TICK-RACCOON ASSOCIATIONS AND THE POTENTIAL FOR LYME DISEASE SPIROCHETE TRANSMISSION IN THE COASTAL PLAIN OF NORTH CAROLINA

Authors: Ouellette, John, Apperson, Charles S., Howard, Peter, Evans, Timothy L., and Levine, Jay F.

Source: Journal of Wildlife Diseases, 33(1) : 28-39

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-33.1.28

Journal of Wildlife Diseases, 33(1), 1997, pp. 28–39

TICK-RACCOON ASSOCIATIONS AND THE POTENTIAL FOR LYME DISEASE SPIROCHETE TRANSMISSION IN THE COASTAL PLAIN OF NORTH CAROLINA

John Ouellette,[1,3] Charles S. Apperson,[1,4] Peter Howard,[2] Timothy L. Evans,[1,5] and Jay F. Levine[2]

[1] Department of Entomology, Box 7647, North Carolina State University, Raleigh, North Carolina 27695-7647, USA
[2] Department of Microbiology, Pathology and Parasitology, Box 8401, North Carolina State University, Raleigh, North Carolina 27695-8401, USA
[3] Current address: Department of Biology, Division of Ecology and Organismal Biology, University of Memphis, Memphis, Tennessee 38152, USA
[4] To whom requests for reprints should be addressed.
[5] Current address: Anderson-Tully Co., P.O. Box 38, Vicksburg, Mississippi 39181, USA

ABSTRACT: Raccoons (*Procyon lotor*) were live-trapped and examined for ticks from July 1990 to July 1993 in the coastal plain of North Carolina on Marine Corps Base, Camp Lejeune, North Carolina (USA). Five species of ixodid ticks were found on 351 (78%) of 449 raccoons. *Amblyomma americanum* was the most abundant tick found on raccoons. *Dermacentor variabilis*, *Ixodes texanus*, and *Ixodes scapularis* were frequently collected, while *Ixodes cookei* were rarely collected from raccoons. Tick burdens were not affected by the age, sex, or trap location of captured raccoons. Ticks parasitizing raccoons had varying seasonal patterns of abundance. *Amblyomma americanum* were generally collected from raccoons year around, but infestation intensities were greatest in summer from June to September. *Dermacentor variabilis* adults were most abundant in mid-summer while peak numbers of larvae were collected in the fall. Infestation intensities of *Ixodes texanus* larvae were greatest in fall and winter months while nymphs were most abundant in winter and spring. No males were collected from raccoons, but females were most frequently collected in the spring and declined in abundance in the summer with no specimens collected in the fall or winter. Numbers of *I. scapularis* adults appeared to reach peak numbers in the fall while larvae and nymphs were most abundant on raccoons in winter. Spirochetes, *Borrelia burgdorferi*, were identified in a small percentage (0.2%) of host-seeking *A. americanum* nymphs and adults, and *I. scapularis* adults by immunofluorescent antibody assays. Similarly, a small percentage (1.9%) of host-associated *A. americanum*, *D. variabilis*, *I. texanus* and *I. cookei* contained *B. burgdorferi*. *Borrelia burgdorferi* spirochetes were cultured from the blood of 23 (26%) of 87 raccoons.

Key words: Raccoons, *Procyon lotor*, *Amblyomma americanum*, prevalence, infestation intensity, *Borrelia burgdorferi*, antibodies.

INTRODUCTION

The interaction of ticks and wildlife is responsible for the maintenance of tick-borne zoonoses, such as Lyme disease. In the northeastern U.S. in disease enzootic areas, *Ixodes scapularis* is infected with the etiologic agent of Lyme disease, *Borrelia burgdorferi*, after feeding on a variety of birds and mammals (Anderson, 1991). The white-footed mouse (*Peromyscus leucopus*), however, is the principal reservoir host (Levine et al., 1985; Mather et al., 1989). Although Lyme disease occurs in the southern U.S., the number of human cases represents a small percentage of the total number of cases reported each year (Centers for Disease Control and Prevention, 1993). Comparatively less information is available on the wildlife and ticks involved in the transmission cycle of the Lyme disease spirochete in the southern U.S.

In geographic locales, where *P. leucopus* is absent or in low abundance, other animals are of primary importance in maintaining transmission of *B. burgdorferi* (Lane et al., 1991). The raccoon (*Procyon lotor*) is widely distributed in the southeastern U.S. in suburban as well as rural environments. This species is often heavily infested by ticks (Zimmerman et al., 1988; Fish and Daniels, 1989; Fish and Dowler, 1990), and in Lyme disease endemic areas appears to contribute to the production of

spirochete-infected ticks (Fish and Dowler, 1990; Anderson et al., 1983).

In 1990, in response to the occurrence of human Lyme disease cases on Marine Corps Base, Camp Lejeune, North Carolina (USA) (Staes, 1989), a comprehensive survey of the interaction of tick and wildlife species that were potentially involved in transmitting *B. burgdorferi* was conducted. On the military base, *Peromyscus* spp. mice were not abundant or heavily parasitized by ticks (Apperson et al., 1993). However, the raccoon population appeared to be high based on nuisance complaints received by the Marine Corps Base's Fish and Wildlife Division. Here we report results of studies of the raccoon population on the Marine Corps Base, Camp Lejeune. Our objectives were to ascertain the species composition and infestation intensities of ticks parasitizing raccoons, and to estimate the proportion of raccoons infected with *B. burgdorferi* as well as determine the presence of spirochetes in ticks infesting them.

MATERIALS AND METHODS

The military base was established in 1941 in the southeastern coastal plain of North Carolina about 60 km north of Wilmington and adjacent to the town of Jacksonville (34°45′N, 76°27′W). Marine Corps Base, Camp Lejeune (MCBCL) has a perimeter of approximately 110 km with 22.5 km of ocean front. The base covers about 610 km^2, including 10.5 km^2 of water (primarily the New River and its tributaries). About 90% of the land area within Camp Lejeune is used for military training. The population of Camp Lejeune averages 63,000 military personnel, their dependents, and civilians. Approximately 13,000 members of the Marine Corps Reserve also receive training at MCBCL each year. This population is primarily concentrated in housing, recreational, and industrial areas which comprise 33.8 km^2 of the base's total area. Topography varies from flat to gently rolling terrain, with elevations ranging from sea level to 21 m. There are 25 different soil series, varying from sandy loams to fine sand. Vegetation in the upland sites consists of pure pine (*Pinus* spp.) and mixed pine and hardwood forests. The bottomlands are typically cypress gum swamps with inland areas dominated by short and tall pocosin habitat.

