Evaluating differences in host preference and phenology of the Lyme disease tick (*Ixodes scapularis)* in middle Tennessee *versus* coastal Michigan

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Abstract:

Lyme disease, caused by the bacterium *Borellia burgdorferi*, is the most commonly reported vector-borne disease in the U.S., with around 20,000 new cases each year (CDC 2007). While Lyme disease is endemic in the northeastern states (Haynes et al. 2005), southern states such as Tennessee have very few reported cases (Apperson et al. 1993). Hypotheses for why Lyme disease has not emerged in the south were explored by identifying differences in the phenology and/or host seeking behavior of *I. scapularis* at one site in middle Tennessee versus endemic areas in the northeastern U.S. *Ixodes scapularis* ticks were collected monthly through dragging and small mammal trapping. Lizard trapping was attempted. These data were then compared with published information and other data collected by our collaborators at Michigan State University. We found that the key vector, *I. scapularis* is present in Tennessee with a different phenology than those at Van Buren State Park, Michigan. However, *Borrelia burgdorferi* was not found in the ticks at our Tennessee study area. In addition, our evaluation of host seeking for lizards did not support this hypothesis for the absence of Lyme disease in the South.

Introduction

Lyme disease, caused by the bacterium, *Borrelia burgdorferi*, is the most commonly reported vector-borne disease in the U.S., with more than 20,000 new cases each year (CDC 2007). While Lyme disease is endemic in the northeastern states (Haynes et al. 2005), southern states such as Tennessee have very few reported cases (Apperson et al. 1993). The key vector of Lyme disease in the eastern US, the blacklegged tick *Ixodes scapularis*, is present in Tennessee, but it has been unclear as to why endemic Lyme disease is absent from the state. Seasonal biology and host preference may explain this absence.

Objectives:

1. To determine whether the phenology (i.e., seasonal abundance of the different life stages) of *Ixodes scapularis* in mid-Tennessee differs from the phenology of this species in Lyme Disease-endemic areas of the U.S.
2. To determine whether the relative host preference of nymphal *I. scapularis* for lizards vs. small mammals in mid-Tennessee differs from the host preference of this species in Lyme Disease-endemic areas of the U.S.

Justification:

Three hypotheses in the literature for why Lyme disease has not established in the south are:

1) Absence of the key vector species – the blacklegged tick *I. scapularis* (Dennis et al. 1998).
2) A higher ratio of lizards (which do not support *B. burgdorferi*) to rodents (which do) in the south that has led to the tick exhibiting different host-seeking behaviors (Apperson et al. 1993).
3) A different seasonal temperature pattern in southern states that alters the seasonal timing of larval, nymphal, and adult stages of *I. scapularis*, which then disrupts pathogen transmission (Clark et al. 1998).

Recent surveys (M. Rosen, pers. comm..) have demonstrated that *I. scapularis* is more widespread and abundant in mid-Tennessee than previously thought – so Hypothesis 1 is unlikely. Therefore, this research focused on Hypotheses 2 and 3.

In southeastern states, there is a higher abundance of lizards than in the northeastern states(CITE). While *I. scapularis* can feed on both lizard and mammal hosts, southern ticks may have adapted behaviorally to feed on lizards rather than on rodents. Since lizards are not competent reservoir hosts for *B. burgdorferi*, this could be a reason why Lyme disease is much less prevalent in the southeastern U.S. (Apperson et al. 1993).

For pathogen transmission of *B. burgdorferi* to occur, the *I. scapularis* larvae and an infected nymph must feed on the same competent host. Because of the two-year tick life cycle in the northeast, the seasonal timing of *I. scapularis* allows nymphs to feed on hosts before larvae. Therefore, the larvae will potentially become infected after feeding on an infected host. This process is best facilitated if the larvae abundance peak right after the nymph abundance peak. However, a warmer climate in the south could lead to a one-year life cycle and the larvae abundance peaking before the nymphal abundance peak (Clark et al. 1998). This would make the transmission cycle of *B. burgdorferi* less likely to occur.

