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Authors: Norment, B. R., Stricklin, L. S., and Burgdorfer, W.

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RICKETTSIA-LIKE ORGANISMS IN TICKS AND ANTIBODIES TO SPOTTED FEVER-GROUP RICKETTSIAE IN MAMMALS FROM NORTHERN MISSISSIPPI¹

B. R. Norment,² L. S. Stricklin,² and W. Burgdorfer³

ABSTRACT: Studies were conducted from April through August during 1974 and 1975 on 30 randomly selected trapping sites in Wall Doxey State Park, Holly Springs National Forest, and 1.6 km south of the park in Marshall County, Mississippi to provide information on the occurrence of ticks involved in the maintenance and transmission of spotted fever-group rickettsiae in nature. Of 365 animals (14 species) collected, 186 (51%) were parasitized by 4,169 ticks. Species of ticks collected included: (1) *Dermacentor variabilis*; (2) *Amblyomma americanum*; (3) *Rhipicephalus sanguineus*; (4) *Haemaphysalis leporispalustris*; and (5) *Ixodes texanus*. Of 2,105 ticks examined, 116 (5.5%) were positive for rickettsia-like organisms.

INTRODUCTION

Many species of wild and domestic animals are known to be susceptible to Rocky Mountain spotted fever (RMSF) and are recognized natural reservoirs in the maintenance of spotted fever-group rickettsiae including *Rickettsia rickettsii*, the causative agent of spotted fever. These animals are also principal hosts of several species of ticks, including *Dermacentor variabilis* (Say), *D. andersoni* Stiles, *D. parumapertus* Neuman, *Amblyomma americanum* (L.), and *Haemaphysalis leporispalustris* (Packard).

The prevalence of RMSF in humans in Mississippi has been highest in the northern and central counties (Sexton and Burgdorfer, 1975). In fact, of 141 cases of RMSF in Mississippi from 1933-1973, only five were reported from the Delta Region, an area which includes over 15% of the state's total population. The major vege-

tative type for the Delta Region is southern floodplain, whereas the northern and north-central areas in Mississippi are principally oak-hickory-pine and oak-hickory. Sonenshine et al. (1972) reported that the distribution of reported cases of spotted fever in the eastern United States during 1951-1971 suggested that the prevalence of reported human cases was related significantly to areas in which the potential dominants are mesic eastern deciduous types, particularly oak-hickory-pine, northeastern coastal oak-pine, Appalachian oak, oak-hickory and mixed mesophytic forest.

Mississippi, like other southern and Appalachian states, is experiencing rapid growth of suburban developments and recreational facilities in areas infested with *Dermacentor variabilis* and *Amblyomma americanum*. Such changes amplify the necessity of expanding and updating our knowledge of the ticks and mammals involved in the natural history of RMSF.

MATERIALS AND METHODS

Mammals were collected from April through August during 1974 and 1975 at randomly selected sites of approximately 8.1 hectares each in Marshall County, Mississippi. Sherman, Tomahawk, and Havahart traps were set in areas conducive to mammal and tick activity; sites included shorelines of creeks and lakes, ecotones between heavily wooded areas, open fields and among low, dense vegetation. Traps were checked and rebaited from Monday

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¹ Publication No. 5692, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, Mississippi 39762, USA.

² Department of Entomology, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, Mississippi 39762, USA.

³ U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840, USA.

TABLE 1. Microagglutination antibody tests in animal sera, Marshall County, Mississippi.