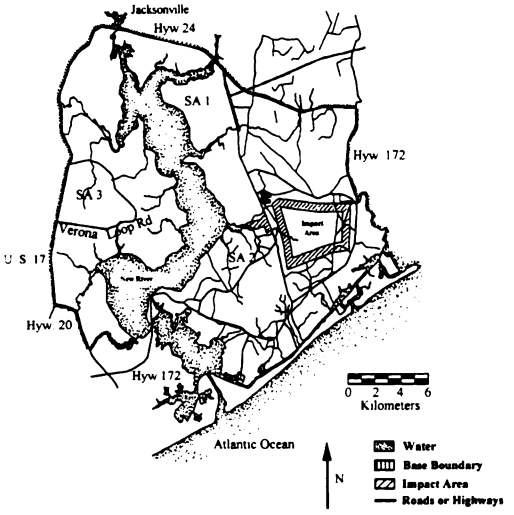

FIGURE 1. Map of Marine Corps Base, Camp Lejeune, North Carolina, with the location of the three study areas (SA 1, SA 2, and SA 3).

Rainfall averages 635 mm per year (Environmental Management Department, 1987).

Our research was divided into two phases. In Phase I, collection efforts on MCBCL were broadly focused. From July 1990 to April 1992, raccoons were trapped in woodland locales where the risk of exposure of military personnel to ticks was high, and at urban and industrial sites in response to nuisance animal complaints. Live traps (Tomahawk 201, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA), baited with canned cat food or peanut butter, were placed adjacent to trash dumpsters or cans in residential or industrial areas and, at dumpsters along roadsides in woodland areas such as the Verona Loop Road (Fig. 1). Traps were set each evening and checked the following morning. Captured raccoons were anesthetized with ketamine hydrochloride (Parke-Davis, Morris Plains, New Jersey, USA) and xylazine (Mobay Corporation, Shawnee, Kansas, USA) (0.5:15 mg/kg), and attempts were made to take a blood sample from the saphenous vein of each animal. Captured animals were then carefully examined and attached ticks were removed and placed in glass vials containing a moist plaster of Paris-powdered charcoal base (Farrell and Wharton, 1948). Blood samples were allowed to clot at 4 C and sera were stored frozen at −18 C.

From May 1992 to July 1993, animals were systematically trapped in three study areas that represented the dominant habitat types on MCBCL; namely, bottomland hardwoods and mesic pine flatwoods habitats. Raccoons

trapped in Phase II were given whole-body inspections to estimate tick infestation intensities. Study Area 1 (SA 1) was located on the eastern side of the base in a woodland (about 518 ha in size) between two urbanized areas (housing communities, schools, and a hospital). Paradise Point bordered the western edge of this woodland, while Berkley Manor-Watkins Village residential communities bordered the eastern side. There were 1,430 housing units clumped together within these communities, providing residences for approximately 4,300 people.

Study Area 2 (SA 2) was used extensively by the Marine Corps for training. It was on the same side of the New River as SA 1, but approximately 10 km south (Fig. 1); it was not urbanized. In SA 2, several permanent streams flowed into the New River to the northwest. The vegetation types in SA 1 and SA 2 were typical of a bottomland hardwoods-blackwater habitat as described in Schafale and Weakley (1990). In SA 1, several permanent streams flowed into Northeast Creek to the north, and the New River to the west.

Study Area 3 (SA 3), located within what is locally known as Verona Loop on the western side of the New River (Fig. 1), was a mesic pine flatwood habitat (Schafale and Weakley, 1990). Verona Loop was a paved road that made a semi-circle, encompassing approximately 800 ha; its western boundary was U.S. highway 17. The area was used year round as a training area for the School of Infantry. The area was subjected to frequent moderate intensity fires which maintained an open pine overstory of longleaf (*Pinus palustris*) and loblolly (*Pinus taeda*) pines dissected by hardwood drainages. Study Area 3 was drained by two creeks, which flowed east into the New River.

A trapping grid, square in configuration and about 4 km^2 in size, was located within each of the three study areas. Grid 1 in SA 1 consisted of 49 live-traps (Tomahawk trap 106, Tomahawk Live Trap Company) placed in a 7×7 configuration at intervals of 333 m. In grids 2 and 3 (SA 2 and SA 3), 25 traps were placed in 5×5 configurations at 500 m intervals. In grid 1, nine traps were placed on residential sites, and the remaining 40 traps were located in woodlands. Trapping effort was consistent across all three grids with grids 2 and 3 sampled concurrently, followed by grid 1. Traps were set for 10 consecutive nights within a 21-day period, after which trapping effort rotated to the next grid. The location of grid 3 had to be shifted 1,000 m to the east to similar habitat in October 1992 because military training interfered with sampling. Traps were baited with a mixture of approximately 25 grams of canned cat and dog food and 3 cc of artificial raspberry flavor (No. 1012, Medallion International, Inc., North Haledon, New Jersey, USA). Traps were set each afternoon and checked the following morning. Ten 10-day trapping cycles were completed in each grid.

Animals were taken to a central location in each grid where they were anesthetized with ketmaine and xylazine, weighed, had their sex determined by external characteristics, had a blood sample taken, and were processed as previously described. Raccoons were assigned to one of five relative age classes based on tooth wear (Grau et al., 1970); these age classes were: I (1 to 14 mo), II (15 to 38 mo), III (39 to 57 mo), IV (58 to 86 mo), and V (>86 mo). Each animal was carefully examined for ticks. Examinations lasted from approximately 10 min to 2.5 hr, depending on tick loads. After being examined, two ear-tags were affixed to each animal. A metal tag (Monel by National Band and Tag Company, Newport, Kentucky, USA) numbered on one side and engraved on the other with the North Carolina State University and base phone numbers was attached on the left ear. A brightly colored, numbered plastic tag (Rototags of Nasco, Ft. Atkinson, Wisconsin) was attached to the right ear. The plastic ear-tags were color coded according to which of the three grids an animal was captured. Nuisance animals not captured within the trapping grids also received color-coded tags. Animals recaptured within 5 days of their last capture were released without examination to minimize stress and the development of trap avoidance behavior. After the examinations were completed, animals were returned to their sites of capture and released.

