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# *Borrelia* sp. in Ticks Recovered from White-tailed Deer in Alabama

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ABSTRACT: Six hundred sixty-five hunter-killed white-tailed deer (*Odocoileus virginianus*) from 18 counties in Alabama (USA) were examined for ticks. Most of the collections were made at state-operated wildlife management areas. Four species of ticks ($n$ = 4,527) were recovered: the lone star tick *Amblyomma americanum* ($n$ = 482); the Gulf Coast tick *A. maculatum* ($n$ = 11); the winter tick *Dermacentor albipictus* ($n$ = 1,242); and the black-legged tick *Ixodes scapularis* ($n$ = 2,792). Fifty-six percent of the ticks ($n$ = 2,555) were examined for *Borrelia* sp. spirochetes using an immunofluorescent, polyclonal antibody assay. Spirochetes were detected in *I. scapularis* (five females, seven males) from Barbour, Butler, Coosa, and Lee counties and *A. americanum* (four males, four nymphs) from Hale, Lee, and Wilcox counties. Area-specific prevalences in ticks were as high as 3.3% for *I. scapularis* and 3.8% for *A. americanum*.

*Key words:* Ticks, *Amblyomma americanum*, *Ixodes scapularis*, Lyme disease, *Borrelia* sp., white-tailed deer, *Odocoileus virginianus*, prevalence, survey.

First recognized in the United States in 1975, Lyme disease (LD) now accounts for the majority of cases of arthropod-borne diseases reported nationwide (Anonymous, 1989). Studies from the northeastern United States indicate that the etiologic agent of Lyme disease, *Borrelia burgdorferi*, is maintained in a complex multihost cycle involving principally the white-footed mouse (*Peromyscus leucopus*), the white-tailed deer (*Odocoileus virginianus*), and the deer tick (*Ixodes dammini*). The number of human cases of LD in the southeastern United States has grown steadily in recent years, but the vector species from this region remain uncertain. The first human case of LD was reported in Alabama in 1986 (Mullen and Piesman, 1987). About 80 additional confirmed cases have been reported since that time according to the Alabama Department of Public Health, with 13 of these cases reported in the literature (Woernle, 1989). Since the appearance of LD in Alabama, statewide efforts have been undertaken to collect and identify ticks parasitizing deer and to examine them for *Borrelia* spp. spirochetes.

During the 1985–86, 1988–89, and 1989–90 hunting seasons (mid-November through January), 665 deer were examined at State-operated hunter check stations and on private properties in 18 counties throughout Alabama (USA; Fig. 1). Most animals were examined at the following Wildlife Management Areas (WMA): Black Warrior WMA (Lawrence County), Skyline WMA (Jackson County), Kinterbish WMA (Sumter County), T. R. Miller WMA (Escambia County), Choccolocco WMA (Cleburne County), Oakmulgee WMA (Hale County), Barbour County WMA, Butler County WMA, Coosa County WMA, and Hollins WMA (Clay County). Animals were examined for ticks by brushing back the fur and visually, as well as tactilely, searching the ears, chin, chest, neck, axillae, escutcheon, back, and inguinal regions. Ticks were removed with forceps, transferred to vials containing slightly moistened cotton, transported to the laboratory, and maintained at 4 C until they were identified. Voucher tick specimens have been deposited with the United States National Tick Collection (Georgia Southern University, Statesboro, Georgia 30460, USA) under accession number RML 119,900.

Live ticks were examined for *Borrelia* sp. using direct and indirect immunofluorescent assays with polyclonal antisera.