Animal	Total sera	No. positive	% Positive	Titer(s)
Domestic dog (<i>Canis familiaris</i>)	13	2	15.4	1:8-1:16
Raccoon (<i>Procyon lotor</i>)	14	3	21.4	1:8-1:256
Opossum (<i>Didelphis marsupialis</i>)	38	1	2.6	1:8
Eastern woodrat (<i>Neotoma floridana</i>)	5	1	20.0	1:32
White-footed mouse (<i>Peromyscus leucopus</i>)	62	2	3.2	1:8
Cotton mouse (<i>Peromyscus gossypinus</i>)	9	1	11.1	1:256
House mouse (<i>Mus musculus</i>)	7	0	0	NR*
Cottontail rabbit (<i>Sylvilagus floridanus</i>)	5	3	60.0	1:16-1:64
Gray squirrel (<i>Sciurus carolinensis</i>)	5	1	20.0	1:16
Domestic cat (<i>Felis domestica</i>)	7	0	0	NR
White-tailed deer (<i>Odocoileus virginianus</i>)	4	1	25.0	1:32

* Non-reactive.

through Friday of each week. New trap sites were selected every 2 wk. Larger mammals were anesthetized with intramuscular injections of ketamine hydrochloride (Ketaset®). Smaller mammals and birds were killed with carbon dioxide. Ticks were removed from the animals, identified to species, placed in glass vials with cotton plugs and stored in a desiccator (saturated potassium chloride) until hemolymph tests were conducted. For rickettsial examination of ticks, hemolymph was used from each adult and nymphal tick by amputating the distal portion of one or more legs and stained as outlined by Burgdorfer (1970); larvae were squashed on a slide and then stained. Slides were examined under oil immersion for the presence of rickettsia-like organisms. Dogs, from which ticks were removed, were categorized as (1) pets, (2) strays and (3) penned hunting dogs. Blood samples were taken by cardiac or venous puncture. Sera were evaluated for antibodies to spotted fever-group rickettsiae by the microagglutination (MA) test according to the method of Fiset et al. (1969). The Sawtooth 92 strain of *Rickettsia rickettsii* was used as an antigen. Sera were considered positive at titers $\geq 1:8$.

The following specimens have been deposited in the Museum Support Center, Smithsonian Institution, Washington, D.C. 20560, USA: *Amblyomma americanum* (RML116970); *Dermacentor variabilis* (RML116971); *Haemaphysalis leporispalustris* (RML116972); *Rhipicephalus sanguineus* (RML116973); *Ixodes texanus* (RML65923).

RESULTS

Of 365 animals collected, 169 serum samples were obtained and tested (Table

1). Of those, 15 (8.9%) had significant titers ($\geq 1:8$) against the Sawtooth 92 strain of *R. rickettsii*. The sera from rabbits (3) and raccoons (3) were the most frequently positive followed by dogs and white-footed mice with two each. Antibodies to spotted fever-group rickettsiae were also detected in opossum, eastern wood rat, cotton mouse, gray squirrel and white-tailed deer. The highest titer (1:256) was detected in the sera of a raccoon and a cotton mouse. There were no significant titers found in domestic cats or house mice.

There were 1,074 specimens of *D. variabilis* collected from 12 different animal species (Table 2). Of this total, 87% were adult ticks from ten different animals, the most common being dogs (hunting > pets > strays), opossum and raccoons. Interestingly, 52 of 58 (90%) opossums and 14 of 18 (78%) raccoons were parasitized by *D. variabilis*. Larval *D. variabilis* were found on three different species; hosts were cotton mice, white-footed mice and eastern wood rats. Nymphs (58 and 11) were found on one of 80 dogs and five of 109 white-footed mice examined; other hosts included cotton mice, domestic cats, raccoons and gray squirrels. Of the three motile life stages of *A. americanum*, nymphs represented the widest range, being found on nine different species. With the exception of 334 nymphs off one white-tailed