After anesthetization, each animal was thoroughly checked for ticks over five body regions: head (ears, snout, and eyes) and neck; shoulder and foreleg; torso; and hind-quarter (including the hind limbs and tail). All life stages of ticks were removed with forceps. Ticks were identified to life stage, and the number of specimens in each life stage was recorded for each body region. If a raccoon had a burden of greater than 50 larvae in a specific body region, the subsampling procedure delineated by Zimmerman et al. (1988) was followed. The animal was divided longitudinally along the midline, and the left side was examined for ticks in each body region. All the adults and nymphs were removed and a maximum of 50 larvae were collected from each region. The remaining larvae were counted, and the number for each body region was recorded. The number of ticks removed from the left side was later doubled for each stage of each species to compensate for the ticks on the right side that were not removed. A readjustment of these counts was

made based on the proportion of each species in the subsample of larvae. Ticks removed from each body region of each animal were placed in separate live-vials, and subsequently identified to species and life stage, and counted. Representative specimens of each species were sent to Dr. J. E. Keirans for confirmation of our identifications. These ticks have been deposited in the U.S. National Tick Collection (entry numbers RML 46102, 46103, 46111, 46113–46115) that is housed in the Institute of Arthropodology, Georgia Southern University, Statesboro, Georgia (USA).

In Phase I, an enzyme-linked immunosorbent antibody assay (ELISA) was used to test blood serum samples for anti-*B. burgdorferi* antibodies (Greene et al., 1988a). Some serum samples with high ELISA values were analyzed by immunoblotting (Western blot) them against *B. burgdorferi* proteins that were separated by polyacrylamide gel electrophoresis (Greene et al., 1988b). In most samples, antibodies reacted with proteins that were not specific for *B. burgdorferi*. Therefore, use of an ELISA to detect *B. burgdorferi*-specific antibodies was discontinued. Serum samples were subsequently analyzed only by immunoblotting (Greene et al., 1988b). Animals whose serum samples reacted with flagellin protein (45 kilodaltons), outer surface protein A (31 kilodaltons) and outer surface protein B (34 kilodaltons) of *B. burgdorferi* were considered to have been exposed to the spirochete.

To culture *B. burgdorferi* from blood samples, a few drops of blood from some raccoons were inoculated by needle directly through the stopper into a tube of Barber-Stoner-Kelly (BSK)-II culture media (Barbour, 1984) in the field. Culture tubes were held in a cooler on a cold pack in the field and then stored at 4 C. Subsequently, these samples were placed in an incubator and held in 5% CO_2 at 35 C. Cultures were periodically examined by darkfield microscopy for spirochetes. Suspect cultures were identified as *B. burgdorferi* by an indirect immunofluorescent antibody assay (IFA) (Levine et al., 1989) using monoclonal antibody H5332 (provided by Alan Barbour, University of Texas Health Science Center, San Antonio, Texas, USA). Due to potential variation in each lot of antibody, checker-board dilutions were made so that an optimal working dilution for each batch of conjugate could be determined. The working dilutions of the monoclonal antibody ranged from 1:5 to 1:25, while the working dilution for the fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin G (IgG) antibody (Kirkegard and Perry Laboratories, Gaithersburg, Maryland, USA) was always 1:200.

In 1990 to 1992, host-seeking ticks were collected by dragging (flagging) a 1 m^2 piece of corduroy cloth over vegetation or leaf litter in bottomland hardwood areas adjacent to creek drainages. Attempts were made to collect ticks year round. Tick collection dates were categorized by season; spring (March through May), summer (June through August), fall (September through November), and winter (December through February). At each site on each sampling date, a minimum of two 10 min collections were usually taken. While walking at a pace of about one m per second, the flag was pulled over vegetation or the woodland floor. Approximately every 30 seconds, the flag was examined for ticks which were removed and placed in live-vials. The vials were placed in a cooler on a cold pack and transported to laboratory facilities for processing. After identification and enumeration, ticks collected by flagging or removed from hosts were dissected and examined by IFA (Levine et al., 1989), using *B. burgdorferi*-specific monoclonal antibody H5332.

A factorial analysis of variance using the General Linear Model procedure (SAS, 1985) was used to determine if variations in the intensity of parasitism of raccoons by some tick species were related to the independent variables studied, which included study area (SA 1 to 3, nuisance raccoons), season (spring, summer, winter, fall), raccoon sex (male, female), age (age class I–V) and body region (head, neck, shoulder, torso, hind-quarter), and tick species (*A. americanum*, or *D. variabilis*, *I. scapularis*, *I. texanus*). One tick species, *I. cookei*, was not collected in sufficient numbers to allow statistical analysis. The data conformed to the Poisson distribution. Variances were normalized by subjecting the numbers of ticks of each life stage for each species to a $\log_{10}(X + 1)$ transformation prior to analysis. To prevent the relatively high prevalence of *A. americanum* from biasing the outcome of statistical analyses, infestation data for *A. americanum* and the other tick species were analyzed separately.

RESULTS

In Phase I, 264 raccoons were captured in 1,365 trap-nights of effort. Seasonal capture rates for raccoons were highly variable, ranging from 0.02 to 0.51 raccoons/trap-night and averaging 0.19 raccoons/trap-night. Generally, raccoons were most active September to November. In Phase II, 168 raccoons (95 males, 73 females,) were captured with 27 animals re-

TABLE 1. Infestation intensities and sites of attachment of ticks on raccoons (*Procyon lotor*) (n = 185) that were live-trapped on Marine Corps Base, Camp Lejeune from May 1992–July 1993.

