Detection and Identification of a Novel Spotted Fever Group Rickettsia in Western Australia

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ABSTRACT: The extent to which rickettsiae are present in Western Australia (WA) is largely unknown. Recently there has been anecdotal evidence of a disease of unknown but possibly rickettsial origin occurring on Barrow Island, WA. Ticks were collected from people and screened using PCR. The rickettsial species was then cultured and its novelty and phylogenetic position examined. The infecting rickettsial species is divergent enough to be classified as a novel species. Sequence data suggest that the evolutionary route for Australian rickettsiae did not progress through a recent common ancestor. The pathogenic potential of the novel species is as yet unknown.

KEYWORDS: spotted fever group rickettsiae; Western Australia; Candidatus Rickettsia gravesii

Rickettsioses have long been recognized to occur in WA when murine typhus was first reported in 1927. Recently, a case of scrub typhus has been reported and preliminary epidemiological studies have detected spotted fever antibodies in human sera. While active research continues on *Rickettsia australis* and *R. honei*, rickettsiae isolated from the eastern states of Australia, there is a limited amount of current information on the nature of rickettsial presence in WA.[1–3]

Barrow Island, located 60 km off the northwest coast of WA, was targeted for sample collection after there was anecdotal evidence of a disease of unknown but possibly rickettsial origin occurring on the island. *Amblyomma triguttatum* ticks were removed from people on the island. DNA was extracted from the ticks using Chelex-100 resin (Bio-Rad Laboratories) and a protocol described

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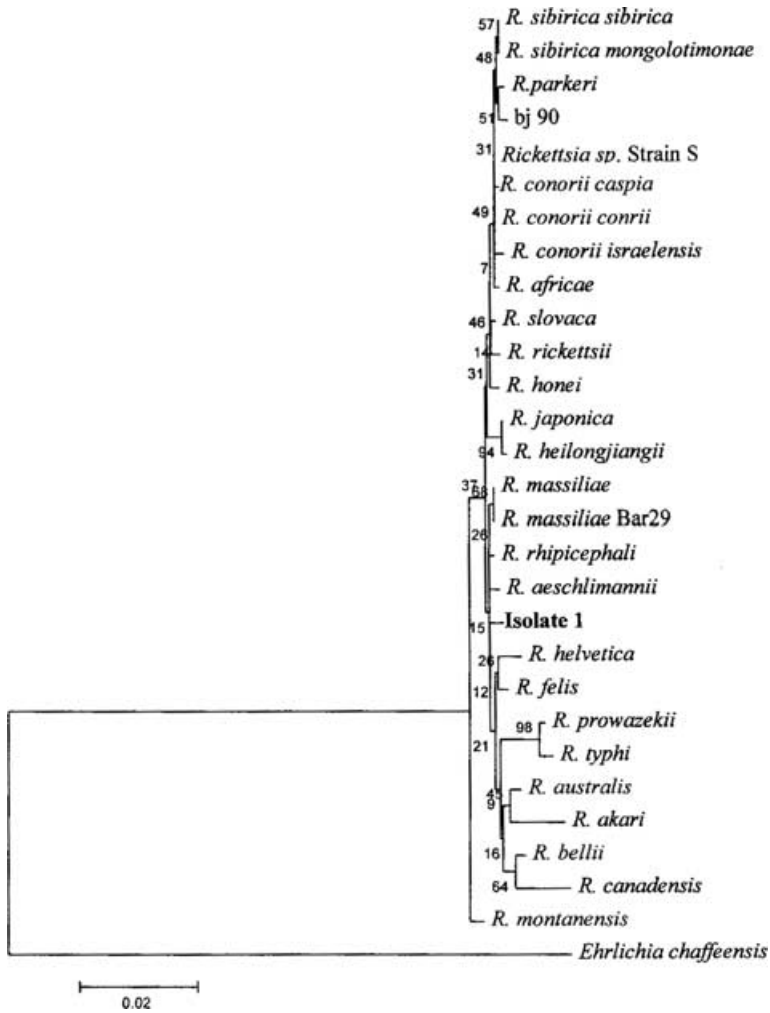

FIGURE 1. Phylogenetic tree of *Rickettsiae* constructed using the neighbor-joining method and based on 16S rRNA sequence comparison. Sequence alignment was performed using the multisequence alignment program CLUSTAL in the BISANCE software package. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1.[6] The novel isolate described in this study is isolate one.

by Guttman *et al.*[4] Screening for the presence of rickettsiae was performed by PCR using *gltA* gene primers. The infecting rickettsial species was cultured in XTC_2 and Vero cell lines and its novelty and phylogenetic position examined by sequencing segments of the 16S rRNA*, gltA, ompA*, 17-kDa antigen, *ompB,* and *sca4*.

Fifteen of the 32 *Amblyomma triguttatum* ticks collected were PCR-positive. The infecting rickettsial species is novel according to the guidelines outlined by Fournier *et al.*[5] It clusters most consistently with the *R. massiliae* subgroup of the spotted fever group (FIG. 1), demonstrating 99.7%, 98.4%, 95.6%, 97.4%, 96.6%, and 99.2% nucleotide sequence similarity to members of the subgroup on the basis of its 16S rRNA, *gltA*, *ompA*, *ompB*, *sca4*, and 17-kDa antigen genes, respectively.

These sequence data suggest that the evolutionary route for Australian rickettsiae did not progress through a recent common ancestor, but is more likely the result of multiple independent introductions of rickettsiae. The next phase of the project will involve further characterization of the isolate and comparison with known pathogenic rickettsiae, investigation into whether a disease with symptoms consistent with a rickettsiosis occurs on the island, and, ultimately, an attempt to detect the isolate from a human sample. This will be done with the aim of determining the pathogenic potential of the isolate.

We propose the creation of one new species, *Candidatus* Rickettsia gravesii (graves.i.i. L. gen. n. *gravesii* after Stephen Graves, an Australian physician and microbiologist who is the founder and director of The Australian Rickettsial Reference Laboratory and has a long history of promoting and contributing to Australian rickettsial research).

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