Study Area:

Henry Horton State Park is located in Chapel Hill, Tennessee, about 40 miles south of Nashville. Its 1140 acres of eastern deciduous forest is home to many wildlife species, including deer, turkey, and small mammals. The park is frequented by visitors and their pets year-round. If Lyme disease were present here, the frequent visitation and close contact between people and wildlife would probably allow for tick bites and consequent disease transmission to people.

Materials and Methods:

To determine the presence and seasonality of *I. scapularis*, we collected ticks and their small mammal hosts at monthly intervals at Henry Horton State Park over the course of one full

year. The sampling methods included vegetation "dragging" and small mammal and lizard trapping. We standardized the drag sampling by creating seven 250m transects, evenly distributed throughout the park. When "dragging" took place, we walked each transect (and back) dragging a 1x1 meter corduroy cloth, checking the cloth for ticks every 20m (Falco and Fish 1992).

We performed live small mammal trapping and attempted lizard trapping in order to determine the host preference and relative abundance of ticks on mice and lizards in the park. Using the dragging transects, one Sherman live trap (3.5" x 3" x 9") was placed every 10 m. These traps were set (with sunflower seeds as bait) at dusk and collected and checked for captures at sunrise the following morning. Dry pitfall and funnel traps were used to attempt lizard trapping.

All animals trapped were identified to species, weighed, sexed, and all ticks, blood, and an ear biopsy were collected. Collected ticks were placed in 70 percent ethanol, brought back to the on-campus lab, identified to species, and *I. scapularis* ticks were tested for the presence of *B. burgdorferi.* Samples were tested by extracting total tick DNA, PCR amplification, gel visualization and DNA sequencing. Data collected from this study were then compared with published information and other data collected by our collaborators at Michigan State University.

Results:

When dragging, we found fewer questing larval, nymph, and adult *Ixodes scapularis* at Henry Horton State Park in Tennessee than at Van Buren State Park in Michigan, as shown in Figs. 1 and 2.

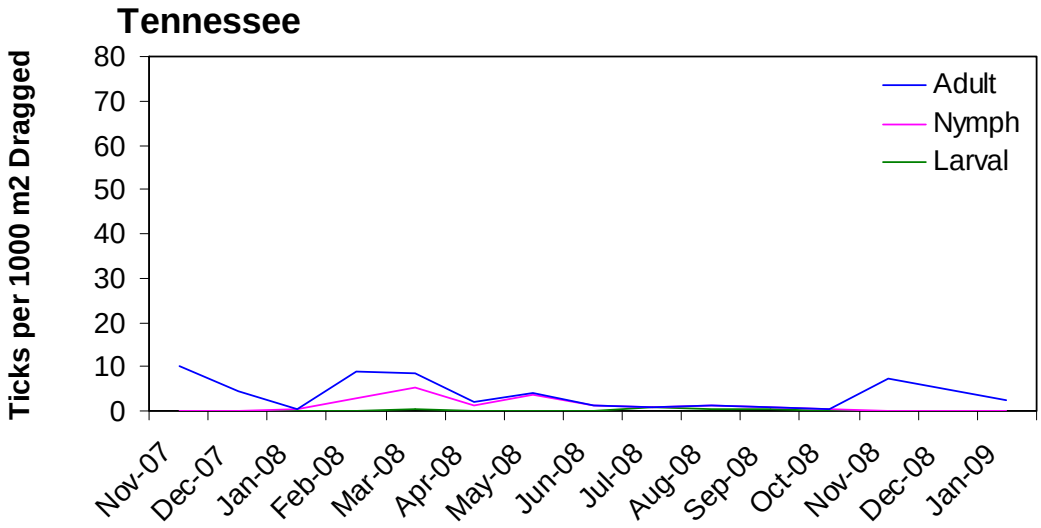

Figure 1: Numbers of *I. scapularis* ticks per 1000 m^2 dragged at monthly intervals at Henry Horton State Park (TN). The highest peaks of larval, nymphal, and adult ticks dragged were 0.71, 4.87 and 10.26, respectively, per 1000 m^2.