TABLE 2. Tick/host records of animals, Marshall County, Mississippi.*

Host	No. ticks collected off host animal														
	<i>Dermacentor variabilis</i>			<i>Amblyomma americanum</i>			<i>Rhipicephalus sanguineus</i>			<i>Haemaphysalis leporispalustris</i>			<i>Ixodes texanus</i>		
	L ^b	N	A	L	N	A	L	N	A	L	N	A	L	N	A
Domestic dogs															
Stray	0	0	125	0	1	50	0	0	0	0	0	0	0	0	0
Pets	0	58	195	0	35	29	0	0	29	0	0	0	0	0	0
Hunting	0	0	208	0	10	136	11	272	476	0	0	0	0	0	0
White-tailed deer	0	0	1	1,026	334	90	0	0	0	0	0	0	0	0	0
White-footed mice	6	11	0	3	2	0	1	0	0	0	0	0	0	3	0
Cotton mice	48	2	0	2	3	0	0	0	0	0	0	0	0	0	0
House mice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eastern wood rats	5	0	6	0	0	0	0	0	0	0	0	0	0	0	0
Opossums	0	0	304	0	2	18	0	0	0	0	0	0	0	0	0
Domestic cats	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0
Cottontail rabbits	0	0	2	7	12	0	0	0	0	46	25	33	0	4	0
Raccoons	0	1	88	203	10	1	0	0	0	0	0	0	0	14	0
Gray squirrels	0	1	7	1	1	0	0	0	0	0	0	0	0	0	0
Moles	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bob-whites (<i>Colinus virginianus</i>)	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Cardinals (<i>Richmondia cardinalis</i>)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

* Unidentified motile stages of *Ixodes* spp. were also collected off white-footed mice (17L), cotton mouse (1L), rabbit (18L, 1N, 14A), and raccoon (35L, 69N, 50A).

^b L = larvae; N = nymph; A = adult.

deer, the preferred hosts included domestic dogs, cottontail rabbits, hunting dogs and raccoons. Larval specimens of *A. americanum* were found on six different mammals with 1,026 removed from three deer and 203 from a single raccoon; rabbits and rodents (white-footed mice, cotton mice and gray squirrels) were the remaining animals parasitized by larvae. Adults were recovered primarily on hunting, stray, and pet dogs and opossums; 90 were collected off a single white-tailed deer.

There was one larval specimen of *Rhipicephalus sanguineus* (Latreille) removed from a white-footed mouse; the remaining hosts were dogs. Both larval, nymphal and adult specimens of *R. sanguineus* were found on hunting dogs whereas only adult ticks were collected off

pet dogs. Of the remaining ticks collected, all motile stages of *H. leporispalustris* were found only on rabbits. Fourteen nymphal stages of *I. texanus* were collected off raccoon, four off cottontail rabbit and three from white-footed mouse. In addition, unidentified motile stages of *Ixodes* spp. were collected off white-footed mouse (17 larvae), cotton mouse (one larva), cottontail rabbit (18 larvae, one nymph, 14 adults) and raccoon (35 larvae, 69 nymphs, 50 adults).

Of 4,169 ticks collected, 2,105 (50.5%) were examined; the remaining ticks died prior to testing (Table 3). One hundred sixteen (5.5%) ticks representing three species (*D. variabilis*, *A. americanum*, and *R. sanguineus*) were positive for rickettsia-like organisms. The 45 positive specimens of *D. variabilis* were all adults (33

TABLE 3. Rickettsial infections of ticks collected from animals in Marshall County, Mississippi.

Host	Total no. examined	No. hosts parasitized					No. hosts with positive ticks	Hemolymph positive ticks		
		Dv*	Aa	Rs	Hp	It		Dv	Aa	Rs
Domestic dogs										
Stray	4	4	4	0	0	0	2	5	2	0
Pets	22	20	12	4	0	0	2	4	0	0
Hunting	54	29	23	20	0	0	8	7	1	22
White-tailed deer	5	1	3	0	0	0	3	0	43	0
White-footed mice	109	10	1	1	0	2	0	0	0	0
Cotton mice	14	6	3	0	0	1	0	0	0	0
Wood rats	19	4	0	0	0	0	0	0	0	0
Opossums	70	53	5	0	0	0	9	19	0	0
Domestic cats	8	2	1	0	0	0	0	0	0	0
Cottontail rabbits	7	1	2	0	5	3	0	0	0	0
House mice	13	0	0	0	0	0	0	0	0	0
Raccoons	18	14	3	0	0	0	2	10	3	0
Gray squirrels	14	2	1	0	0	5	0	0	0	0
Moles	2	0	0	0	0	0	0	0	0	0
Bob-whites	2	1	0	0	0	0	0	0	0	0
Cardinals	4	1	0	0	0	0	0	0	0	0

