repercussions for human health? Given that 95%–99% of humans possibly exposed to such a reservoir are Duffy negative, and therefore resistant to the parasite, these would appear to be slight. However, as humans encroach more frequently into ape habitats, the chances of humans encountering the parasite will increase. In the short term, the risks are probably limited to Duffy-positive persons who enter areas where apes are present, such as tourists and migrant workers.

Richard Leighton Culleton and Pedro Eduardo Ferreira

Author affiliations: Nagasaki University, Nagasaki, Japan (R.L. Culleton, P.E. Ferreira); University of Algarve, Faro, Portugal (P.E. Ferreira); and Karolinska Institutet, Stockholm, Sweden (P.E. Ferreira)

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Address for correspondence: Richard Leighton Culleton, Malaria Unit, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan; email: richard@nagasaki-u.ac.jp

Rickettsia parkeri and *Candidatus* Rickettsia andeanae in Gulf Coast Ticks, Mississippi, USA

To the Editor: *Rickettsia parkeri*, a spotted fever group *Rickettsia* (SFGR) bacterium, is transmitted by *Amblyomma maculatum*, the Gulf Coast tick (*1*). The prevalence of *R. parkeri* in Gulf Coast ticks has been reported as <42% in the United States, which is higher than reported rates of *R. rickettsii* (the cause of Rocky Mountain spotted fever) in *Dermacentor* species ticks. Misdiagnosis among SFGR infections is not uncommon, and *R. parkeri* rickettsiosis can cause symptoms similar to those for mild Rocky Mountain spotted fever (*1*). We evaluated infection rates of *R. parkeri* and *Candidatus* Rickettsia andeanae, a recently identified but incompletely characterized SFGR, in Gulf Coast ticks in Mississippi, USA.

During May–September of 2008–2010, we collected adult Gulf Coast ticks from vegetation at 10 sites in Mississippi. We extracted genomic DNA from the ticks using the illustra tissue and cells genomicPrep Mini Spin Kit (GE Healthcare Life Sciences, Piscataway, NJ, USA). We tested amplifiable tick DNA by PCR of the tick mitochondrial 16S rRNA gene (*2*). We tested for molecular evidence of any SFGR species by nested PCR of *rompA* (rickettsial outer membrane protein A gene) (*1*). Samples positive for SFGR were subsequently tested by using species-specific *rompA* PCR for *R. parkeri* (*3*) and *Candidatus* R. andeanae (*4*). All PCRs included 1) a positive control of DNA from cultured *R. parkeri*– (Tate's Hell strain) or *Candidatus* R. andeanae–infected Gulf Coast ticks and 2) a negative control of water (nontemplate). PCR products were purified by using Montage PCR Centrifugal Filter Devices (Millipore, Bedford, MA, USA) and sequenced by using Eurofins MWG Operon (Huntsville, AL, USA). We generated consensus sequences using ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2/) alignment and identified the sequences using GenBank BLAST searches (www.ebi.ac.uk/Tools/clustalw2/).

Proportions of ticks infected with SFGR, by region and year, were compared separately by using Fisher exact test followed by pairwise comparisons with a Bonferroni

Table. PCR results for adult *Rickettsia parkeri*– and *Candidatus* Rickettsia andeanae–infected Gulf Coast ticks (*Amblyomma maculatum*) collected from 10 sites in Mississippi, USA, 2008–2010*

Location (no. collection sites)	No. ticks	No. (%; 95% CI) SFG *rompA*	No. (%; 95% CI) *R. parkeri* only	No. (%; 95% CI) *Candidatus* R. andeanae only	Expected no. (%) co-infected ticks	No. (%; 95% CI) co-infected ticks
North (4)	257	49 (19.1; 14.5–24.4)†	48 (18.7; 14.1–24)‡	0§	0.19 (0.07)	1 (0.4; 0–2.1)¶
Central (1)	38	4 (10.5; NA)	1 (2.6; NA)	2 (5.3; NA)	0.16 (0.42)	1 (2.6; NA)
South (5)	403	75 (18.6; 14.9–22.8)†	57 (14.1; 10.9–17.9)‡	8 (2.0; 0.9–3.9)§	2.99 (0.74)	10 (2.5; 1.2–4.5)¶
Total (10)	698	128 (18.3; NA)	106 (15.2; NA)	10 (1.4; NA)	3.65 (0.52)	12 (1.7; NA)

*The estimated value of co-infection caused by chance alone (E) was calculated by using the formula E = (a + b)(a + c) / (a + b + c + d) (5), where a = no. ticks infected with both *Rickettsia* species, b = no. ticks infected only with *R. parkeri*, c = no. ticks infected only with *Candidatus* R. andeanae, and d = no. ticks not infected with either *Rickettsia* species. SFG *rompA*, spotted fever group rickettsial outer membrane protein A gene. NA, not applicable.
†p = 0.9187.
‡p = 0.1275.
§p = 0.0257.
¶p = 0.0578 (comparison of prevalence from northern and southern sites only).

adjustment (PROC FREQ, SAS for Windows, V9.2; SAS Institute, Cary, NC, USA). For all analyses, $p<0.05$ was considered significant. An index of co-infection was calculated by using the formula IC = ([O – E]/N) × 100, in which IC is index of co-infection, O is number of co-infections, E is expected occurrence of co-infection caused by chance alone, and N is total number of ticks infected by either or both *Rickettsia* species. A χ^2 test was used to determine statistical significance (*5*).

