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Rickettsiales in Ticks Removed from Outdoor Workers, Southwest Georgia and Northwest Florida, USA

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We determined the prevalence of selected Rickettsiales in 362 ticks removed from outdoor workers in southwest Georgia and northwest Florida, USA. Persons submitted an average of 1.1 ticks/month. We found *Ehrlichia chaffeensis* in an *Amblyomma maculatum* tick, and Panola Mountain *Ehrlichia* sp. in 2 *A. maculatum* ticks and 1 *Dermacentor variabilis* tick.

The southeastern United States has multiple tick species that can transmit pathogens to humans. The most common tick species, *Amblyomma americanum*, is the vector for the causative agents of human ehrlichioses and southern tick-associated rash illness, among others (1). *Dermacentor*

variabilis ticks can transmit the causative agent of Rocky Mountain spotted fever, and *Ixodes scapularis* ticks can transmit the causative agents of Lyme disease, babesiosis, and human granulocytic anaplasmosis (1). Although less common in the region, *A. maculatum* ticks are dominant in specific habitats and can transmit the causative agent of *Rickettsia parkeri* rickettsiosis (1).

Persons who have occupations that require them to be outside on a regular basis might have a greater risk for acquiring a tickborne disease (2). Although numerous studies have been conducted regarding risks for tickborne diseases among forestry workers in Europe, few studies have been performed in the United States (2,3). The studies that have been conducted in the United States have focused on forestry workers in the northeastern region (2). However, because of variable phenology and densities of ticks, it is useful to evaluate tick activity and pathogen prevalence in various regions and ecosystems.

Burn-tolerant and burn-dependent ecosystems, such as pine (*Pinus* spp.) and mixed pine forests commonly found in the southeastern United States, have unique tick dynamics compared with those of other habitats (4). The objective of this study was to determine the tick bite risk and tickborne pathogen prevalence in ticks removed from forestry workers working in pine and mixed pine forests in southwest Georgia and northwest Florida, USA.

During June 2009–December 2011, forestry workers in southwestern Georgia (7 counties) and northwestern Florida (1 county) submitted ticks crawling on or attached to them. We identified ticks and tested them for selected pathogens (Appendix, <https://wwwnc.cdc.gov/EID/article/25/5/18-0438-App1.pdf>). Immature forms of the same species from the same day and person were pooled (≤ 5 nymphs and ≤ 20 larvae) for testing.

A total of 53 persons submitted 362 ticks (Table). Excluding larvae, the most common tick species submitted was *A. maculatum*, followed by *A. americanum*, *I. scapularis*, and *D. variabilis*. On 4 occasions, 1 person submitted *A. tuberculatum* ticks (3 batches of larvae and 1 batch of nymphs) from a longleaf pine site in Baker County, Georgia. Average submissions per person were 2.6 ticks (median 1 tick), but 1 person submitted 100 ticks. A total of 24 persons submitted ticks more than once, and they submitted an average of 0.08–6.5 ticks/month (overall average submission rate of 1.1 ticks/month). Three ticks were engorged (1 *D. variabilis* adult, 1 *A. americanum* nymph, and 1 *Amblyomma* sp. nymph); only the *Amblyomma* sp. nymph was positive for a pathogen (*R. amblyommatis*).

Rickettsia spp. prevalence was 36.4% in adult, 27.9% in nymphal, and 20% in larval *A. americanum* ticks; *R. amblyommatis* was the only species identified (Table). *Rickettsia* spp. were detected in 23% of *A. maculatum* adults; *R. amblyommatis* was most common (6.0%), followed by

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Table. Prevalence of *Ehrlichia chaffeensis*, PME, and *Rickettsia* spp. in ticks submitted by outdoor workers, southwestern Georgia and northwestern Florida, USA*