Species	Life stage	Number of animals infested	Number of ticks	Mean number of ticks per raccoon (±SD)	Mean number of ticks per body region			
					Head	Shoulder	Torso	Hind-quarters
Amblyomma americanum	Larvae	54	7,367	45.2 (164.4)	22.1	6.7	6.6	9.8
	Nymphs	139	12,813	78.6 (130.5)	48.3	8.8	6.7	14.8
	Males	24	51	0.3 (1.0)	0.2	0.01	0.01	0.05
	Females	14	36	0.2 (0.9)	0.2	0.01	0.01	0.02
Dermacentor variabilis	Larvae	10	134	0.8 (5.0)	0.8	0.0	0.01	0.0
	Nymphs	6	14	0.08 (0.4)	0.07	0.01	0.0	0.0
	Males	32	96	0.6 (1.9)	0.6	0.02	0.01	0.01
	Females	49	110	0.7 (2.2)	0.5	0.2	0.01	0.0
Ixodes scapularis	Larvae	8	59	0.4 (2.9)	0.4	0.0	0.0	0.0
	Nymphs	23	90	0.6 (2.4)	0.5	0.01	0.01	0.0
	Males	7	10	0.06 (0.4)	0.06	0.0	0.0	0.0
	Females	5	8	0.05 (0.3)	0.05	0.0	0.0	0.0
Ixodes texanus	Larvae	14	218	1.3 (8.6)	1.3	0.0	0.0	0.02
	Nymphs	36	209	1.3 (4.2)	1.1	0.1	0.01	0.04
	Males	0	0	0.0 (0)	0.0	0.0	0.0	0.0
	Females	31	135	0.8 (3.3)	0.5	0.3	0.0	0.0

captured two or more times in 9,900 trap-nights. Capture rates averaged 0.017 raccoons per trap-night.

In Phase I, attached ticks were found on 188 (71%) of 264 raccoons examined, while in Phase II, 163 (88%) of 185 raccoons were tick-infested. The five species of ticks found were *Amblyomma americanum*, *Dermacentor variabilis*, *I. scapularis*, *I. texanus*, and *I. cookei*. In Phase II, no differences in tick infestation intensity on raccoons were found between the three study areas for *A. americanum* (df = 2; F = 0.05; P = 0.83) or the other tick species (df = 6; F = 1.69; P = 0.15). Tick burdens for *A. americanum* varied seasonally (df = 3; F = 3.0; P = 0.029) and by raccoon body region for each tick life stage (df = 9; F = 3.63; P = 0.0002). Similarly for *D. variabilis*, *I. scapularis*, and *I. texanus*, seasonal differences were found between species for attachment site for each tick life stage (df = 8; F = 2.28; P = 0.031). No variation in tick infestation intensities were found between raccoons of different ages for *A. americanum* (df = 1; F = 3.04; P = 0.082) or any of the other four tick species (df = 2; F = 1.25; P = 0.30).

Amblyomma americanum was the predominant species, representing 20,267 (95%) of the 21,364 ticks collected from raccoons and, infesting 148 (80%) of the 185 animals examined in Phase II. Immature forms constituted over 99% of the total number of *A. americanum* removed from hosts. *Ixodes texanus* represented about 3.0% of the total number of ticks collected, while the remaining three species constituted <2.0% of the total (Table 1). *Dermacentor variabilis*, *I. texanus*, and *I. scapularis* infested 66 (35%), 30 (16%), and 63 (34%) of the 185 raccoons examined, respectively.

Mean (± SD) burdens of *A. americanum*, *D. variabilis*, *I. scapularis*, and *I. texanus* were 119.1 (± 256.9), 2.2 (± 6.2), 1.1 (± 4.2), and 3.4 (± 10.7) ticks per raccoon, respectively (Table 1). Larvae (45.2 ± 164.4) and nymphs (78.6 ± 130.5) of *A. americanum* were considerably more abundant on and frequently collected from raccoons than were males (0.3 ± 1.0) and

TABLE 2. Seasonal variation in the abundance of *Amblyomma americanum* on raccoons (*Procyon lotor*) (n = 185) on Marine Corps Base, Camp Lejeune, North Carolina, from May 1992 to July 1993.

	Number of raccoons infested per number examined	Mean (±SD) and median number per raccoon							
		Larvae		Nymphs		Males		Females	
May 1992	28/28	0.6 (1.7)	0.0	50.0 (51.7)	34.5	0.1 (0.3)	0.0	0.1 (0.4)	0.0
Jun.	49/49	<0.1 (0.3)	0.0	84.4 (76.4)	58.0	0.3 (0.7)	0.0	0.3 (0.8)	0.0
Jul.	4/4	528.5 (426.2)	555.5	253.8 (269.5)	179.0	0.8 (1.0)	0.5	0.0 (0)	0.0
Aug.	17/17	209.8 (347.6)	22.0	147.1 (202.1)	58.0	0.5 (1.5)	0.0	0.3 (0.7)	0.0
Sept.	5/5	88.2 (64.2)	117.0	179.0 (324.6)	34.0	1.4 (2.6)	0.0	1.2 (1.8)	0.0
Oct.	16/22	22.9 (51.3)	3.5	16.2 (45.3)	1.0	0.0 (0)	0.0	0.0 (0)	0.0
Nov.	3/6	9.5 (23.3)	0.0	0.5 (0.8)	0.0	0.0 (0)	0.0	0.0 (0)	0.0
Dec.	1/4	0.0 (0)	0.0	8.0 (16.0)	0.0	0.0 (0)	0.0	0.0 (0)	0.0
Jan. 1993	0/5	0.0 (0)	0.0	0.0 (0)	0.0	0.0 (0)	0.0	0.0 (0)	0.0
Feb.	1/6	0.0 (0)	0.0	0.8 (2.0)	0.0	0.0 (0)	0.0	0.0 (0)	0.0
Mar.	9/9	0.0 (0)	0.0	9.0 (12.2)	4.0	0.0 (0)	0.0	0.0 (0)	0.0
Apr.	18/18	0.6 (1.6)	0.0	38.7 (58.4)	15.0	0.3 (0.7)	0.0	0.1 (0.3)	0.0
May	1/1	0.0 (0)	0.0	85.0 (0)	85.0	1.0 (0)	0.0	0.0 (0)	0.0
Jun.	11/11	10.6 (23.7)	0.0	70.7 (44.8)	67.0	0.5 (0.8)	0.0	0.2 (0.4)	0.0

females (0.2 ± 0.9) (Table 1). In contrast, few nymphs of *D. variabilis* were collected relative to other stages. Mean infestation intensities for larvae (0.8 ± 5.0) were higher than for males (0.6 ± 1.9) and females (0.7 ± 2.2) but larvae (and nymphs) were collected less frequently from raccoons than were adults. For *I. scapularis*, mean infestation intensities for larvae (0.4 ± 2.9) and nymphs (0.6 ± 2.4) were about equal, but nymphs were collected more frequently from raccoons. Adults of *I. scapularis* were rarely collected from raccoons. Likewise, adults of *I. texanus* were infrequently collected. On average, larvae (1.3 ± 8.6) and nymphs (1.3 ± 4.2) of *I. texanus* were 2.5 times more abundant than were females (0.8 ± 3.3). No males of *I. texanus* were removed from raccoons.

