concentration of 200 nM. The PCR cycling conditions consisted of denature DNA at 95°C for 3 min followed by 40 cycles of 95°C for 10 s, and 60°C for 1 min on a C1000 Touch thermal cycler with a CFX96 real time system (BioRad).

Drop-off Study and Vector Competence. In order to infect mice, a total of five nymphal *I. scapularis* infected with the B31 strain of *B. burgdorferi* were allowed to feed on 4- to 5-wk-old mice ad libitum until repletion. At 3 weeks postnymphal exposure, an ear biopsy was obtained and cultured in Barbour-Stoenner-Kelly (BSK) to determine whether the animal was infected as previously described (Sinsky and Piesman 1989). All mice serving as hosts for larval ticks had positive ear biopsies on examination by darkfield microscopy. Mice were exposed to test larval ticks from different locations at 4 weeks postnymphal exposure. Logistically, we could not conduct this experiment all at once due to the number of mice involved. Therefore, three replicates (trials) for each location were performed; however, replicates were always run with northern (CT) ticks and one southern strain (MS or LA). In each of these trials, larval ticks were placed on mice and allowed to feed ad libitum. Larvae were not counted prior to being placed on hosts. Exact larval counts prior to infestation would have required prehandling and separating larvae into small batches for application. Prior experience in our lab has shown that prehandling larval batches notably reduces viability. At the time of these experiments, we only had small numbers of flat larvae available to us, especially the Louisiana larvae. Therefore, we made the decision not to precount larvae. Nonetheless, by a rough visual count, a minimum of 150 and a maximum of 350 larvae were placed on individual mice depending on the number of larvae available. Larval drop-off was assessed at least twice daily, and numbers of replete ticks found were counted and charted as to days postapplication to each mouse. Replete larval ticks were held in desiccators with saturated humidity at 22°C. At 10 days post-larval repletion, ≥5 larvae were tested for spirochetes by PCR.

For vector competence studies, in two separate experiments, groups of five nymphs (at least 2 mo postmolt) resulting from the above feedings were placed on test mice and allowed to feed to repletion, comparing the ability of MS and LA ticks to transmit *B. burgdorferi* to mice as compared with CT ticks (Note: in the case of LA ticks, three mice received less than five nymphs each due to low numbers available; Table 1). No effort was made to compare nymphal drop-off times of ticks from different locations due to the small numbers of nymphs used. Replete nymphs were subsequently tested for the presence of spirochetes by PCR. Exposed mice were then tested for

transmission of spirochetes by culturing ear, bladder, and heart at 1-mo postnymphal exposure (Piesman and Happ 1997). Each organ cultured in BSK was examined by darkfield microscopy weekly for 1 mo to detect live spirochetes.

Statistical Analysis. Pearson's Chi-squared test was used to evaluate statistical significance of differences in tick drop-off rates in northern versus southern ticks (Table 2). Specifically, this test was used to evaluate whether location and tick drop-off times were independent or related.

Table 1. Experiments assessing ability of nymphal *I. scapularis* from different locations to transmit *B. burgdorferi* to mice

Mouse[a]	No. placed on mouse	No. fed on mouse	No. ticks PCR ++	Mouse culture for Bb[b]
MS-1	5	4	4	Pos
MS-2	5	2	2	Pos
MS-3	5	1	1	Pos
MS-4	5	4	4	Pos
CT-1	5	4	2	Pos
CT-2	5	5	3	Pos
CT-3	5	5	1	Pos
CT-4	5	5	4	Pos
Second experiment				
LA-1	5	2	2	Pos
LA-2	5	2	2	Pos
LA-3	4	4	4	Pos
LA-4	3	1	1	Pos
LA-5	3	1	1	Pos
LA-6	3	1	1	Neg
CT-1	5	1	1	Pos
CT-2	5	4	3	Neg
CT-3	5	1	1	Pos
CT-4	5	4	3	Pos

[a] Mouse number with ticks from three locations—either Mississippi, Connecticut, or Louisiana.
[b] Cultured for *B. burgdorferi*.