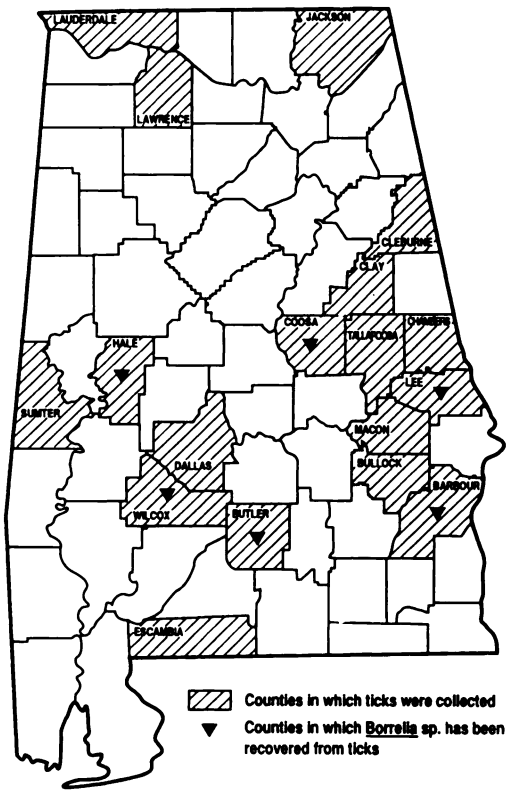


FIGURE 1. Map of Alabama depicting counties in which ticks were collected from white-tailed deer and those counties in which ticks infected with *Borrelia* sp. were recovered.

The rationale for using polyclonal, rather than monoclonal, antisera was that we do not known how reliably the southern strains of *B. burgdorferi* respond to available monoclonal antibodies (e.g., H5332). The use of polyclonal antibody tests thus reduces the prospect of overlooking *Borrelia* sp. infections even when they are present. Portions of midgut and salivary gland tissue were removed from each tick and teased apart on a glass slide. Dissections were air-dried and fixed with cold acetone. Direct assays were performed as described by Piesman et al. (1986). Prepared slides and control slides of cultured spirochetes were overlaid with fluorescein-labelled, high-titered rabbit anti-*B. burgdorferi* polyclonal conjugate (Guilford strain), incubated, washed, and examined using fluorescence microscopy. Indirect assays also

TABLE 1. Deer ticks examined for *Borrelia* sp. using direct fluorescent antibody assays by site and date of positive tick recovery, 1988–90.

| Date | Site | Number of deer examined | Ticks examined | | | | Positive ticks |
|---|---|---|---|---|---|---|---|
| | | | *Amblyomma americanum* N, M, F[a] | *Ixodes scapularis* N, M, F | *Dermacentor albipictus* N, M, F | *Amblyomma maculatum* N, M, F | |
| 16 Jun 88 | Lee County | 1 | 14, 4, 0 | 0 | 0 | 0 | 1 N *Amblyomma americanum* |
| 18 Jun 88 | Lee County | 1 | 11, 1, 0 | 0 | 0 | 0 | 3 N *Amblyomma americanum* |
| 29 Nov 88 | Lee County | 1 | 0 | 0, 6, 5 | 0 | 0 | 1 M *Ixodes scapularis* |
| 2 Dec 88 | Lee County | 1 | 0 | 0, 4, 5 | 0, 1, 0 | 0 | 1 M *Ixodes scapularis* |
| 25 Jan 89 | Lee County | 1 | 0, 1, 0 | 0, 4, 5 | 0 | 0 | 1 M *Amblyomma americanum* |
| 26 May 89 | Wilcox County | 1 | 2, 8, 2 | 0 | 0 | 0 | 1 M *Amblyomma americanum* |
| 13 Dec 89 | Coosa WMA[b] | 61 | 1, 13, 1 | 0, 39, 82 | 0 | 0, 2, 0 | 2 M, 2 F *Ixodes scapularis* |
| 16 Dec 89 | Barbour WMA | 92 | 0 | 0, 164, 150 | 0 | 0 | 1 F *Ixodes scapularis* |
| 12 Jan 90 | Barbour WMA | 27 | 0 | 0, 224, 171 | 0 | 0 | 1 M, 1 F *Ixodes scapularis* |
| 27 Jan 90 | Oakmulgee WMA | 39 | 7, 29, 16 | 0, 36, 30 | 0 | 0 | 1 F *Ixodes scapularis* |
| | | | | | | | 2 M *Amblyomma americanum* |
| 31 Jan 90 | Butler WMA | 18 | 5, 8, 2 | 1, 58, 40 | 0 | 0 | 2 F *Ixodes scapularis* |

[a] N, nymphs; M, males; F, females.
[b] WMA, wildlife management area.

