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SHORT REPORT: THAI TICK TYPHUS, *RICKETTSIA HONEI,* AND A UNIQUE *RICKETTSIA* DETECTED IN *IXODES GRANULATUS* (IXODIDAE: ACARI) FROM THAILAND

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Abstract. A unique *Rickettsia* species (*Rickettsia thailandii* sp. nov.) was identified in an *Ixodes granulatus* by use of polymerase chain reaction heteroduplex mobility assay by use of segments of the citrate synthase gene. This tick was collected from *Rattus rattus* from Nakhon Ratchasima province in 1970. Another *I. granulatus* was infected with Thai tick typhus strain TT-118, *Rickettsia honei* sp. nov. Stenos, Roux, Walker & Raoult; this tick was removed from a *R. rattus* collected 4 years later from the same province. *Ixodes granulatus* is the first tick species in Australasia shown to be infected with *R. honei* and the unique *Rickettsia* species.

Little is known of the distribution and vectors of spotted fever group *Rickettsia* (SFGR) in Thailand. The only isolate of SFGR from ticks in Thailand was from pooled immature *Rhipicephalus* and *Ixodes* of unknown species.[1] This isolate was named Thai tick typhus, TT-118 *Rickettsia* sp. Therefore, the specific tick species infected with SFGR is unknown. The first clinical cases of spotted fever group rickettsiosis in Thailand were identified serologi-

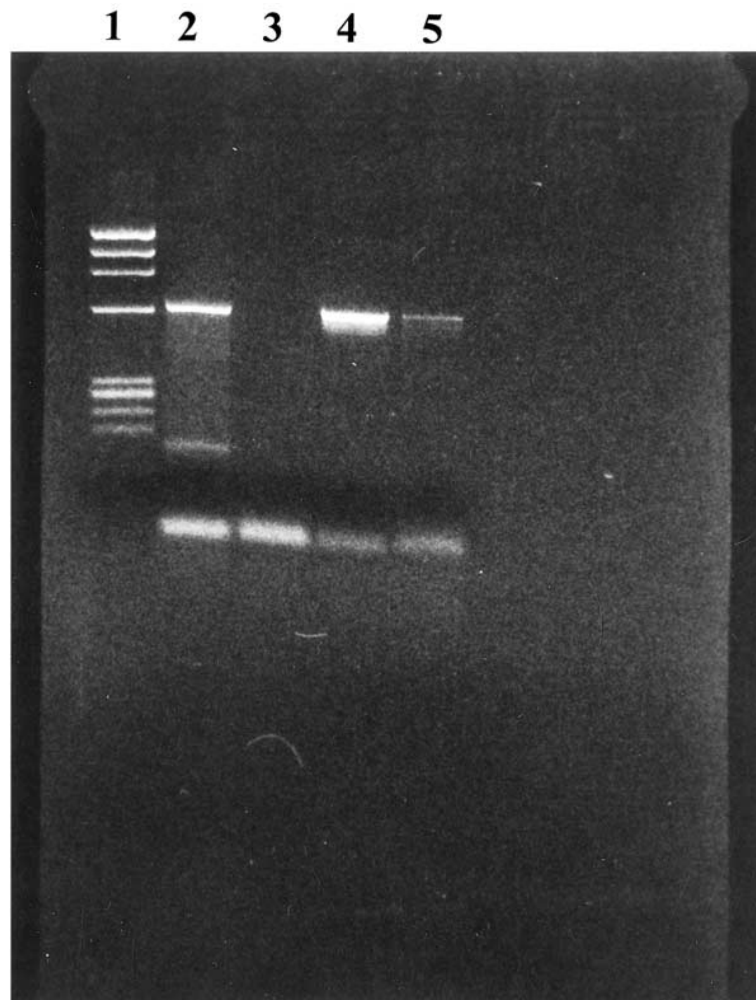

FIGURE 1. *Ixodes granulatus* positive by polymerase chain reaction for a 617-bp segment of the citrate synthase gene of spotted fever group *Rickettsia.* **Lane 1,** molecular weight ladder. **Lane 2,** positive control (*R. montana*). **Lane 3,** negative control. **Lane 4,** GMP 2540. **Lane 5,** SP 2464.