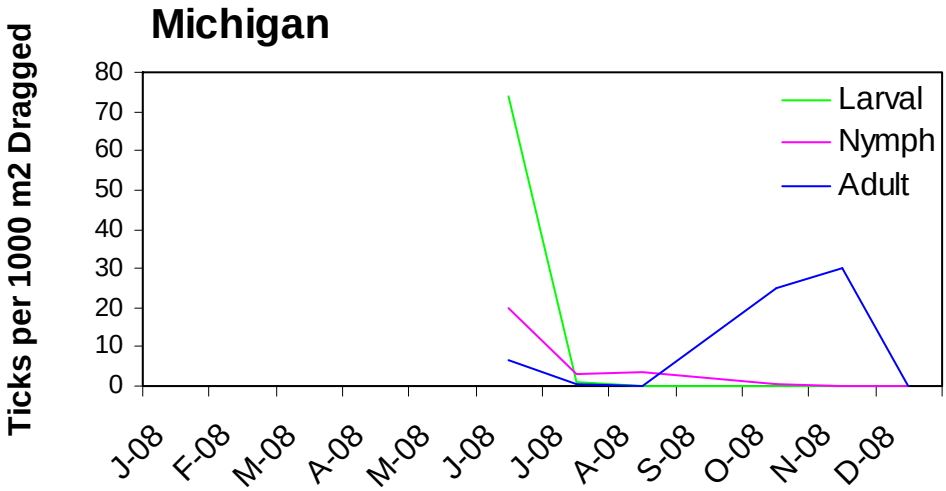

Figure 2: Numbers of *I. scapularis* ticks per 1000 m^2 dragged at monthly intervals at Van Buren State Park (MI) in 2008. The highest peaks of larval, nymphal, and adult ticks dragged were 74.09, 20.0 and 30.12, respectively, per 1000 m^2.

Van Buren State Park (MI) had higher mouse densities and a higher mean mouse catch index from June through October than did Henry Horton State Park (TN) at the same time of year (Fig. 3).