Tick species and stage	Months submitted	No. positive ticks/no. tested (%)			
		<i>E. chaffeensis</i>	PME	<i>Rickettsia</i> spp.	<i>Rickettsia</i> spp. †
<i>Amblyomma americanum</i> , adults	Feb–Sep	0/11 (0)	0/11 (0)	4/11 (36.4)	2 <i>R. amblyommatis</i>
<i>A. americanum</i> nymphs‡	Mar–Sep	0/43 (0)	0/43 (0)	12/43 (27.9)	9 <i>R. amblyommatis</i>
<i>A. americanum</i> larvae‡	Apr and Oct	0/5 (0)	0/5 (0)	1/5 (20.0)	1 <i>R. amblyommatis</i>
<i>Amblyomma</i> sp. nymphs	Jun and Oct	0/3 (0)	0/3 (0)	1/3 (33.3)	1 <i>R. amblyommatis</i> §
<i>Amblyomma</i> sp. larvae	Oct	0/5 (0)	0/5 (0)	0/5 (0)	
<i>A. maculatum</i> adults	May–Oct	1/83 (1.2)	2/83 (2.4)¶	18/83 (21.7)	5 <i>R. amblyommatis</i> ,§ 4 <i>R. parkeri</i> ,§ 1 <i>Rickettsia</i> sp. TR-39/TX125, 2 <i>Candidatus</i> <i>R. andeanae</i>
<i>A. tuberculatum</i> nymphs‡	Apr	0/5 (0)	0/5 (0)	1/5 (5.0)	1 novel SFG <i>Rickettsia</i> sp.
<i>A. tuberculatum</i> larvae‡	Feb#	0/182 (0)	0/182 (0)	10/182 (5.5)**	10 novel SFG <i>Rickettsia</i> sp.**
<i>Dermacentor variabilis</i> adults	Jun–Aug	0/10 (0)	1/10 (10.0)	2/10 (20.0)	1 <i>R. amblyommatis</i> §
<i>Ixodes scapularis</i> adults	Oct–Mar	NT	0/15 (0)	7/15 (46.7)	4 <i>Rickettsia</i> sp. TR-39, 3 <i>R. buchneri</i>

*All *Rickettsia* spp. were identified by sequencing unless otherwise noted. NT, not tested; PME, Panola Mountain *Ehrlichia* sp., SFG, spotted fever group.

†*Rickettsia* spp. for whom amplicons did not provide high-quality bidirectional sequences were categorized as unknown *Rickettsia* spp.

‡Minimum infection prevalence is no. positive tick pools/no. ticks tested.

§The following *R. amblyommatis* samples were identified by restriction fragment length polymorphism analysis: for 1 *D. variabilis* adult, 5 *A. maculatum* adults, and 1 *Amblyomma* sp. nymph; for *A. americanum*, 1 adult, 2 nymphs, and 1 larva. Three *A. maculatum* adults were also identified as containing *R. parkeri* positive by restriction fragment length polymorphism analysis.

¶Data included in Loftis et al. (5).

#Date was known only for 1 submission of 20 larvae. Dates for others were not provided when submitted.

**Data included in Zemtsova et al. (6).

R. parkeri (4.8%). A previously detected novel *Rickettsia* sp. was identified in 10 of 11 *A. tuberculatum* larval pools and was reported by Zemtsova et al. (6). An additional pool of *A. tuberculatum* nymphs was tested in this study and also was positive for the novel *Rickettsia* sp. *E. chaffeensis* was detected in 1 *A. maculatum* adult (prevalence 1.2%), and Panola mountain *Ehrlichia* sp. was detected in 2 *A. maculatum* adults (prevalence 2.4%) and 1 *D. variabilis* adult (prevalence 10%). No ticks were positive for *Borrelia* spp., *E. ewingii*, or *Anaplasma phagocytophilum*.

Thus, forestry workers were found to encounter ticks on a regular basis, and peak encounter rates reflected previously reported tick seasonality in this region (4). Only 3 (0.8%) of the ticks submitted were engorged, indicating prompt removal of most ticks and thus low risk for pathogen transmission. *A. maculatum*, a fairly uncommon tick in the southeastern United States, was the most commonly submitted tick. However, *A. maculatum* ticks dominate in regularly burned pine ecosystems (4), which is where most of these workers spent their time.

We observed several unique findings related to pathogens during this study. Larvae and nymphs of *A. tuberculatum* ticks were submitted on multiple occasions, a tick rarely reported on humans (7). These findings, in conjunction with the identification of a novel *Rickettsia* sp. (6), suggest that additional research is warranted. This study also identified *E. chaffeensis* and Panola Mountain *Ehrlichia* in *A. maculatum* ticks. Although *A. americanum* ticks are considered the primary vector of *Ehrlichia* spp., these pathogens have been occasionally reported in questing *A. maculatum* ticks, suggesting that this tick might be involved in their transmission cycles (5,8). We also de-

tected Panola Mountain *Ehrlichia* in 1 *D. variabilis* tick. Thus, further research regarding these alternative tick species as potential vectors of these pathogens is warranted, particularly in the case of *A. maculatum* ticks, which were a common species on forestry workers and are widespread in this region (4).

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At the time of this study, Dr. Gleim was a research scientist at the University of Georgia, Athens, GA. She is currently a disease ecologist at Hollins University, Roanoke, VA. Her research interests include wildlife and zoonotic diseases with a particular emphasis on tickborne diseases.

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Hepatic Brucelloma Diagnosis and Long-Term Treatment, France

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We report a case of hepatic brucelloma in France. This diagnosis may be suspected in any patient who has a liver abscess after traveling to a brucellosis-endemic area. *Brucella* spp. may be detected by PCR in the liver tissue or suppuration. Abscess drainage and prolonged antimicrobial therapy help achieve healing.