A total of 707 adult Gulf Coast ticks were collected during the 3 years (350 in 2008, 194 in 2009, and 163 in 2010). Tick mitochondrial 16S rRNA gene was detected in 698 (98.7%), of which 128 (18.3%) were positive for SFGR DNA, comprising 106 (15.2%) positive only for *R. parkeri*, 10 (1.4%) positive only for *Candidatus* R. andeanae, and 12 (1.7%) co-infected with *R. parkeri* and *Candidatus* R. andeanae (Table). Positive test results from 22 ticks singly or co-infected with *Candidatus* R. andeanae were confirmed by sequencing.

Most (94.6%) ticks were from northern (n = 260) and southern (n = 409) Mississippi (online Technical Appendix, Figure, wwwnc.cdc.gov/EID/article/18/10/12-0250-F1.htm). No significant difference in the number of *R. parkeri*–infected ticks between northern and southern Mississippi was observed (p = 0.13) (Table). However, significantly more ticks were singly infected with *Candidatus* R. andeanae in southern sites than in northern sites (p = 0.03). The infection rate for co-infected ticks in southern sites was higher than that in northern sites (p = 0.06). Among the 3 collection years for northern and southern sites, only the prevalence of *R. parkeri* in singly infected ticks differed significantly (p = 0.01) (data not shown); the infection rate was significantly greater during 2010 than during 2009 (p = 0.003, α/3 = 0.02). The overall index of co-infection with *R. parkeri* and *Candidatus* R. andeanae was 6.5, statistically higher than expected by chance alone (Table) ($p<0.0001$).

The overall prevalence of infection with SFGR species in Gulf Coast ticks sampled was 18.3%; 15.2% of ticks were singly infected with *R. parkeri*, and 1.7% were infected with *R. parkeri* and *Candidatus* R. andeanae. As reported, the frequency of *R. parkeri* in Gulf Coast ticks is generally high, ranging from ≈10% to 40% (*3*,*4*,*6*–*8*). We found approximately 1 *R. parkeri*-infected Gulf Coast tick for every 6 ticks tested, suggesting that infected Gulf Coast ticks are commonly encountered in Mississippi. Because Gulf Coast ticks are among the most common human-biting ticks in Mississippi (*9*), awareness of *R. parkeri* rickettsiosis should be increased in this state. We identified *Candidatus* R. andeanae in ≈3% of Gulf Coast ticks in Mississippi; this frequency is similar to those reported in other studies of Gulf Coast ticks in the southern United States (*4*,*6*). Our finding of co-infected Gulf Coast ticks is at a frequency significantly higher than expected from chance alone. The biologic role of co-infections of Gulf Coast ticks with *R. parkeri* and *Candidatus* R. andeanae remains to be determined.

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Flavia A.G. Ferrari, Jerome Goddard, Christopher D. Paddock, and Andrea S. Varela-Stokes

Author affiliations: Mississippi State University, Mississippi State, Mississippi, USA (F.A.G. Ferrari, J. Goddard, A.S. Varela-Stokes); and Centers for Disease Control and Prevention, Atlanta, Georgia, USA (C.D. Paddock)

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Address for correspondence: Andrea S. Varela-Stokes, Mississippi State University, Wise Center, 240 Wise Center Dr, Mississippi State, MS 39762, USA; email: stokes@cvm.msstate.edu

Attributing Cause of Death for Patients with *Clostridium difficile* Infection

To the Editor: Hota et al. report that for deceased patients who had *Clostridium difficile* infection (CDI), agreement is poor between causes of death reported on death certificates and those categorized by a review panel (*1*). Our data support the difficulty of attributing cause of death for patients with CDI.

In 2004 in Quebec, Canada, a mandatory CDI surveillance program was implemented. Deaths that occurred within 30 days after CDI diagnosis were classified as 1) directly attributable to CDI (e.g., toxic megacolon, septic shock), 2) having a CDI contribution (e.g., acute decompensation of chronic heart failure), or 3) unrelated to CDI (e.g., terminal cancer) (*2*). To determine accuracy of the surveillance classifications, we compared cause-of-death classification of 22 deceased CDI patients reported to surveillance by 1 hospital in 2007 with causes of death reported by 13 external reviewers who examined summaries of medical files of the deceased patients. Reviewers were 11 infectious disease and 2 public health physicians involved with CDI surveillance at their respective hospitals but not this hospital. The median (minimal, maximal) κ statistics for comparison of external reviews with surveillance classification were 0.495 (0.252, 0.607) for directly attributable, 0.182 (–0.091, 0.182) for contributed, and 0.321 (0.124, 0.614) for unrelated. Comparison within external reviewers yielded 0.697 (0.394, 1.0), 0.233 (–0.294, 0.703), and 0.542 (0.154, 0.909), respectively. Complete agreement was found for only 6 cases (4 directly attributable and 2 unrelated) (Figure).

