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LETTER TO THE EDITOR

Detection of *Rickettsia amblyommii* in ticks collected from Missouri, USA

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Dear Editor,

In September 2012, we collected ticks from Missouri with the goal of isolating the novel phlebovirus, Heartland virus (HRTV). HRTV was described in two farmers from northwestern Missouri who presented with thrombocytopenia and severe febrile illness.[1] These patients were both bitten by ticks 5–7 days before the onset of their clinical symptoms. Our hypothesis was that we would isolate HRTV from ticks collected in Missouri by inoculation of cell culture and/or by detection of viral RNA on polymerase chain reaction (PCR) assay. Furthermore, we used this field surveillance study as an opportunity to screen for other potential viral and bacterial pathogens in the tick samples we collected.

Information from the published literature[1] was used to identify three geographically relevant collection sites across the central and western region of the state (Supplementary Figure S1A). One thousand two hundred and sixty-nine total ticks were collected from the three locations. Of these, 1191 (93.9%) were *Dermacentor albipictus*, 74 (5.8%) were *Amblyomma americanum* and four (0.3%) were *Ixodes scapularis* (Supplementary Figure S1B). Two nymphs were collected at location 2, but all other ticks collected during this study were larvae.

The speciated ticks were pooled into groups of twenty and screened for tick-borne pathogens (Supplementary Methods and Supplementary Table S1). Using the primers specific for HRTV,[1] Powassan virus[2] and deer tick virus,[3] we were unable to generate any positive PCR amplicons in the viral PCR screening. At location 2, one larval pool and one nymphal pool of ticks generated positive PCR amplicons when screened with the *Rickettsia*-specific primers for the outer membrane protein A (*ompA*) and citrate synthase (*gltA*) genes.[4]

Sequence analysis demonstrated that the two *Rickettisa*-positive samples aligned with the *ompA* and *gltA* genes of *Candidatus Rickettsia amblyommii* [GenBank: 378930552]. *ompA* gene sequences for *R. amblyommii*, *R. raoultii*, *R. slovaca* and *R. rickettsii* were obtained from GenBank. These sequences were trimmed and then underwent ClustalW alignment in the MegAlign program. Specifically, *ompA*-positive samples from both the larval and nymphal pools shared 100% sequence identity across a 431 bp segment of the *ompA* gene of *Candidatus Rickettsia amblyommii* [GenBank: 378930552] (Figure 1A). Additional sequence analysis demonstrated that there was 100% sequence identity between both the larval and nymphal *gltA*-positive samples and *Candidatus Rickettsia amblyommii* [GenBank: 378930552] across a 628 bp segment of the *gltA* gene (Supplementary Figure S2). Furthermore, the presence of *Rickettsia* in a larval pool of ticks collected at location 2 indicates the occurrence of transovarial transmission of *R. amblyommii*.

To further confirm our molecular identification of *R. amblyommii*, we screened the tick samples with *Rickettsia*-specific primers for the outer membrane protein B (*ompB*) gene[5] and for the 17 kDa gene.[6] The tick samples aligned perfectly with the *Candidatus Rickettsia amblyommii* [GenBank: 378930552] *ompB* gene sequence (Supplementary Figure S3). Our *R. amblyommii*-positive samples also completely aligned with the *Candidatus Rickettsia amblyommii* [GenBank: 378930552] 17 kDa gene sequence (Supplementary Figure S4).

No cytopathic effect was detected in any of the cell lines inoculated with the tick homogenates. However, we used an immunofluorescence assay (Supplementary Methods) to confirm the detection of *R. amblyommii* antigens in the *A. americanum* tick homogenates (Figure 1B). Tick mitochondrial 16S rRNA sequence analysis confirmed that the *R. amblyommii*-positive samples were isolated from *A. americanum* ticks. The 16S rRNA sequence from our *Rickettsia*-positive tick pools shared 100% sequence identity with *A. americanum* 16S rRNA (Supplementary Figure S5).

This field surveillance study was unable to isolate HRTV in any of the ticks we collected from Missouri. The lack of HRTV found in this study may be the result of our small sample sizes; only 5.8% of the total collected ticks were *A. americanum*. After our field collection of ticks was completed, another group published their findings of detecting HRTV from *A. americanum* collected in Missouri during 2012.[7] This group conducted tick collections ranging from April to early-August 2012. As our collection did not occur until mid-September, the majority of ticks collected in our study were larvae, as would be expected.[8]

We did confirm the presence of *R. amblyommii* in two pooled *A. americanum* tick homogenates. To date, no definitive role has been defined for *R. amblyommii* in human pathogenesis, but a recent study has shown that *A. americanum* ticks parasitizing humans are frequently infected with *R. amblyommii*.[9] Two *A. americanum* ticks collected in Kansas were found to be concurrently infected with *R. rickettsii*, which causes Rocky Mountain spotted fever, and with *R. amblyommii*.[10] The co-infection of these *A. americanum* ticks with *R. rickettsii* and *R. amblyommii* raises interesting questions about the epidemiology of spotted fever group rickettsiae and Rocky Mountain