Brucellosis is a zoonosis found worldwide (1,2) caused by gram-negative, facultative intracellular bacteria of the genus *Brucella*. Approximately 500,000 new infections are diagnosed annually, mainly in the Mediterranean basin, the Middle East, Latin America, and Asia (1–3). Brucellosis is a rare and mainly imported disease in other countries, including France (1,4). *Brucella* infection usually occurs after contact with infected animals or consumption of contaminated unpasteurized dairy products. Hepatic brucelloma (HB) is a chronic form of brucellosis arising up to 40 years after initial infection (1,3,5). Only 60 cases (1%–2% of all brucellosis infections) have been reported in English-language literature since 1904 (1,3,5,6). HB is associated with nonspecific systemic clinical symptoms (e.g., fever, malaise, weight loss, upper abdominal pain), moderate biologic abnormalities, and typical hypodense hepatic lesion with peripheral enhancement and central calcification (1–3,5,6).

In April 2015, a previously healthy 55-year-old woman was referred to Grenoble University Hospital after 7 days of fever, asthenia, and weight loss. She had lived in France for 20 years, but had been born in and had traveled every year to Algeria. Her clinical examination was unrevealing. Blood tests showed moderate inflammation and anicteric cholestasis (Table). Hepatic ultrasound (HUS) and computed tomography (CT) confirmed a defect 60 mm in diameter in liver segments IV and VIII with several subcapsular liquid collections and central calcification (Appendix Figure, panel A, <https://wwwnc.cdc.gov/EID/article/25/5/18-0613-Appl.pdf>).

Blood cultures remained sterile. Serologic test results were negative for HIV, amebiasis, and echinococcosis, but positive for *Yersinia enterocolitica* serotype O:9 and *Brucella* sp. (Table). HUS-guided drainage of the abscess yielded thick purulent fluid. Fluid cultures were negative, but we detected *Brucella melitensis* DNA by PCR amplification and sequencing of the 16S rRNA-encoding gene. Histological findings of liver tissue were compatible with a chronic abscess. We confirmed diagnosis on 2 occasions by PCR detection of *Brucella* DNA in the liver abscess, as previously reported (1,3,5–7). The serologic profile was suggestive of chronic brucellosis combining low IgM but strong IgG *Brucella* antibody titers (1,3,5,7). However, *Brucella* serologic diagnosis is poorly specific, due to antigenic cross-reactions (e.g.,

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Technical Appendix

Sample Acquisition

For tick collections, submissions were accepted from forestry and wildlife workers who worked throughout the region (Baker, Calhoun, Stewart, Thomas, Decatur, Dooly, Macon Counties in Georgia and Gladsden County in Florida). We notified participants about the project by emails and meetings. Workers were given tubes of 70% ethanol and asked to store and submit ticks in these tubes if they wished to participate in the project. Workers were instructed to separate ticks by date and location (if possible). The date(s) and location(s) where workers had obtained the tick(s) were recorded. No other information (e.g., time spent outdoors, past information on tick infestations or tick bites, etc.) was provided.

Tick Identification and Pathogen Testing

We morphologically identified nonlarval *Dermacentor variabilis* and *Amblyomma* spp. ticks. *Amblyomma* larvae and *Ixodes* spp. (all stages) were identified by using PCR targeting the 16S rRNA gene and sequencing (1). Ticks submitted in 2009 and 2010 were tested for *Rickettsia* spp., *Ehrlichia chaffeensis*, and *E. ewingii* by using a multiplex quantitative PCR targeting the 17-kDa gene of *Rickettsia* spp. and the 16S rRNA gene of *Ehrlichia* spp. (2).

Samples positive for *Rickettsia* spp. were analyzed by using a restriction fragment length polymorphism assay targeting the major outer membrane protein A gene by using primers RR190.70 and RR190.701R (3), followed by digestion with restriction enzymes *RsaI* and *PstI* (4). Ticks collected in 2011 were tested for *Rickettsia* spp., *Ehrlichia chaffeensis*, and *E. ewingii* by using nested PCRd specific for the 17-kDa (*Rickettsia* spp.) or 16S rRNA genes (*Ehrlichia* spp.) (4). Any samples positive for *Rickettsia* spp. that were not successfully identified by 17-kDa sequencing were then tested by using PCR and sequencing of the outer membrane protein A

gene (5). For all ticks, the *gltA* gene of Panola Mountain *Ehrlichia* sp., the *fla* gene of *Borrelia* spp., and the *msp2* gene of *Anaplasma phagocytophilum* were targeted (1).

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