There were significant differences in the number of ticks removed from different body regions of the raccoons examined. Most (59%) ticks parasitizing raccoons were attached to the head and neck in areas where pelage was thin, such as the snout, around the eyes, and on the ears (Table 1). Fewer ticks were attached in other body regions. Generally, in these other regions, ticks were removed from areas of thin pelage, in the axis of limbs, on teats or scrotum, and around the anus, or in areas where the animal may have lost hair due to an injury. All stages of *A. americanum* were primarily attached on the head (50%), followed by the hind quarter (22%), shoulder and forelimbs (15%), and torso (13%) (Table 1). *Dermacentor variabilis* (91%), *I. texanus* (74%), and *I. scapularis* (99%) were predominantly removed from the head. The hindquarter was infested with 19% of the total numbers of ticks removed from raccoons, while the shoulder and torso contained 12% and 10%, respectively, of the total number of ticks parasitizing raccoons (Table 1).

Seasonal variations in infestation intensities for each tick life stage were found. For *A. americanum*, considerable variation between the mean and median numbers of ticks per animal was found within and between monthly collections (Table 2). Larvae were not collected from December to March. The abundance of larvae on raccoons increased dramatically from <0.1 larvae/animal in June to peak numbers reaching >500 larvae/raccoon in July. In contrast, some *A. americanum* nymphs were found on raccoons in December,

February, and March. A marked increase in the prevalence of nymphs on raccoons occurred in March and peak numbers exceeding an average of 250 nymphs per raccoon were collected in July. Males and females were much less abundant on raccoons with average numbers rarely exceeding one tick/animal. Adults were not collected from October to March. *Dermacentor variabilis* adults were most abundant in July while peak numbers of larvae were collected in October. Infestation intensities of *Ixodes texanus* larvae were greatest from October to January while nymphs were most abundant on raccoons from January to April. No males were collected from raccoons, but females were most frequently collected in the April and declined in abundance from June to August, with no specimens collected from October to February. Numbers of *I. scapularis* adults appeared to reach peak numbers in October while larvae and nymphs were most abundant on raccoons in January.

To collect host-seeking ticks, 18 sites were visited on at least one occasion but a majority of sites were repeatedly sampled. These areas were bottomland hardwood or pine-hardwood woodlands located in or adjacent to the study areas. *Amblyomma americanum* nymphs were collected from April to July (Fig. 2), but they were most abundant in early May when an average of over 200 ticks per ten min drag sample was collected. In comparison to nymphs, few males and females were collected. Generally, by June, comparable numbers of nymphs and adults were collected. After July, few *A. americanum* of any stage except larvae were collected by flagging. Larvae were not sampled because of the inordinate amount of time required to remove and count the thousands of larvae that were collected on each drag cloth. After October, few ticks were collected by flagging vegetation or dragging leaf litter.

Three species of ticks were collected from vegetation or leaf litter. *Amblyomma americanum* and *I. scapularis* were the only species collected in appreciable numbers. For *A. americanum*, almost 10 times as many nymphs as adults were tested; however, for *I. scapularis*, only adults were collected and screened for *B. burgdorferi*. Small numbers of spirochete-containing specimens of both of these species were collected (Table 3). For all stages of *A. americanum*, 0.2% of the specimens examined contained *B. burgdorferi* while 0.3% of black-legged tick adults contained spirochetes. Few spirochetes were found in individual specimens.

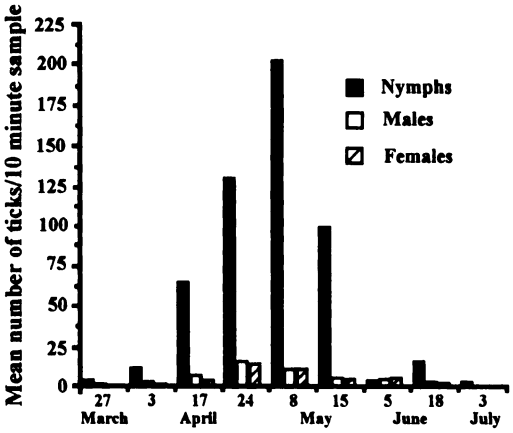

FIGURE 2. Seasonal abundance of lone star ticks (*Amblyomma americanum*) collected by flagging vegetation and leaf litter at 18 sites on Marine Corps Base, Camp Lejeune, North Carolina, in 1990 and 1992. Hyw is highway.

In Phase I, four species of ticks collected from raccoons were found to contain *B. burgdorferi* (Table 4). The overall percent of specimens with spirochetes was comparable for all species, ranging from a low of 0.7% for *I. texanus* to a high of 1.9% for *A. americanum*. In Phase II, most (93%) ticks removed from raccoons and examined for *B. burgdorferi* were *A. americanum* (Table 4). Two of the five species removed from raccoons contained *B. burgdorferi*. Spirochete prevalence in ticks was 1.7 and 21% for *A. americanum* and *D. variabilis*, respectively. Eleven of the 12 larval *D. variabilis* which contained *B. burgdorferi* were removed from the same raccoon.

TABLE 3. *Borrelia burgdorferi* detected in questing ticks collected by flagging vegetation and leaf litter on Marine Corps Base, Camp Lejeune, from 1 July 1990 to 30 June 1992. An indirect fluorescent antibody assay using monoclonal antibody H5332 was used to identify spirochetes found in the midgut of ticks.

Tick species	Nymphs			Males			Females			Overall		
	Total number	Number positive	% positive	Total number	Number positive	% positive	Total number	Number positive	% positive	Total number	Number positive	% positive
Amblyomma americanum	2,442	5	0.2	271	0	0	272	1	0.4	2,985	6	0.2
Dermacentor variabilis	0	0	0	6	0	0	2	0	0	8	0	0.0
Ixodes scapularis	0	0	0	105	0	0	200	1	0.5	305	1	0.3
Overall	2,442	5	0.2	382	0	0	474	1	0.2	3,298	7	0.2

In Phase I, only nine of 115 (7.8%) serum samples tested were serologically positive for antibody to *B. burgdorferi*. In Phase II, 50 of 103 serum samples were analyzed using Western blot procedures but no samples contained antibodies to the Lyme disease spirochete. Samples of blood collected from 87 raccoons were placed in culture media. Of this number, 23 (26%) yielded positive cultures of spirochetes. These cultures were identified to be *B. burgdorferi* senso lato by immunofluorescent antibody testing using monoclonal antibody H5332.

