Virginia Journal of Science
Volume 47, Number 4
Winter 1996

Abundance of Adult *Ixodes scapularis* and Infection with *Borrelia burgdorferi* in Eastern Virginia

Michael Casteel and **Daniel E. Sonenshine**
Department of Biological Sciences, Old Dominion University,
Norfolk, Virginia 23529

ABSTRACT

Field studies to determine the relative abundance of adult blacklegged ticks, *Ixodes scapularis* and rates of infection of adult ticks with *Borrelia burgdorferi* were done in three localities in eastern Virginia. At the time of the Fall seasonal peak, tick capture frequency was greatest, (37.5 ± 4.3 (S.E) ticks/100 minutes, on Assateague Island (Chincoteague National Wildlife Refuge), a peninsula along the Atlantic Ocean near the Maryland border. Tick capture frequency was considerable lower, 21.7 ± 5.3 (S.E.) ticks/100 minutes, at the Cheatham Annex Naval Supply Station, an inland locality near Williamsburg. Ticks were virtually absent at the Back Bay National Wildlife Refuge, a coastal locality in southeastern Virginia near the North Carolina border. Rates of *Borrelia burgdorferi* infection in adult ticks were similar at the Assateague Island (7.7%) and Cheatham Annex sites (9.1%). No evidence of infection was found in the two ticks examined from Back Bay.

Key words: Blacklegged tick, *Ixodes scapularis*, abundance, *Borrelia burgdorferi*, infection, Lyme borreliosis.

INTRODUCTION

First described in 1977 (Steere et al., 1977) Lyme disease is now considered the most prevalent vector-borne disease in humans in the United States. The disease is caused by a spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Stierwalt and Brenner, which is transmitted by the bite of a black-legged tick, *Ixodes scapularis* Say. Thousands of cases have been reported in the United States each year (Anon., 1993). Although the numbers of cases of Lyme disease that occur in the southeastern states are not as high as in the northeastern and north central regions of the United States (Ginsberg, 1993; Luckhart et al., 1991; Amerasinghe et al., 1993), the number of cases from the former region have increased greatly since the 1980's. In Virginia, case numbers of Lyme disease for 1992, 1993 and 1994 which met the case definition of the Center for Disease Control and Prevention (Atlanta, GA) were 115, 95 and 131, respectively. According to the Virginia State Health Department, the risk of infection for humans is greatest in the eastern part of the state, especially the northeastern and coastal counties. Studies of ticks and wildlife native to Virginia demonstrated that *B. burgdorferi* occurs in wildlife and ticks (Levine et al., 1991; Sonenshine et al., 1995). *B. burgdorferi* was isolated (and cultured) from 5 species of small mammals in a coastal site near Chincoteague and an inland site between Williamsburg and Yorktown. In addition, *B. burgdorferi* infection was demonstrated by immunofluorescence assay in ticks, mostly *I. scapularis* in these same localities (Sonenshine *et. al.*, 1995). These

reports suggest that Lyme borreliosis (i.e., spirochete infection in wildlife and ticks) is established in eastern Virginia. However, most of the evidence concerning infection in *I. scapularis,* the major vector, was based on immature ticks collected from vertebrate hosts. Few records were obtained from unfed adult black-legged ticks.

Although nymphal black-legged ticks are considered to be the most important vectors of *B. burgdorferi* to humans (Lane *et al.*, 1993), adult ticks also represent a serious threat to hunters, hikers and other people enjoying outdoor recreational activities in the fall and early spring. In the northeastern United States, *I. scapularis* is abundant and rates of natural infection in unfed adults frequently range from 20 - 100% (Burgdorfer *et al.*, 1982; Anderson, 1989). Consequently, the risk of human infection from contact with unfed adults in that region is considerable. Less is known about *I. scapularis* abundance or rates of *B. burgdorferi* infection in adult ticks in the southeastern part of the United States. In Virginia, rates of infection in *I. scapularis* adults, based on small sample numbers, were 23.5% near the coast (Chincoteague) and 0.2% 64 kilometers inland (Williamsburg/Yorktown). Elsewhere in the southeastern region, reports of natural infection in adult *I. scapularis* ranged from 1 - 3% (Magnarelli *et al.*, 1986; Luckhart *et al.*, 1991). No estimates of adult *I. scapularis* abundance in this region have been reported.

Knowledge of tick abundance and *B. burgdorferi* infection rates are important for defining high risk areas for Lyme disease. Consequently, this study was undertaken to compare the relative abundance of adult, unfed *I. scapularis* and to determine infection rates of *B. burgdorferi* in this species at three contrasting localities in eastern Virginia.