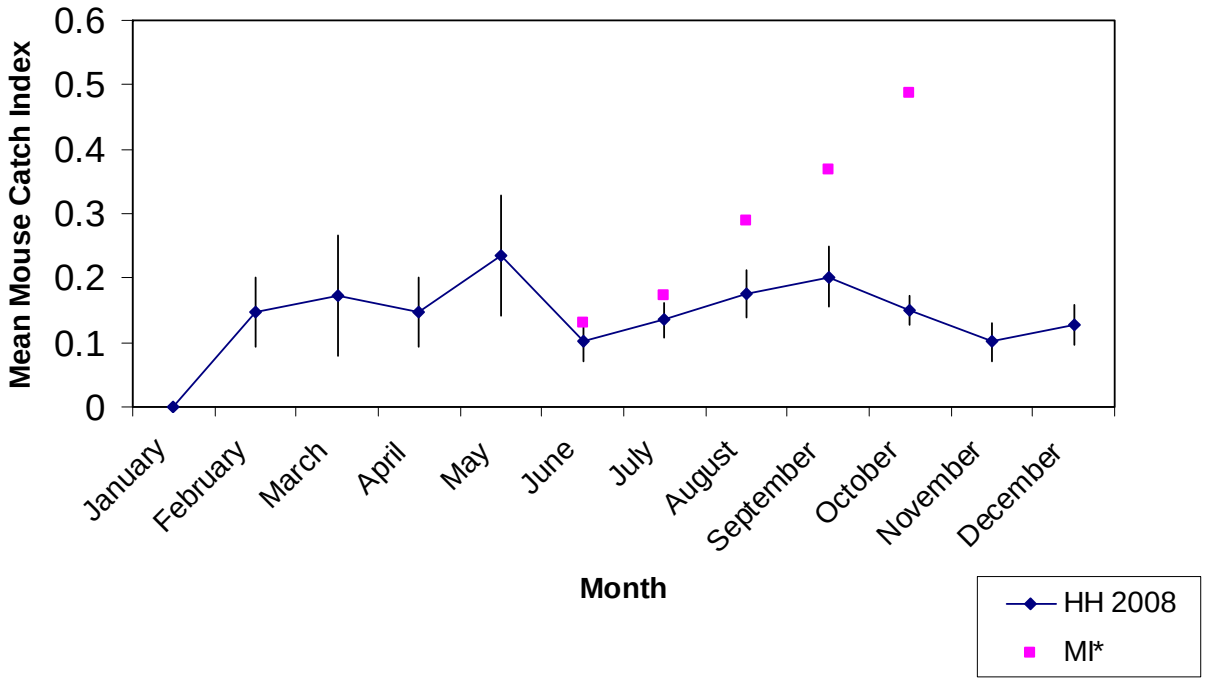

Figure 3: Mean mouse catch indices at Tennessee (HH) and Michigan (MI) study sites. The mean mouse catch index indicates the number of mice caught per adjusted trap night. *Michigan data is an average of each month during years 2004 through 2008 (October & September data are only from 2008).

Peak abundance of the larval and nymphal life stages of *I. scapularis* attached to mice occurred during different months between the two sites: the larval abundance peak at Henry Horton State Park (TN) occurred during May and the nyphal abundance peak occurred in July (Fig.4); whereas at Van Buren State Park (MI) the laraval and nymphal peaks both occurred in June (Fig. 5).

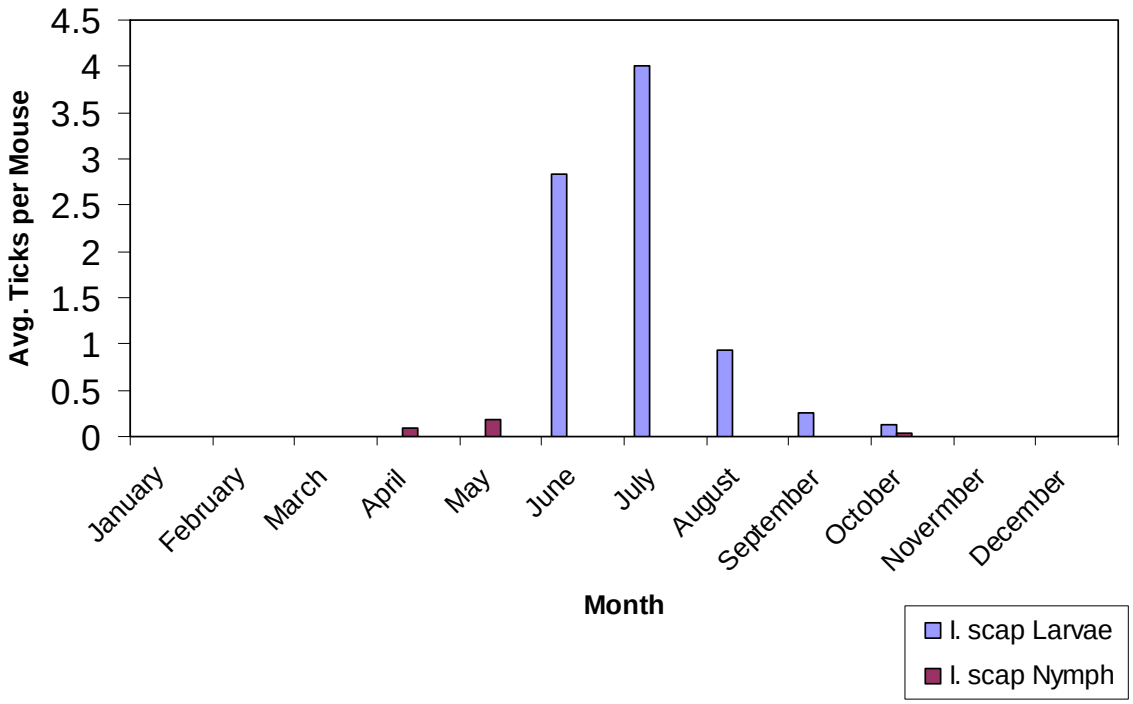

Figure 4: Average Number of *Ixodes scapularis* Ticks per Mouse at Henry Horton State Park in 2008.