DISCUSSION

Most (351 of 449) raccoons examined in our study were tick-infested. Raccoons trapped and examined from June to August were heavily parasitized by ticks, especially *A. americanum*. Although all raccoons captured in Phase II were subjected

TABLE 4. Ticks removed from raccoons (*Procyon lotor*) that were tested for *Borrelia burgdorferi* using a monoclonal antibody (H5332) immunofluorescence antibody assay.

Tick species	Phase I (n = 179)			Phase II (n = 203)			Overall		
	Number negative	Number positive	% positive	Number negative	Number positive	% positive	Number negative	Number positive	% positive
Amblyomma americanum	650	12	1.9	1,999	33	1.7	2,694	45	1.7
Larvae	71	3	4.1	211	26	11.0	282	28	9.0
Nymphs	568	9	1.6	1,759	7	0.4	2,327	16	0.7
Males	8	0	0.0	15	0	0.0	23	0	0.0
Females	3	0	0.0	14	0	0.0	17	0	0.0
Dermacentor variabilis	136	2	1.5	52	14	21.2	188	16	7.8
Larvae	33	0	0.0	15	13	86.7	48	13	21.3
Nymphs	3	0	0.0	15	1	6.7	18	1	5.2
Females	38	1	2.6	21	0	0.0	59	1	1.7
Males	62	1	1.6	0	0	—	62	1	1.6
Ixodes cookei	88	1	1.1	9	0	0.0	97	1	1.0
Larvae	5	0	0.0	3	0	0.0	8	0	0.0
Nymphs	81	1	1.2	6	0	0.0	87	1	1.1
Females	2	0	0.0	0	0	—	2	0	0.0
Ixodes texanus	277	2	0.7	91	0	0.0	368	2	0.5
Larvae	1	0	0.0	38	0	0.0	39	0	0.0
Nymphs	146	2	1.4	8	0	0.0	154	2	1.3
Females	130	0	0.0	45	0	0.0	175	0	0.0
Overall	1,151	17	1.5	2,151	47	2.1	3,302	64	1.9

to thorough whole-body searches, infestation intensities (Table 1) most likely represented underestimates of the numbers of ticks parasitizing raccoons. Fish and Daniels (1990) suspended raccoons over pans of water to collect engorged *I. scapularis* larvae and collected nine times more engorged larvae from the water pans than were counted in initial examinations of raccoons.

Amblyomma americanum was the predominant tick species on raccoons on MCBCL. The observed seasonal patterns of occurrence and abundance of *A. americanum* in our study areas were also reported by Davidson et al. (1994) for an area of central Georgia (USA). In their investigation, peak numbers of *A. americanum* nymphs, as assessed by dragging vegetation, occurred in May, and the abundance of larvae markedly increased in July. In our investigation, too few *D. variabilis* adults were collected by flagging to establish seasonal patterns of abundance. Small numbers of *I. scapularis* adults were collected in winter months, and no immatures of this species were sampled by flagging. Likewise, *I. cookei* and *I. texanus* were not collected by flagging vegetation.

Amblyomma americanum was the most abundant species of tick parasitizing raccoons. The high abundance and frequency of occurrence of *A. americanum* on raccoons is evidence for a predilection for feeding on this animal (Tugwell and Lancaster, 1962; Koch and Dunn, 1980; Zimmerman et al., 1988). On MCBCL, the seasonal occurrence of raccoon-associated *A. americanum* larvae in July (Table 2) coincided with the observed activity pattern of host-seeking larvae. The infestation intensities of larvae on raccoons declined in September concurrent with the decrease in the numbers of larvae collected by flagging leaf litter. In contrast, the number of nymphs per raccoon peaked in July but the abundance of host-seeking nymphs collected from leaf litter was highest in May. This difference probably reflected temperature-induced changes in host-seeking activity. Night-time air temperatures in early spring likely declined to levels where the host-seeking activity of nymphs was suppressed when nocturnally-active raccoons were foraging in tick habitats. During the day, air temperatures increased sufficiently to allow nymphs to host-seek since we collected large numbers on cloth drags. In summer, as night-time air temperatures increased, nymphs would continue to host-seek for an extended period each day and would consequently be found in increased numbers on raccoons. Numbers of *A. americanum* nymphs and adults collected by flagging generally were congruent with the relative abundance of these stages on raccoons.

In contrast to *A. americanum*, *Ixodes scapularis*, an established vector of *B. burgdorferi* (Lane et al., 1991), was collected in low numbers from raccoons. On MCBCL, an average of <1 *I. scapularis* per raccoon was found. In a previous study, *Peromyscus* spp. mice, preferred hosts of *I. scapularis* (Mather et al., 1989), were not abundant on MCBCL (Apperson et al., 1993) and found to be infrequently parasitized (three of 107) by *I. scapularis* immatures. In contrast, larvae and nymphs of *I. scapularis* were removed from 49 of 92 skinks (*Eumeces inexpectatus*, *Eumeces laticeps*, and *Scincella lateralis*) (Apperson et al., 1993). Apperson et al. (1993) suggested that skinks might be interfering with transmission of *B. burgdorferi* by deflecting *I. scapularis* from feeding on *Peromyscus* spp. However, skinks can be infected with *B. burgdorferi* by tick bite, and the spirochete can be xenodiagnostically recovered from skinks with *I. scapularis* larvae (Levin et al., 1996). The relevance of this finding to the epidemiology of Lyme disease on MCBCL has not been established, but it is evidence that ticks on MCBCL could potentially acquire *B. burgdorferi* from hosts other than white footed mice or raccoons. *Ixodes scapularis* parasitizes a variety of mammalian, avian, and reptilian species (Anderson, 1991).

Results of our investigation are paradoxical in that a large proportion of the raccoon population on MCBCL appears to be infected with *B. burgdorferi*; however, ticks removed from culture or antibody-positive ticks were not found to contain *B. burgdorferi*. Likewise, *B. burgdorferi* antibodies were not detected in any culture-positive raccoons. The small percentage of host-seeking ticks that contained *B. burgdorferi* (Table 3) was congruent with the low percentage of host-associated ticks with *B. burgdorferi* (Table 4). Nevertheless, finding a small percentage (64 of 3,302; 1.9%) of *B. burgdorferi*-positive, host-associated ticks was surprising given the large percentage (23 of 87; 26%) of raccoons from which spirochetes were cultured.