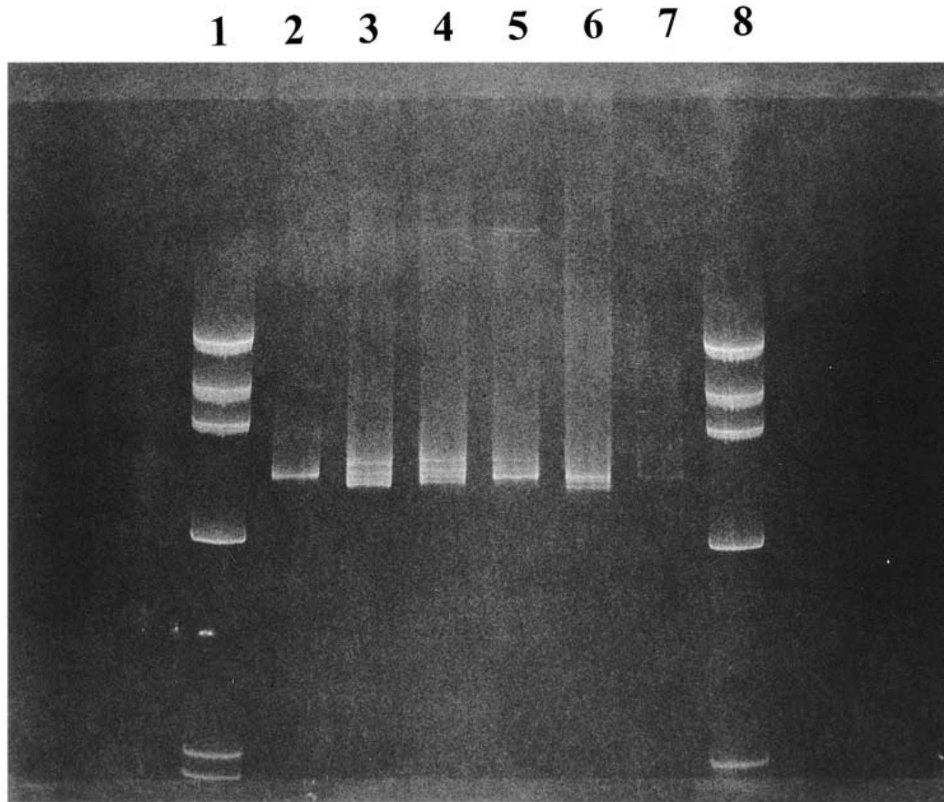

FIGURE 2. Heteroduplex mobility assay of *Ixodes granulatus* of 617-bp segment of the citrate synthase gene of spotted fever group *Rickettsia.* **Lanes 1, 8,** molecular weight ladder. **Lanes 2, 7,** hybrid control (*R. montana*). **Lane 3,** Thai tick typhus agent TT-118 (*R. honei*). **Lane 4,** GMP 2540. **Lane 5,** SP 2464. **Lane 6,** *Rickettsia montana* strain from *I. scapularis.*

cally in 3 people from Chiang Mai in 1994. All 3 patients had antibodies to *Rickettsia,* in descending order of titer: TT-118, *R. conorii,* and *R. prowazekii.*[2]

Museum specimens of *I. granulatus* from the Department of Entomology, U.S. Army Medical Component, Armed Forces Research Institute of Medical Science, Bangkok, Thailand (USAMC-AFRIMS), were tested for SFGR by use of the polymerase chain reaction (PCR).

We designed primers to amplify a 617-bp segment of the citrate synthase gene from various SFGR by use of GenBank. These primers were RrCS.372 (5′-TTTGTAGCTCTTCTCATCCTATGGC-3′) and RrCS.989 (5′-CCCAAGTTCCTTTAATACTTCTTTGC-3′). We tested these primers with *R. montana* (ATCC vr-611) and *R. japonicum* (ATCC vr-1363) as SFGR-positive controls and *Ehrlichia sennetsu* (ATCC vr-367), *Orientia tsutsugamushi* (Karp strain), and *R. typhi* (ATCC vr-144) as negative controls.

Museum specimens of *I. granulatus* ($n = 83$), preserved in ethanol, from the Department of Entomology, USAMC-AFRIMS, were tested. These ticks were collected from a variety of small mammals during a 30-year period from provinces throughout Thailand. Ticks were tested for SFGR by means of the following methods. Individual ticks were placed separately into small plastic bags and then smashed with a hammer. Nuclease-free water (1 mL) was used to extract the soft tissue from the bag after gentle mixing. DNA was extracted from the soft tissue by use of the procedures of the Ready Amp Genomic DNA Purification System (Promega, Madison, WI).