MATERIALS AND METHODS

Intensive sampling for ticks was done in selected study sites in three localities in eastern Virginia between 4 October and 22 November, 1994, the period of maximum adult *I. scapularis* seasonal activity. The study sites were located at 1) Assateague Island, part of the Chincoteague National Wildlife Refuge (CNWR), adjacent to the Atlantic Ocean; 2) the Cheatham Annex Naval Supply Station (CANSS) near the York River between Williamsburg and Yorktown; and 3) at the Backbay National Wildlife Refuge (BBNWR) in Virginia Beach, with study sites located on Long Island in Backbay and on the adjacent mainland (Fig. 1). The CNWR and CANSS study sites were chosen because of their previous use for intensive field studies of small mammals, immature *I. scapularis* activity and spirochete infection studies over a three year period (Sonenshine *et al.*, 1995).

Description of Study Sites. The dominant vegetation in the CNWR study site consisted of closely-spaced bayberry bushes (*Myrica cenifera*) (most less than 3 m high) on sandy dunes, with scattered loblolly pine (*Pinus taeda*) and Virginia pine (*P. virginiana*) distributed among them. Ground cover was relatively sparse within the bayberry thickets, but masses of greenbrier (*Smilax*), thistle (*Cardus sp.*) and various vines (*Cuscuta sp.*) dominated the understory. Surrounding the thickets were grassy meadows covered predominantly by salt grass (*Distichlis spicata*) and other grasses but also containing blackberry (*Rubus cuneifolius*), greenbrier, thistle and various weeds. Brackish marshes dominated by marsh grass (*Spartina alterniflora*) were interspersed among the meadows and bayberry thickets. Further inland, about 0.5 to 1 km from the beach, there was a transition to mixed deciduous-pine second growth

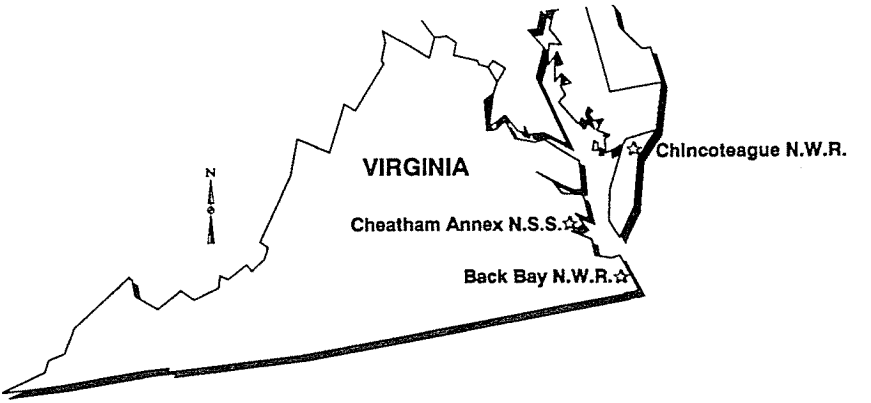

FIGURE 1. Map of Virginia showing the location of the three different study localities. Sampling at the Chincoteague National Wildlife Refuge (CNWR) was done along two transacts established in or adjacent to bayberry thickets on Assateague Island, near the Atlantic Ocean. Sampling at the Cheatham Annex Naval Supply Station (CANSS) was done along four transects established in or adjacent to upland forest communities. Sampling at the Back Bay National Wildlife Refuge (BBNWR) was done along transects established in or adjacent to forest habitat.

forests dominated by loblolly pine, red maple (*Acer rubrum*), tulip (*Liriodendron tulipifera*) and white oak (*Quercus alba*) with a dense, almost impenetrable understory of blueberry (*Vaccinium crassifolium*), masses of greenbrier (*Smilax spp*) and other vines. Previous studies showed large numbers of *I. scapularis* immatures on small mammals in this habitat, but not in drier, oak-pine thickets further inland. Consequently, the adult tick sampling effort was limited to the bayberry thickets and adjacent meadows.

The dominant vegetation at the CANSS was deciduous forest, mostly white oak, loblolly pine, Virginia pine, red maple, sweet gum (*Liquidambar styricflua*), American beech (*Fagus grandifolia*), tulip poplar and river birch (*Betula nigra*). In the understory, greenbrier, Japanese honeysuckle, Virginia creeper, blackberry and other vines were common, especially near the margins of the woodlands or along trails.