With the exception of one raccoon, all seropositive raccoons were sampled in the late summer (August) or early winter (December). Whereas, all culture-positive raccoons were trapped in the winter and spring from January to the beginning of April. Spirochete-containing ticks (host-seeking and host-associated) were generally collected in late summer or fall from August to November when raccoons were antibody-positive but not culture-positive.

Our results for wild raccoons were similar to the findings of an attempt to experimentally infect raccoons with *B. burgdorferi* (Norris et al., 1996). Five raccoons were exposed to the JD1 and Wisconsin 210 Wise strains of *B. burgdorferi* by feeding infected *I. scapularis* nymphs on them. Spirochetes were not recovered from these raccoons by xenodiagnosis using *I. scapularis* larvae; however, *B. burgdorferi* was cultured from skin and blood from one raccoon and the skin of another raccoon. Norris et al. (1996) speculated that wild raccoons in Lyme disease endemic areas are potentially bitten by thousands of *I. scapularis* each season, based on tick burdens reported by Fish and Daniels (1990) for field-collected raccoons, and that raccoons may require repeated and prolonged exposure to spirochete-infected-ticks before being able to infect feeding ticks. In our investigation, raccoons were parasitized by relatively few *I. scapularis* (Table 1), and none of the eight female ticks tested contained *B. burgdorferi*. However, small numbers of *A. americanum*, *D. variabilis*, *I. cookei* and, *I. texanus* removed from raccoons did contain *B. burgdorferi* (Table 4). Few spirochetes were observed in most specimens, and spirochete-containing ticks were removed from six or less animals in each phase of our investigation. The small percentage of ticks that acquired spirochetes and the low number of spirochetes in individual specimens is evidence that transfer of the spirochete to these species of ticks might be inefficient. With the exception of *I. texanus* (which has not been tested), these ticks have repeatedly failed to transmit *B. burgdorferi* in laboratory vector competence trials (Piesman and Sinsky, 1988; Ryder et al., 1992).

The prevalence of antibody provides an index of exposure to *B. burgdorferi*. Norris et al (1996) found that four of five experimental raccoons developed an antibody response, but only after being fed upon by two cohorts of spirochete-infected *I. scapularis* nymphs. For wild raccoons trapped in Lyme disease endemic areas of Connecticut (USA), antibody prevalence rates ranged from 9% to 23% (Magnarelli et al., 1984a, b; 1991). In North Carolina (USA) on coastal barrier islands, 15 (38%) of 40 raccoons had antibody to *B. burgdorferi* (Magnarelli et al., 1991). In contrast, less than 5% (nine of 165) of the raccoons sampled on MCBCL had antibodies to *B. burgdorferi*. By our definition, only animals whose sera reacted to Osp A and Osp B were considered to be antibody-positive. Magnarelli et al. (1995) reported the presence of antibody to both proteins in free-ranging raccoons trapped in Connecticut; however in their study, sera from all raccoons designated as seropositive lacked reactivity to both proteins. Therefore, the criteria used in our investigation may provide a conservative estimate of the preva-

lence of antibody in the raccoon population on MCBCL.

Our results are evidence that transmission of *B. burgdorferi* among raccoons on MCBCL may be maintained by means that do not involve ticks as intermediate hosts of the spirochete. Tick infection rates were low, and raccoons were predominantly parasitized by tick species that do not successfully transmit *B. burgdorferi*. Recently, Ryan (1996) compared two *B. burgdorferi* strains (one derived from *I. scapularis* the other from a MCBCL raccoon) for infectivity in black legged ticks (*I. scapularis*), marsh rice rats (*Oryzomys palustris*), and laboratory white mice (*Mus musculus*). The infectious doses (ID_{50s}) for the mammal-derived strain were 100-fold less by needle inoculation (a mechanical method) in mice and rats relative to the tick-derived strain. In contrast, the percentage of *I. scapularis* ticks infected after feeding on *B. burgdorferi*-infected rice rats was three times higher for the tick-derived strain. Ryan (1996) concluded that observed differences in strain infectivity possibly reflected a divergence in the manner in which the two spirochete strains were transmitted with the raccoon-derived strain becoming relatively more effective in mammal-to-mammal transmission, perhaps through contaminated urine. In this regard, Lord et al. (1994) recently found *Peromyscus* spp. in western Pennsylvania (USA) to be infected with *B. burgdorferi* in the absence of ticks. They suggested that *B. burgdorferi* might be transmitted among mice directly through oral or sexual contact.

We conclude that raccoons are presently of minor importance as reservoir hosts in the maintenance of *B. burgdorferi* transmission to humans in the coastal plain of North Carolina, and perhaps in the southeastern U.S. as well.

ACKNOWLEDGMENTS

Partial financial support for our research was provided by the U.S. Marine Corps, the Centers for Disease Prevention and Control through a subcontract with the Virginia Department of Health, and the Agricultural Research Service and College of Veterinary Medicine at North Carolina State University. The assistance of personnel of MCBCL Environmental Management Dept., Fisheries and Wildlife Section is appreciated.

LITERATURE CITED

ANDERSON, J. F. 1991. Epizootiology of Lyme borreliosis. Scandinavian Journal of Disease (Supplement) 77: 23–34.

———, L. A. MAGNARELLI, W. BURGDORFERI, AND A. BARBOUR. 1983. Spirochetes in *Ixodes dammini* and mammals from Connecticut. American Journal of Tropical Medicine and Hygiene 32: 818–824.

APPERSON, C. S., J. F. LEVINE, T. L. EVANS, A. BRASWELL, AND J. HELLER. 1993. Relative utilization of reptiles and rodent as hosts by immature *Ixodes scapularis* (Acari: Ixodidae) in the coastal plain of North Carolina, USA. Experimental and Applied Acarology 17: 719–731.

BARBOUR, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. Yale Journal of Biology and Medicine 57: 521–525.

CENTERS FOR DISEASE CONTROL AND PREVENTION. 1993. Lyme disease surveillance—United States, 1991 and 1992. Morbidity and Mortality Weekly Report 42: 345–348.

DAVIDSON, W. R., D. A. SIEFKEN, AND L. H. CREEKMORE. 1994. Seasonal and annual abundance of *Amblyomma americanum* (Acari: Ixodidae) in central Georgia. Journal of Medical Entomology 31: 67–71.