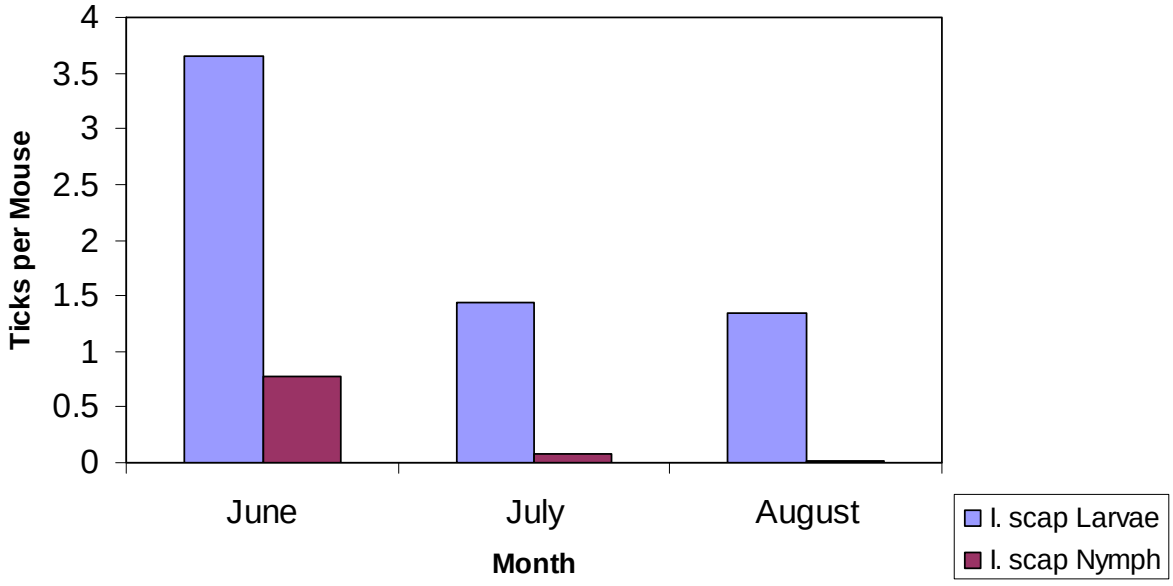

Figure 5: Average Number of *Ixodes scapularis* Ticks per Mouse in Van Buren County, MI Site. The ticks per mouse is an average of the data each month from years 2006 through 2008.

Our attempts at lizard trapping were not successful - we did not capture any lizards during this study, nor were any observed in the vicinity of the field site

Discussion:

During this study, we did find *I. scapularis* ticks, both through dragging and on captured rodents. This confirms M. Rosen's (unpublished) findings that the vector of Lyme disease is present in Tennessee.

The presence of *I. scapularis* on captured mice indicates that the ticks are utilizing rodents as a host. The unsuccessful lizard trapping suggests that the lizard population at Henry Horton State Park in Tennessee is at low enough levels to not play a significant role in disrupting the Lyme disease cycle. However, it is possible that other hosts are playing a role in the cycle, such as birds or shrews.

At Van Buren State Park (MI), the peak abundances of larval and nymph *I. scapularis* fell within the same month. This was found with both the peak abundance on mice as well as with dragging. However, at Henry Horton State Park (TN), both peak abundances fell in different months. This separation in time between the larval and nymph peaks of abundance may be enough to disrupt the Lyme disease cycle in Tennessee.

Two of the published hypotheses for the absence of Lyme disease in the south, absence of *I. scapularis* ticks and *I. scapularis* host seeking preference for lizards, were not supported by our findings. Further fieldwork should be undertaken at HHSP to explore the possibility of other host seeking preferences. Differences in seasonality and mouse abundance were apparent between the two regions, and factors such as mast years, precipitation, and temperature should be investigated further.

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