Variation among reviewers suggested that categorizations reported to surveillance were inaccurate. Number of deaths among patients with CDI, regardless of the cause of death, seemed to better indicate CDI severity. Since 2008, only the crude numbers of deaths, not subjected to individual interpretation, have been reported to surveillance. A questionnaire addressing concurrent medical conditions, prognosis, level of care, and circumstances of death is being implemented in Quebec hospitals participating in CDI surveillance and should help determine the role of CDI in deaths.

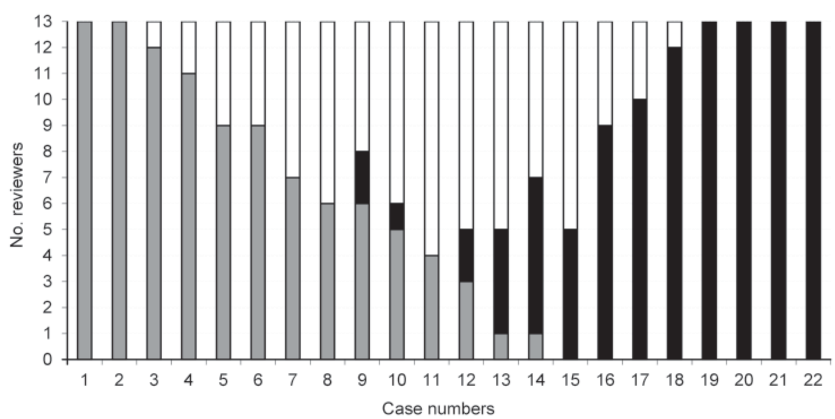

Figure. Classification of cause of death among 22 patients with *Clostridium difficile* infection (CDI), by 13 external reviewers, Quebec, Canada, 2007. Bars indicate the number of reviewers who assigned each category. Gray bars indicate that CDI was unrelated to death, white bars indicate that CDI contributed to death, and black bars indicate that death was directly attributable to CDI.

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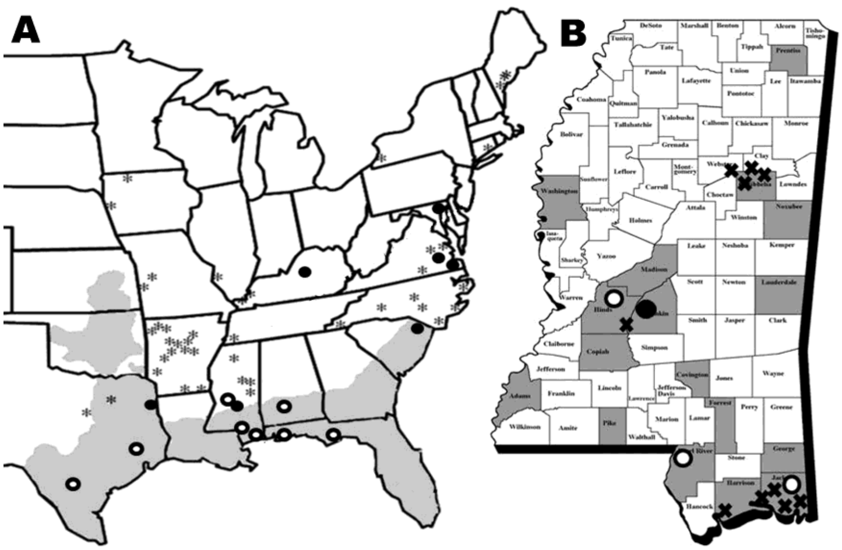
Technical Appendix



Technical Appendix Figure. Distribution of Gulf Coast ticks and cases of rickettsiosis. Solid circles indicate confirmed cases of *Rickettsia parkeri* rickettsiosis, and open circles indicate probable cases of *R. parkeri* rickettsiosis noted by Paddock et al. (*1*) and Cragun et al. (*2*). A) Gray area indicates the most established distribution of Gulf Coast ticks (*Amblyomma maculatum*); asterisks (*) indicate documented collection of these ticks in incidental reports (may or may not reflect permanent populations). B) Gray shading indicates Mississippi counties where Goddard and Paddock reported the occurrence of Gulf Coast ticks and locations of previously reported cases of *R. parkeri* rickettsiosis in Mississippi (*3*). X indicates tick collection sites for this study. Map adapted with permission from Teel et al. (*4*).

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