TABLE 2. Area-specific prevalence of ticks infected with *Borrelia* sp. in Alabama, 1989–90.

| Date | Site | Number of ticks examined | Number of ticks positive | Prevalence (%) |
|---|---|---|---|---|
| | *Ixodes scapularis* | | | |
| 13 Dec 89 | Coosa WMA* | 121 | 4 | 3.3 |
| 16 Dec 89 | Barbour WMA | 314 | 1 | 0.3 |
| 12 Jan 90 | Barbour WMA | 395 | 2 | 0.5 |
| 27 Jan 90 | Oakmulgee WMA | 66 | 1 | 1.5 |
| 31 Jan 90 | Butler WMA | 99 | 2 | 2.0 |
| | *Amblyomma americanum* | | | |
| 27 Jan 90 | Oakmulgee WMA | 52 | 2 | 3.8 |

* WMA, wildlife management area.

were performed using unlabeled rabbit anti-*B. burgdorferi* antiserum and fluorescein-labeled goat antirabbit antiserum as described (Luckhart et al., 1991).

Four thousand five hundred twenty seven nymphal and adult ticks of four species were recovered from the deer examined. The majority of ticks collected were *Ixodes scapularis* ($n$ = 2,792; 62%), followed by *Dermacentor albipictus* ($n$ = 1,242; 27%), *Amblyomma americanum* ($n$ = 482; 11%), and *A. maculatum* ($n$ = 11; 0.2%). Two thousand five hundred fifty-five ticks (56%) were dissected and examined for spirochetes.

Twenty ticks (0.8%) were found infected with spirochetes which were IFA-positive and morphologically indistinguishable from cultured *B. burgdorferi* on control slides: five male and seven female *I. scapularis* from Barbour, Butler, Coosa, and Lee counties, and four nymphal and four male *A. americanum* from Hale, Lee, and Wilcox counties (Table 1). Five of these infected ticks were collected from deer at the same study site in Lee County where *I. scapularis* from cotton mice (*Peromyscus gossypinus*) previously were found to be infected with *B. burgdorferi* as confirmed using *B. burgdorferi*-specific murine monoclonal antibody H5332 (Luckhart et al., 1991). Efforts to isolate and culture spirochetes at this site have not been unsuccessful.

Area-specific prevalence was calculated for the December 1989 and January 1990 wildlife management area collections by dividing the number of infected ticks of one species from an area on a specific date by the total number of that species recovered and assayed from that area on the same date. Because only individual deer were examined on the dates indicated in Lee and Wilcox counties, no attempt was made to report prevalence of infection for these collections.

Prevalence of infection at the wildlife management areas ranged from 0.3 to 3.3% for *I. scapularis* and was 3.8% for the single IFA-positive *A. americanum* collection (Table 2). These findings are consistent with observations made in North Carolina by Magnarelli et al. (1986). They reported infection prevalences of 0.5% and 4.8% for *I. scapularis* and *A. americanum*, respectively, recovered from deer in Onslow County in December 1984.

In our previous field studies, *B. burgdorferi*-infected *I. scapularis* immatures were recovered from rodents in areas of confirmed human cases of Lyme disease in Alabama (Luckhart et al., 1991). This supports the belief that *I. scapularis* plays a role in at least the maintenance cycle of *B. burgdorferi* among wild reservoir hosts. However, there is no conclusive evidence documenting the involvement of this tick in actual transmission of the Lyme disease agent to humans in the southeastern United States. In fact, *I. scapularis* is not commonly collected on humans in Alabama. Among more than 200 cases of human in-

festations with ticks in Alabama in 1990–91, only eight involved *I. scapularis*; in contrast, 132 of those cases (65%) involved *A. americanum* (G. R. Mullen, unpubl. data). The detection of *B. burgdorferi*-like spirochetes in *A. americanum* therefore continues to raise the prospect that this species plays a role in transmission of *B. burgdorferi* among wild hosts and to domestic animals and humans in the southeastern United States.

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