The vegetation at the BNWR consisted of small areas of forest, mostly mixed pine and oak species, and grassy meadows (especially on Long Island). The dominant forest vegetation included Virginia live oak (*Quercus virginiana*), southern prickly ash (*Xanthoxylum clavaherculis*), sassafras (*Sassafras albidum*), loblolly pine and white swamp oak (*Quercus bicolor*) with an understory of greenbrier, other vines, bayberry, Virginia inkberry (*Ilex glabra*) and others. The dominant meadow grasses included switchgrass (*Panicum virgatum*), saltgrass and beachgrass (*Ammophila breviligulata*) interspersed with scattered shrubs. Brackish marshes were also present but were not included in the sampling areas.

Adult Tick Sampling Procedures. Ticks were collected with a one m^2 denim cloth tick flag attached to a 1.2 m wooden dowel as described by Sonenshine (1993). Flagging was done along measured transects selected at random (from compass bearings) for a total of 120 min (excluding time to remove ticks) at two transects at the

CNWR (Assateague Island), four at the CANSS and two at the BNWR. A stopwatch was used to record the time actually spent during flagging and to exclude the time required for examining the flag, collecting the ticks and transferring them to numbered vials. The number of ticks captured at each interval that the flag was examined was used to determine the mean number of ticks ± S.D. captured on each sampling date. Markers were placed at regular intervals along each transect to guide the flagging process. Based on measurements of the time required to flag 10 m (13.7 ± 0.2 s, n = 18), the average area sampled at each of the three locations, CNWR, CANNS, and BNWR, was 5,256 m^2 (0.53 ha). At Assateague Island, the two transects were in the (predominantly) bayberry thickets approximately 1 km apart from one another and adjacent to the dunes that separated these habitats from the Atlantic Ocean. At the CANSS, two of the four transects were at the edge of old fields surrounded by dense woodlands; the remainder were in dense forest. At the BNWR, one of the transects was in a grassy area at the edge of wooded habitat on a small (280 ha) island; the other was in wooded habitat along the bay shore. The area sampled at each of the three locations was 5,256 m^2 (approximately 0.53 ha). Captured ticks were identified (species and sex), the date and site of collection recorded, and the specimens taken to the laboratory for examination. Sampling was done from 4 October to 24 November, 1994, after which the refuges were closed for deer hunting.

Laboratory Procedures. Surviving ticks were surfaced sterilized and examined for *B. burgdorferi* antigens by the immunofluorescence assay (IFA) using monoclonal antibodies H5332 and H6831 in accordance with techniques described by Bissett and Hill (1987). Ticks were surface sterilized (0.5% sodium hypochlorite, 70% ethanol), washed with phosphate-buffered saline (PBS), dissected, the midgut contents smeared onto slides and allowed to air dry. Slides were fixed in acetone (20 min), rinsed 3 X with PBS, treated with 10 μL of monoclonal antibody H5332 or H6831 (gift from Alan Barbour, University of Texas Health Center, San Antonio, TX) and incubated for 45 min at 37° C. Following staining with fluorescein isothyiocyanate-labeled anti-mouse immune antibodies, the slides were covered and examined with epifluorescence microscopy at 400X with an Vanox AH2 microscope (Olympus) for evidence of spirochetes. Smears made from cultures of the B31 strain of *B. burgdorferi* were used as positive controls while smears of *B. anserina* spirochetes were used as negative controls. The control specimens were obtained from the American Type Culture Collection (Washington, D.C.).

RESULTS

Relative Abundance of *I. scapularis*. At Assateague Island, a total of 171 adult *I. scapularis* (76 males, 95 females) were collected in the two transects on six dates between 4 October and 22 November. Tick capture success at the three different study localities, expressed as the mean number of ticks ± S.E./100 minutes, is summarized in Table 1 and in Fig. 2. The seasonal activity peak occurred on 4 November. Tick numbers captured increased from only 1.7 ± 1.0 (3.8 ticks/ha) on 4 October to 37.5± 4.1 ticks/100 min (85.6 ticks/ha) on 4 November. Tick numbers captured declined thereafter, down to 24.2 ± 5.6 ticks/100 min (55.2 ticks/ha) when sampling was terminated on 22 November. It is not known how much longer ticks would have remained active after sampling ended. At the CANSS, a total of 99 adult *I. scapularis*

TABLE 1. Frequency of collection and relative abundance of adult *Ixodes scapularis* at three contrasting localities in eastern Virginia.