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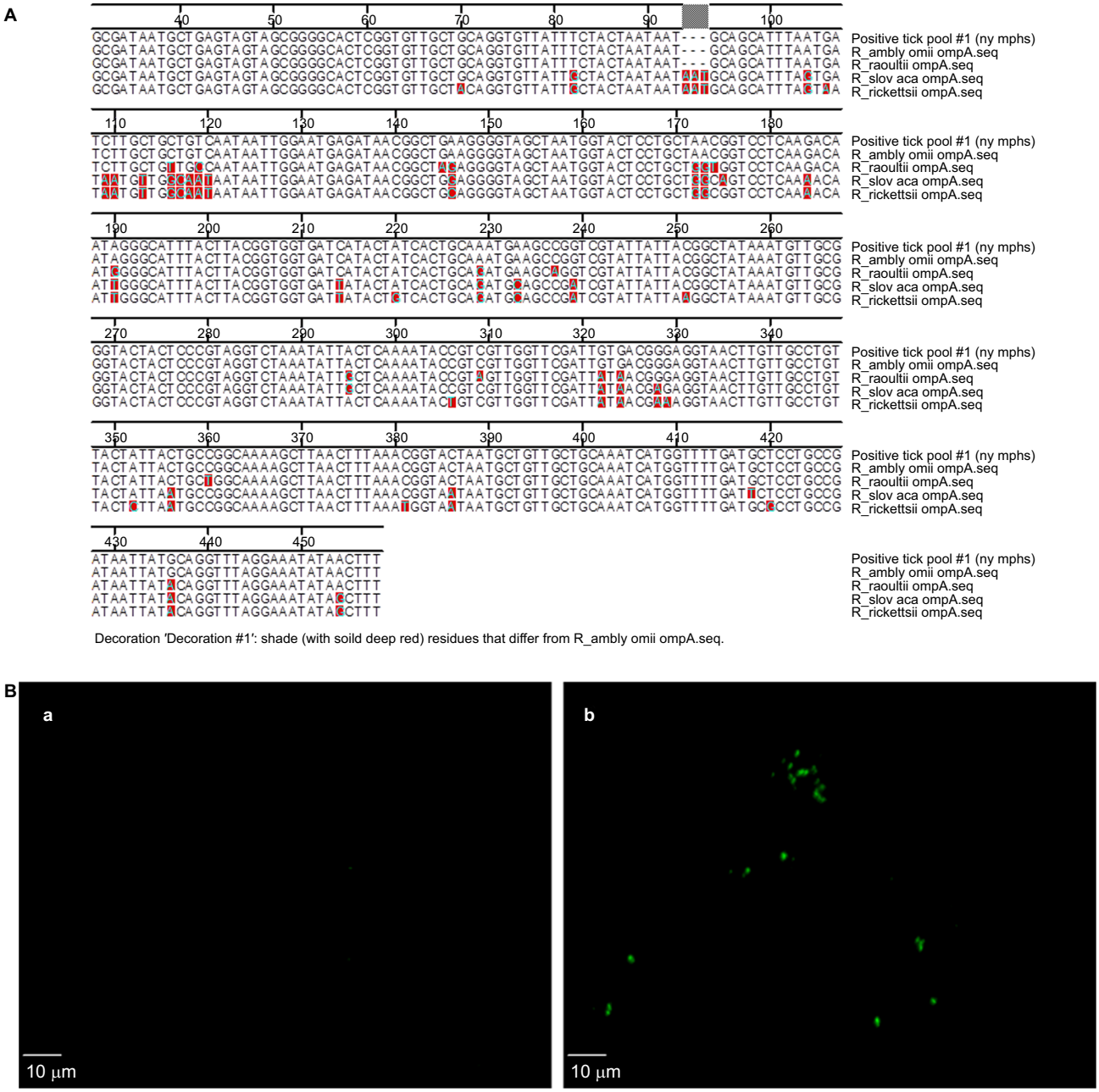

Figure 1 Molecular detection of *R. amblyommii*. (**A**) Multiple sequence alignment of the outer membrance protein A (*ompA*) gene. Sequence ruler applies to *R. amblyommii omp A* sequence. (**B**) *R. amblyommii* polyclonal antibody-stained tick homogenate. Tick homogenates positive or negative for *R. amblyommii* were further confirmed by immunofluorescence using guinea pig immune serum specific for *R. amblyommii*. Tick homogenate pools that were not positive for *R. amblyommii* by molecular detection were used as a control. (a) Negative tick homogenates; (b) positive tick homogenates.

spotted fever. A recent study in North Carolina screened ticks for spotted fever group rickettsiae and found that there was a high prevalence of *A. americanum* ticks infected with *R. amblyommii*.[11] As this tick species is relatively aggressive and readily parasitizes humans, the authors suggested that some cases of rickettsiosis diagnosed as Rocky Mountain spotted fever in North Carolina may instead be caused by *R. amblyommii*. Because other *Rickettsia* species, such as *R. parkeri*, were initially thought to be endosymbionts but were later shown to be pathogenic, it is important to continue evaluating the potential public health threat that *R. amblyommii*-infected *A. americanum* ticks pose to the humans they parasitize.

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Supplementary Information for this article can be found on *Emerging Microbes and Infections*' website (http://www.nature.com/emi/)