ENVIRONMENTAL MANAGEMENT DEPARTMENT. 1987. Executive summary—Multiple-use natural resources management plan: United States Marine Corps, Camp Lejeune, North Carolina. U.S. Marine Corps, Camp Lejeune, North Carolina, 8 pp.

FARRELL, C. E., AND G. W. WHARTON. 1948. A successful method for shipping larval trombiculids (chiggers). The Journal of Parasitology 34: 71.

FISH, D., AND T. J. DANIELS. 1990. The role of medium-sized mammals as reservoirs of *Borrelia burgdorferi* in southern New York. Journal of Wildlife Diseases 26: 339–345.

———, AND R. C. DOWLER. 1989. Host associations of ticks (Acari: Ixodidae) parasitizing medium-sized mammals in a Lyme disease endemic area of southern New York. Journal of Medical Entomology 26: 200–209.

GRAU, G. A., G. C. SANDERSON, AND J. P. ROGERS. 1970. Age determination of raccoons. The Journal of Wildlife Management 34: 364–372.

GREENE, R. T., J. F. LEVINE, E. B. BREITSCHWERDT, R. L. WALKER, H. A. BERHOFF, J. CULLEN, AND W. L. NICHOLSON. 1988a. Clinical and serolog-

ical evaluations of induced *Borrelia burgdorferi* infection in dogs. American Journal of Veterinary Research 49: 752–757.

———, R. L. WALKER, W. L. NICHOLSON, H. W. HEIDNER, J. F. LEVINE, E. C. BURGESS, M. WYAND, E. B. BREITSCHWERDT, AND H. A. BERKHOFF. 1988b. Immunoblot analysis of immunoglobulin G response to the Lyme disease agent (*Borrelia burgdorferi*) in experimentally and naturally exposed dogs. Journal of Clinical Microbiology 26: 648–653.

KOCH, H. G., AND J. E. DUNN. 1980. Ticks collected from small and medium-sized wildlife hosts in Leflore county, Oklahoma. Southwestern Entomologist 5: 214–221.

LANE, R. S., J. PIESMAN, AND W. BURGDORFER. 1991. Lyme borreliosis: Relation of its causative agent to its vectors and hosts in North American and Europe. Annual Review of Entomology 36: 587–609.

LEVIN, M., J. F. LEVINE, S. YANG, P. HOWARD, AND C. S. APPERSON. 1996. Reservoir competence of the southeastern five-lined skink (*Eumeces inexpectatus*) and the green anole (*Anolis carolinensis*) for *Borrelia burgdorferi*. American Journal of Tropical Medicine and Hygiene 54: 92–97.

LEVINE, J. F., M. L. WILSON, AND A. SPIELMAN. 1985. Mice as reservoirs of the Lyme disease spirochete. American Journal of Tropical Medicine and Hygiene 34: 355–360.

———, C. S. APPERSON, AND W. L. NICHOLSON. 1989. The occurrence of spirochetes in ixodid ticks in North Carolina. Journal of Entomological Science 24: 594–602.

LORD, R. D., V. R. LORD, J. G. HUMPHREYS, AND R. G. MCLEAN. 1994. Distribution of *Borrelia burgdorferi* in host mice in Pennsylvania. Journal of Clinical Microbiology 32: 2501–2504.

MAGNARELLI, L. A., J. F. ANDERSON, W. BURGDORFERI, AND W. A. CHAPPEL. 1984a. Parasitism by *Ixodes dammini* (Acari: Ixodidae) and antibodies to spirochetes in mammals at Lyme disease foci in Connecticut, USA. Journal of Medical Entomology 21: 52–57.

———, ———, AND W. A. CHAPPEL. 1984b. Geographic distribution of humans, raccoons and white-footed mice with antibodies to Lyme disease spirochetes in Connecticut. The Yale Journal of Biology and Medicine 57: 619–626.

———, J. H. OLIVER, H. J. HUTCHESON, AND J. F. ANDERSON. 1991. Antibodies to *Borrelia burgdorferi* in deer and raccoons. Journal of Wildlife Diseases 27: 562–568.

———, ———, R. A. FLAVELL, AND E. FIKRIG. 1995. Reactivity of white-footed mouse and raccoon sera to whole cell and recombinant antigens of *Borrelia burgdorferi*. Journal of Wildlife Diseases 31: 339–344.

MATHER, T. N., M. L. WILSON, S. I. MOORE, J. M. C. RIBEIRO, AND A. SPIELMAN. 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). American Journal of Epidemiology 130: 143–150.

NORRIS, D. E., J. F. LEVINE, M. MENARD, K. NAGAGAKI, P. HOWARD, AND C. S. APPERSON. 1996. Experimental infection of the raccoon (*Procyon lotor*) with *Borrelia burgdorferi*. Journal of Wildlife Diseases 32: 300–314.

PIESMAN, J., AND R. J. SINSKY. 1988. Ability of *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). Journal of Medical Entomology 25: 336–339.

RYAN, J. R. 1996. Host-associated genomic and antigenic variance in diverse strains of *Borrelia burgdorferi*, the etiologic agent of Lyme disease. Ph.D. Dissertation, North Carolina State University, Raleigh, North Carolina, 145 pp.

RYDER, J. W., R. R. PINGER, AND T. GLANCY. 1992. Inability of *Ixodes cookei* and *Amblyomma americanum* nymphs (Acari: Ixodidae) to transmit *Borrelia burgdorferi*. Journal of Medical Entomology 29: 525–530.

SAS. 1985. Statistical analysis system: User's guide. SAS Institute, Inc., Cary, North Carolina, 965 pp.

SCHAFALE, M. P., AND A. S. WEAKLEY. 1990. Classification of the natural communities of North Carolina: Third approximation. North Carolina Natural Heritage Foundation, Inc., Raleigh, North Carolina, 325 p.

STAES, C. 1989. Lyme disease in North Carolina. Epi Notes Report Number 89-6, North Carolina Division of Health Services, Epidemiology Section, Raleigh, North Carolina, 9 pp.

TUGWELL, P., AND J. L. LANCASTER, JR. 1962. Results of a tick host study in northwest Arkansas. Journal of the Kansas Entomological Society 35: 202–211.

ZIMMERMAN, R. H., G. R. MCWHERTER, AND S. R. BLOEMER. 1988. Medium-sized mammal hosts of *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae) at Land Between the Lakes, Tennessee, and effects of integrated tick management on host infestations. Journal of Medical Entomology 25: 461–466.

Received for publication 17 August 1995.