Dates (1994)	Assateague Is. (CNWR) Mean ± S.E Ticks/100 min.	Avg. No./ha[1]	CANSS Mean ± S.E. Ticks/100 min.	Avg. No./ha[1]	BNWR Mean ± S.E. Ticks/100 min.	Avg. No./ha[1]
4 - 6 Oct	1.7 ± 1.0	3.8	4.2 ± 1.8	9.5	0.00	0.00
11 - 13 Oct	21.7 ± 5.2	49.5	15.0 ± 3.0	34.3	2.0 ± 1.2	3.8
29 - 31 Oct	31.6 ± 4.3	72.3	11.7 ± 2.9	26.6	0.00	0.00
4 - 6 Nov	37.5 ± 4.1	85.6	18.3 ± 3.6	41.9	1.0 ± 0.8	1.9
15 -17 Nov	25.8 ± 3.0	59.0	21.7 ± 5.3	49.5	0.00	0.00
22 -24 Nov	24.2 ± 5.6	55.2	11.7 ± 3.1	26.6	0.00	0.00
Mean ± (S.E.) all samples	23.8 ± 33.4	53.8	13.8 ± 19.7	31.1	3.0 ± 2.0	0.9

1 Estimated number of ticks that would have been collected if one hectare had been sampled. Each value represents the actual number collected in each 120 min sampling period x the conversion of sampling area 0.53 ha to 1 ha.

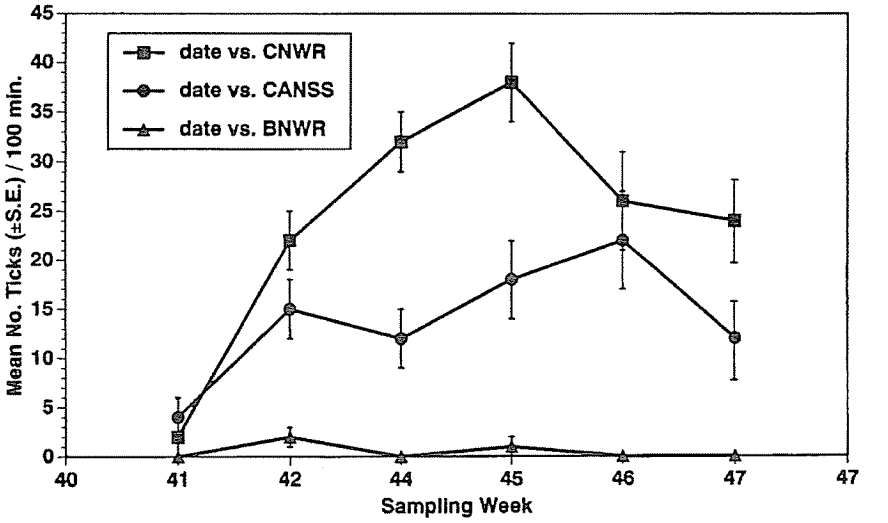

FIGURE 2. Graph illustrating adult tick *(Ixodes scapularis)* sampling success at the three different study sites in eastern Virginia during the fall, 1994 sampling period.

TABLE 2. Rates of infection of *Borrelia burgdorferi* in adult *Ixodes scapularis* at three localities in eastern Virginia.

Life stage	Assateague Is./ CNWR No. examined	Assateague Is./ CNWR No.(%) positive	CANSS No. examined	CANSS No.(%) positive	BNWR No. examined	BNWR No.(%) positive
Males	46	3 (6.5)	41	4 (9.8)	2	0 (0)
Females	58	5 (8.6)	25	2 (8.0)	0	NA[1]
Totals	104	8 (7.7)	66	6 (9.1)	2	0 (0)

1 Not applicable.

(62 males, 37 females) were collected on approximately the same dates as above. Most of the ticks (71.7%) collected came from the forested transects. The seasonal peak occurred on 15 November when captures reached 21.7 ± 5.3 ticks/100 min (49.5 ticks/ha). At the BNWR, only three ticks were collected during the sampling period, all from the island transect. Capture success at the three different localities was significantly different from one another ($F = 13.108, 15, 17, p < 0.001$; 1-way analysis of variance). If the BNWR, where ticks were virtually absent, is excluded, capture success at the CNWR (Assateague Island) was significantly greater than at the CANSS ($F = 3.21, 10, 11, p < 0.05$, 1-way analysis of variance).

