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

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DOI: https://doi.org/10.3201/eid2505.180438

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 eastern ehrlichiosis 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. amblyomma*).

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

Acknowledgments
We thank the persons whom submitted ticks for this study and members of the Yabsley and Levin laboratories for providing laboratory assistance.

This study was supported by the Centers for Disease Control and Prevention/University of Georgia (UGA) collaborative grant (#8212, Ecosystem Health and Human Health: Understanding the Ecological Effects of Prescribed Fire Regimes on the Distribution and Population Dynamics of Tick-Borne Zoonoses); the Oxford Research Scholars Program at Oxford College of Emory University; the Joseph W. Jones Ecological Research Center, the Warnell School of Forestry and Natural Resources (UGA); the Southeastern Cooperative Wildlife Disease Study (UGA) through the Federal Aid to Wildlife Restoration Act (50 Statute 917); and Southeastern Cooperative Wildlife Disease Study sponsorship from fish and wildlife agencies of member states.

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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 | E. chaffeensis | PME | Rickettsia spp. | Rickettsia spp.† |
|------------------------|------------------|----------------|-----|----------------|-----------------|
| Amblyomma americanum, adults | Feb–Sep | 0/11 (0) | 0/11 (0) | 4/11 (36.4) | 2 R. amblyommatis |
| A. americanum nymphs‡ | Mar–Sep | 0/43 (0) | 0/43 (0) | 12/43 (27.9) | 9 R. amblyommatis |
| A. americanum larvae‡ | Apr and Oct | 0/5 (0) | 0/5 (0) | 1/5 (20.0) | 1 R. amblyommatis |
| Amblyomma sp. nymphs | Jun and Oct | 0/3 (0) | 0/3 (0) | 1/3 (33.3) | 1 R. amblyommatis§ |
| Amblyomma sp. larvae | Oct | 0/5 (0) | 0/5 (0) | 0/5 (0) | |
| A. maculatum adults | May–Oct | 1/83 (1.2) | 2/83 (2.4)‡ | 18/83 (21.7) | 5 R. amblyommatis,§ 4 R. parkeri,‡ 1 Rickettsia sp. TR-39/TX125, 2 Candidatus R. andeanae |

| A. tuberculatum nymphs‡ | Apr | 0/5 (0) | 0/5 (0) | 1/5 (5.0) | 1 novel SFG Rickettsia sp. |
| A. tuberculatum larvae‡ | Feb# | 0/182 (0) | 0/182 (0) | 0/182 (5.5)‡ | 10 novel SFG Rickettsia sp.‡ |
| Dermacentor variabilis adults | Jun–Aug | 0/10 (0) | 1/10 (10.0) | 2/10 (20.0) | 1 R. amblyommatis§ |
| Ixodes scapularis adults | Oct–Mar | NT | 0/15 (0) | 7/15 (46.7) | 4 Rickettsia sp. TR-39, 3 R. buchneri |

*All Rickettsia spp. were identified by sequencing unless otherwise noted. NT, not tested; PME, Panola Mountain Ehrlichia sp.; SFG, spotted fever group. ‡Rickettsia sp. 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. #Date was known only for 1 submission of 20 larvae. Dates for others were not provided when submitted. **Data included in Loftis et al. (5).
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 suppurated 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 (I–3). Brucellosis is a rare and mainly imported disease in other countries, including France (4,5). *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 (I,3,5). Only 60 cases (1%–2% of all brucellosis infections) have been reported in English-language literature since 1904 (I,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 (I–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-app1.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 (I,3,5–7). The serologic profile was suggestive of chronic brucellosis combining low IgM but strong IgG *Brucella* antibody titers (I,3,5,7). However, *Brucella* serologic diagnosis is poorly specific, due to antigenic cross-reactions (e.g.,

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Hepatic Brucelloma Diagnosis and Long-Term Treatment, France

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DOI: https://doi.org/10.3201/eid2505.180613
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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 Gladesden 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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