

Gulf Coast Ticks (*Amblyomma maculatum*) and *Rickettsia parkeri*, Ohio, USA, 2020

Appendix

Additional Methods

We conducted drags and flags in 2020 with 1m² muslin cloths checked for ticks every 10-m at minimum, whereas publicly submitted ticks were collected and received in 2021. We immediately stored ticks in 70% ethanol then identified stage, sex, and species by examining morphological features using light microscopy in accordance with an established key (1). In 2023, we dried ticks identified as *A. maculatum* of ethanol and homogenized using a bead mill (Thermo Fisher Scientific, <https://www.thermofisher.com>) before extracting their DNA using the PureLink Genomic DNA Kit (Invitrogen, <https://www.thermofisher.com>). We screened DNA for *R. parkeri* using a real-time PCR assay with previously published primers and probes (2). We included 5 µL of DNA in a 20 µL reaction using TaqMan Master Mix (Applied Biosystems, <https://www.thermofisher.com>) with an initial denaturation of 95°C for 3 minutes, then 45 cycles of 95°C for 15 seconds followed by 60°C for 1 minute. We considered positive any samples with a cycle threshold ≤40 with a characteristic curve. We included PCR-grade water and a synthetic gBlock gene fragment (Integrated DNA Technologies, <https://www.idtdna.com>) of *R. parkeri* (KF782321.1) as controls.

References

1. Keirans JE, Litwak TR. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. J Med Entomol. 1989;26:435–48. [PubMed](https://doi.org/10.1093/jmedent/26.5.435)
<https://doi.org/10.1093/jmedent/26.5.435>
2. Jiang J, Stromdahl EY, Richards AL. Detection of *Rickettsia parkeri* and *Candidatus Rickettsia andeanae* in *Amblyomma maculatum* Gulf Coast ticks collected from humans in the United

States. Vector Borne Zoonotic Dis. 2012;12:175–82. [PubMed](#)
<https://doi.org/10.1089/vbz.2011.0614>