* Dv = *Dermacentor variabilis*, Aa = *Amblyomma americanum*, Rs = *Rhipicephalus sanguineus*, Hp = *Haemaphysalis leporispalustris* and It = *Ixodes texanus*.

males; 12 females) collected from nine opossums, one raccoon, two domestic and two hunting dogs. There were also 49 positive specimens of *A. americanum* (three female adults, 45 larvae, and one nymph) from one raccoon, one stray and one hunting dog and three white-tailed deer. Rickettsia-like organisms were detected in 19 nymphs and three adult male specimens of *R. sanguineus* from eight hunting dogs. All specimens of *H. leporispalustris* and *I. texanus* tested in this study were negative for rickettsiae.

DISCUSSION

Information available on the significance of various mammals in the maintenance cycle of Rocky Mountain spotted fever in Mississippi is limited. Although the significance of vertebrate hosts as reservoirs for spotted fever-group rickettsiae cannot be supported by our data, the fact that the majority of these animals occur in suburban environments reinforces their

importance as a transport mechanism for infected ticks. Magnarelli et al. (1979), who tested 1,093 mammalian sera, presented evidence that eight of 49 raccoons, 14 of 549 deer, eight of 470 of white-footed mice and one of two gray squirrels had titers >1:8 against spotted fever-group rickettsiae. Similarly, evidence has been reported of spotted fever-group rickettsiae exposure for numerous mammals and birds that serve as a blood source for ticks (Burgdorfer et al., 1966, 1974; Lundgren and Thorpe, 1966; Bozeman et al., 1967; Lundgren et al., 1968; Sexton et al., 1976; Lissman and Benach, 1980; Lane et al., 1981; Magnarelli et al., 1982).

Our data agree with those of Cooney and Burgdorfer (1974), who found that 13 of 19 animal species were parasitized by immature and adult specimens of *D. variabilis*. They observed that the medium-sized animals (dogs, raccoons, opossums, woodchucks, red fox) were the principal hosts of the adult ticks and that dogs, gray

squirrels, raccoons, opossums and prairie voles (*Microtus ochrogaster*) were the species parasitized by larval and nymphal stages. Coultrip et al. (1973) found 17 of 18 mammalian species parasitized by specimens of *D. variabilis*. The medium-sized animals [opossum, gray fox (*Urocyon cinereoargenteus*), skunk (*Mephitis mephitis*), and bobcat (*Felis rufus*)] were the preferred hosts of the adult stage, whereas eight rodent species, brush-rabbits, ground squirrels and domestic cats were the principal hosts of the immature stages. Similarly, Sonenshine and Stout (1971) and Sonenshine and Levy (1971) reported that of seven species of medium-sized animals [raccoons, striped skunk, opossums, red (*Vulpes fulva*) and gray fox, gray squirrels and woodchuck (*Marmota monax*)], the raccoon was the dominant host of *D. variabilis*.

The lone star tick, *A. americanum*, was associated with nine of 14 animal species collected in our study. The five major hosts included white-tailed deer (three of five), dogs (39 of 80), opossum (five of 70) and raccoons (three of 18). Previously, Cooney and Burgdorfer (1974) revealed that the bobcat, white-tailed deer and fallow deer were the preferred hosts of adult stages of *A. americanum*, whereas, gray fox, raccoon and woodchuck appeared to be principal hosts for the immature stages. A report by Sonenshine and Stout (1971) indicated that *A. americanum* was the least abundant of four tick species infesting medium-sized animals in the Piedmont area of Virginia; however, *A. americanum* was the most abundant species associated with raccoons and opossums in the Coastal Plains of Virginia.

Collections of specimens of *R. sanguineus* in our study were similar to previous reports (Bishopp and Trembley, 1945; Clifford et al., 1961; Keh, 1964; Rhodes and Norment, 1979) in that this tick occurred almost exclusively on dogs. Although we cannot completely discount the possibility that larval and/or nymphal *R. sanguineus*

feed on other hosts, this study and previous ones strongly suggest that dogs are the primary host animal.