Results and Discussion

Drop-off Study. Despite our efforts to roughly place equal numbers of tick larvae on each mouse, the numbers feeding to repletion on experimental mice were widely disparate—339 MS ticks; 75 LA ticks; and 516 CT ticks (Fig. 1). However, because actual larval numbers were not counted, no conclusions about the relative numbers of ticks recovered per location will be made here. Future studies are warranted to more accurately determine feeding success on mice of immature *I. scapularis* ticks derived from different locations. As for drop-off times, CT ticks fed to repletion much faster than MS and LA ticks: overall, 73.6% of CT ticks had dropped off mice at Day 3 compared with only 1.7% and 6.6% of ticks dropped off for MS and LA

Table 2. Statistical analysis of tick drop-off data

A

	Day 2	Day 3	Day 4	Day 5
MS–LA ticks	0	11	267	136
CT ticks	158	225	100	33

$\chi^2 = 485.4705$, df = 3, $P < 2.2e\text{-}16$

B

	Day 3	Day 4
MS–LA ticks	11	267
CT ticks	225	100

$\chi^2 = 265.2742$, df = 1, $P < 2.2e\text{-}16$

C

	Day 4	Day 5
MS–LA ticks	267	136
CT ticks	100	33

$\chi^2 = 3.2955$, df = 1, $P < 0.06947$

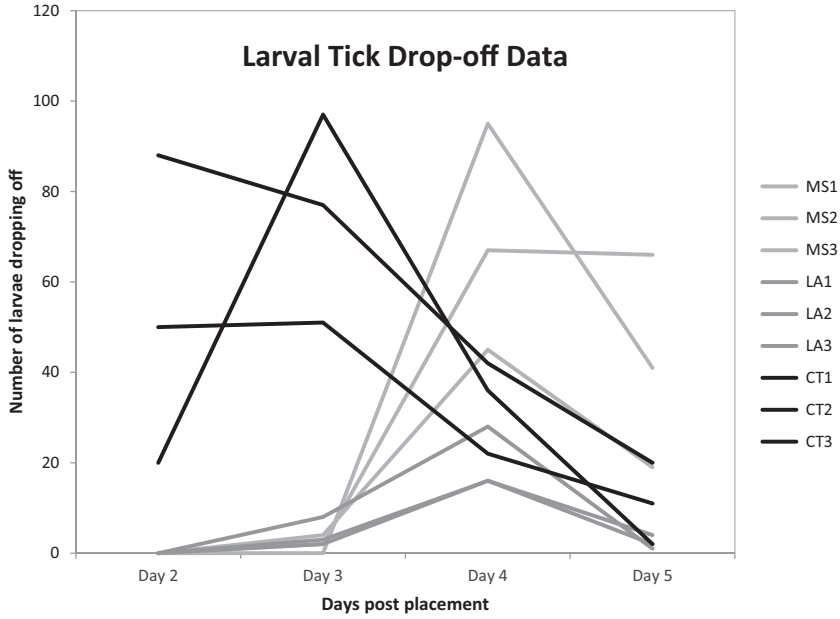

Fig. 1. Larval *I. scapularis* drop-off times from three different locations, repeated three times (three trials of each).

ticks at that same time point (Fig. 1). Statistical analysis of the data showed that location (tick origin) and time until tick drop-off were related ($P < 0.001$, Table 2). Further analysis showed that the timeframe of days 3–4 for tick drop-off was significantly related to location ($P < 0.001$, Table 2B), while the timeframe of days 4–5 for tick drop-off was not significantly related to location ($P > 0.05$, Table 2C). It is important to note that our study included only two (F and O) of several genetic haplotypes of northern and southern populations of *I. scapularis*, thereby limiting the conclusions we can make to these two particular haplotypes. Despite this limitation, there were marked differences in feeding success (as measured by drop-off times) between northern and southern larval ticks. Interestingly, even southern haplotype F (from Mississippi) did not have the same drop-off rate as northern haplotype F (from Connecticut). Perhaps there is selection pressure on southern populations of *I. scapularis* immatures to feed on lizards and thus they are not adapted to feeding rapidly on rodents. In fact, previous studies of small rodents in Mississippi have found few, if any of them infested with immature *I. scapularis* (Norment et al. 1985, Clark and Durden 2002, Moraru et al. 2012, 2013).

Vector competence. All *I. scapularis* ticks, regardless of geographic origin, easily transmitted *B. burgdorferi* to mice (Table 1). In the first experiment, 4 of the 4 mice in each case (MS or CT) that had been fed on by infected nymphs tested positive for *B. burgdorferi.* In the second experiment, 5 of the 6 mice tested positive for *B. burgdorferi* after exposure to infected LA ticks as compared with 3 of the 4 mice exposed to infected CT ticks. These data demonstrate that there is no difference in northern and southern populations of *I. scapularis* in their ability to serve as vectors of *B. burgdorferi* and points to lack of anthropophily of local immature ticks as a reason for scarcity of LB in southern states. Certainly, there may be many ecological or host-preference differences in tick populations that indirectly affect Lyme disease epidemiology in the southern United States, but southern populations of *I. scapularis* are indeed able to acquire and transmit the agent of Lyme disease.

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