Rates of *B. burgdorferi* infection in *I. scapularis*. Infection was found in eight (7.7%) of 104 adult *I. scapularis* examined from Assateague Island (Table 2). These included three males (6.5% of 46 examined) and five females (8.6% of 58 examined). Infection was found in six (9.1%) of 66 adult *I. scapularis* examined from the CANSS, including four males (9.8% of 41 examined) and two females (8.0% of 25 examined). No evidence of infection was found in two ticks that were examined from the BNWR.

DISCUSSION

This study was the first opportunity to systematically compare adult tick relative abundance and *B. burgdorferi* infection rates in populations of *I. scapularis* in different localities of eastern Virginia. A previous study (Sonenshine *et al.*, 1995) compared larval and nymphal *I. scapularis* on wild-caught small mammals and infection rates in these immatures, but did not systematically sample adult ticks. Although limited in scope, the results of the present study suggest that *I. scapularis* is more abundant near the Atlantic Ocean, as represented by Assateague Island (CNWR) than further inland in Virginia, as represented by the CANSS. This finding is consistent with an earlier report (Sonenshine et al., 1995) that found the tick to be more abundant on Assateague Island than further inland. Whether the larger *I. scapularis* population on Assateague Island is representative of the eastern coast or an isolated focus of high tick density is unknown.

Studies of *I. scapularis* occurrence in Maryland have shown that the ticks are most numerous on white-tailed deer in the eastern part of the state, near the Atlantic Ocean (Amerasinghe et al., 1992, 1993). Similarly, a four year statewide survey of ticks on white-tailed deer in North Carolina showed that 46% of all *I. scapularis* were collected in the eastern coastal plain region (Apperson et al., 1990). Thus, it is not surprising that tick relative abundance in Virginia also appears to be greater near the Atlantic Ocean than further inland. However, at Back Bay, along the southeast coast and Parramore Island, a barrier island bordering the Atlantic Ocean, *I. scapularis* was virtually absent (this paper; Levine *et al.* 1991; Sonenshine *et al.* 1995). The infrequent occurrence of *I. scapularis* on the barrier islands or coastal habitats south of Assateague Island is puzzling. White-tailed deer and small mammal hosts occur in these localities, although white-footed mice (*Peromyscus leucopus*) are absent from several of the barrier islands. However, studies on Assateague Island have shown that a variety of other small mammals can support the immature stages of this tick in the absence of *P. leucopus* (Sonenshine *et al.*, 1995). South of Assateague Island, the vegetation changes and the waxmyrtle thickets that provide excellent tick habitats at the former locality are rare or absent on the other barrier islands. Assateague Island (actually a peninsula extending from the Maryland mainland) is more representative of eastern Maryland, where *I. scapularis* is abundant, than the Virginia barrier islands or other coastal communities. However, except for our study at the CANSS near Williamsburg, nothing is known about the occurrence of *I. scapularis* or its relative abundance on the mainland of eastern Virginia. The relatively high incidence of Lyme disease cases in Accomack county, where Assateague Island is located (189.3 cases/100,000 population for the five-year period, 1990 - 1994) (Virginia State Health Department, unpublished) suggests that *I. scapularis* may be more abundant on the mainland than on the coastal islands. This possibility should be investigated further.

Despite differences in tick abundance, infection rates in unfed adults at CNWR (7.7%) and CANSS (9.1%) were similar. This is quite different from the rates reported for immature ticks from these two localities; for nymphs collected from small mammals, the rates for the same two localities, CNWR and CANSS, were 22.1% and 0%, respectively (Sonenshine *et al.*, 1995). Both studies support the conclusion that *B. burgdorferi* infection rates in ticks(*I. scapularis*) in eastern Virginia are substantially lower than in ticks in the northeastern United States.

ACKNOWLEDGEMENTS

We thank Dr. Robert Ratzlaff. Dr. Ralph Stevens and Ms. Jill Troyer, Old Dominion University, for use of their laboratory facilities and their assistance in conducting the laboratory studies. We thank Mr. Irving Ailes, CNWR, Mr. B. Hoag, CANSS, and Mr. John Gallegos, BNWR, for access to the field sites. We thank Dr. Alan Barbour, University of Texas Health Sciences Center, San Antonio, TX, for the supply of MAB H5332 and H6831. Finally, we thank Dr. Suzanne Jenkins, Division of Epidemiology, Virginia State Health Department, Richmond, Virginia, for providing the statistics on cases of Lyme disease in Virginia.

Mr. Casteel carried the field work and performed the laboratory diagnostic assasy under the overall supervision of Dr. Sonenshine. Dr. Sonenshine assisted with some of the field work and wrote the manuscript.

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