Although the animal sample was low, an infestation prevalence of 71% (104 ticks off five rabbits) was indicative of meaningful *H. leporispalustris* populations in the area. Previously Coultrip et al. (1973) found that *H. leporispalustris* was host specific on rabbits; however, Cooney and Burgdorfer (1974) also reported *H. leporispalustris* from raccoons and Sonenshine and Clifford (1973) from 24 species of birds.

The first reported collections of *Ixodes texanus* in Mississippi were made in this study. Although previous reports did not list this tick in Mississippi, evidence of its occurrence in surrounding states has been documented (Clifford et al., 1961; Lancaster, 1973; Cooney and Burgdorfer, 1974). We found *I. texanus* associated with raccoons, white-footed mice, and cottontail rabbits. Although raccoons and rabbits have been reported previously as hosts for *I. texanus*, the white-footed mouse represents a new host record for the nymph of this species (Goddard and Norment, 1983). Sonenshine and Stout (1971) found the raccoon to be the sole host of *I. texanus* in the Piedmont area of Virginia; however, Cooney and Burgdorfer (1974) found nymphal stages associated with medium-sized mammals (gray fox, opossums, and raccoons); larvae and adults were observed only on raccoons.

Prevalence of ticks (116 of 2,105 = 5.5%) with rickettsia-like organisms was similar to that reported by Burgdorfer et al. (1974) who observed that 51 of 931 (5.5%) adult specimens of *D. variabilis* were infected with spotted fever-group rickettsiae. They also noted rickettsia-like organisms antigenically related to the spotted fever group in 64 of 545 specimens of *A. americanum*. These rickettsiae, however, did not produce detectable infections or antibodies in meadow voles. Similarly, various studies have revealed

spotted fever-group rickettsiae associated with *D. variabilis*, *A. americanum* and other species of ticks from Massachusetts, Connecticut, South Carolina, Mississippi, New York, and California (Sexton et al., 1976; Benach et al., 1977; Loving et al., 1978; Magnarelli et al., 1979, 1982; Feng et al., 1980; Lane et al., 1981).

Interestingly, of the 14 mammal and bird species from which ticks were collected in our study, only four, dogs, white-tailed deer, raccoon and opossum, were parasitized by ticks with rickettsia-like organisms. More specifically, a comparison of prevalences in ticks varied from 3.7% (41 of 1,106) to 13.6% (43 of 316) collected from dogs and white-tailed deer, respectively. Of the ticks collected from raccoons and opossums, prevalences of 6.2% (13 of 211) and 6.8% (19 of 281), respectively, were determined. In a recent study, Nause and Norment (1984) determined that 31 of 4,473 ticks (0.7%) removed from dogs in three counties in Mississippi possessed rickettsia-like organisms. Moreover, 94% of the positive ticks came from Lafayette County which adjoins Marshall County, the site of our study. However, the observed differences in prevalences are not readily apparent.

The antibody surveys in our study revealed an exposure to spotted fever-group rickettsiae in nine of 11 animal groups. Although this does not incriminate these animals as reservoirs of infection, it suggests previous infections. Burgdorfer et al. (1980) reported that rickettsial concentrations in the peripheral blood of cottontails infected experimentally were sufficient to infect ticks; however, infection prevalences were low and were suggestive that cottontail rabbits do not serve as efficient reservoirs for infecting ticks.

In the present studies, determinations were not made as to whether the organisms observed in the 45 specimens of *D. variabilis* and 49 specimens of *A. americanum* were *Rickettsia rickettsii* or related spotted fever-group rickettsiae. Like-

wise, whether the rickettsia-like organisms in the 22 specimens of *R. sanguineus* represent *Rickettsia rhipicephali* [a new spotted fever group agent first reported by Sexton et al. (1976) and subsequently described by Burgdorfer et al. (1978)] or related organisms was not determined. Our observations, however, confirm prior reports incriminating the ticks *D. variabilis*, *A. americanum* and *R. sanguineus* as hosts of rickettsia-like organisms.

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