PCR amplification was conducted by use of the following conditions: amplification volume was 50 μL containing 10× buffer, 200 μM each dNTP, 2.5 U Taq polymerase, 50 pmol of each primer (Gene Amp DNA amplification Reagent kit; Perkin-Elmer Cetus, Norwalk, CT), and 5 ng of DNA template. Amplification conditions were: denaturation (60 sec at 95°C), annealing (60 sec at 58°C), and extension (60 sec at 72°C) for 40 cycles. The molecular weight ladder was *Hae* III digest of ϕX 174 (GIBCO, Gaithersburg, MD) DIG. PCR amplification of DNA was verified by electrophoresis on a 2% agarose gel at 100 V for 30 min.

Briefly, heteroduplex mobility assay[3] of SFGR to differentiate the products of the RrCS.372 and RrCS.989 PCR amplification from ticks. *Rickettsia montana* (ATCC vr-611) was used as the hybrid strain, and *R. montana* strain from an *I. scapularis* individual collected in Missouri was used as a positive control. The heteroduplex mobility assay can be more sensitive than restriction fragment length polymorphisms and is sensitive enough to detect a single base pair mismatch between hybridized PCR products.[3] For heteroduplex formation, 8 μL of wild-type PCR product was mixed with 8 μL of *R. montana* PCR product. The mixture was heated to 95°C for 5 min and allowed to cool to room temperature. After addition of standard loading dye (95% formamide, 20 mM ethylenediamine tetra-acetic acid, 0.05% bromophenol blue, and 0.05% xylene cyanol), the mixture was loaded on 1.0-mm-thick 8% polyacrylamide vertical gels (Hoefer SE 600).

Gels were run in tuberculin bacillary emulsion buffer at 230 V for 4 hr at room temperature. The gels were then stained with ethidium bromide for visualization and photography.

Two of the 83 *I. granulatus* tested were positive for SFGR (Figure 1). Both ticks were collected from *Rattus rattus* from Nakhon Ratchasima province, Pak Thong Chai district, in central Thailand, ~ 200 km northeast of Bangkok. Tick specimen SP 2464 was collected in 1970, and tick specimen GMP 2540 was collected in 1974. The heteroduplex pattern of SFGR from tick GMP 2540 was identical to TT-118 and the second, from tick SP 2464, had a different genotypic pattern (Figure 2). The occurrence of more than one SFGR in a tick species is not without precedent. For example, *R. belli, R. montana,* and *R. rickettsii* have been identified in *Dermacentor variabilis.*[4,5]

Previously, the evidence supported an endemic cycle of SFGR in northern Thailand in the provinces of Chiang Mai and Chiang Rai, ~ 700 km north of Bangkok. In 1962, the pooled *Rhiphicephalus* and *Ixodes* larval ticks, from which TT-118 was isolated, were collected in Chiang Mai. The only human cases of spotted fever group rickettsioses reported in Thailand were also from this district.[2] Positive serology on farmers, but not clinical diagnoses, for SFGR were made in Chiang Rai.[6] In the serosurvey of the farmers, 8.2% were only positive to TT-118 antigen, 2.5% were only positive to a Japanese spotted fever group–*R. sibirica* antigen mixture, and 25% were cross-reactive to both antigens. Further research and isolation are needed to determine if the unique *Rickettsia* sp. (*Rickettsia thailandii* sp. nov.) is responsible for the positive SFGR serologies that are not TT-118.

TT-118 type SFGR appear to occur on at least 3 continents. In the United States, a SFGR with similar sequence homology to TT-118 was described from *Amblyomma cajennense.*[7] Recently it was proposed that Flinders Island SFGR in Australia be assigned the name *R. honei.*[8] Molecular similarities between *R. honei* and TT-118 led Stenos and others[8] to suggest that TT-118 is a strain of *R. honei.* It appears that at least 2 species of SFGR, *R. honei* strain TT-118 and a new *Rickettsia* sp., occur in Thailand. *Rickettsia honei* is pathogenic to humans.[2,8] However, whether TT-118 is pathogenic to humans is unknown because no isolations have been made. In addition, the pathogenicity of the new *Rickettsia* is unknown. DNA sequencing of the unique *Rickettsia* species is being conducted. Further investigations into the prevalence of SFGR in other tick species and rodent hosts occurring in Thailand are also being conducted.

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