Rickettsia honei

A Spotted Fever Group Rickettsia on Three Continents

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ABSTRACT: *Rickettsia honei* (also known as strain TT-118) has been detected on three continents. Originally isolated in Thailand in 1962 (and confirmed in 2001), it has also been detected on Flinders Island (Australia) in 1993 and in Texas (USA) in 1998. On each continent it has been associated with a different species of tick. The original isolate (Thai Tick Typhus strain TT-118) was from a pool of larval *Ixodes* and *Rhipicephalus* ticks. Later it was detected in *I. granulatus* from *Rattus rattus*. Its pathogenicity for humans has not yet been confirmed in Thailand, but it is possibly responsible for the Spotted Fever Group human rickettsiosis in Thailand. The strain from Texas (USA) was isolated from *Amblyomma cajennense* ticks taken from cattle. Its pathogenicity for humans has not yet been confirmed in Texas. However, this tick is known to bite humans. The strain from Flinders Island (Australia) described as *R. honei*, has been isolated from patients with "Flinders Island Spotted Fever" and from *Aponomma hydrosauri* ticks taken from blue-tongue lizards (*Tiliqua nigrolutea*), tiger snakes (*Notechis ater humphreysi*), and copperhead snakes (*Austrelaps superbus*) on Flinders Island. The ecology of *R. honei* in this location is unusual in that reptiles, rather than mammals, are the vertebrate hosts.

KEYWORDS: rickettsia; spotted fever; *Rickettsia honei*; ticks

INTRODUCTION

Rickettsia of the Spotted Fever Group (SFG) are found world-wide (with the possible exception of New Zealand). Most SFG rickettsiae are tick-transmitted with the exception of *R. akari* (transmitted by the mouse mite) and *R. felis* (transmitted by cat and possum fleas).

Most isolates of SFG rickettsia are found in a particular geographic location. They have relatively minor antigenic and genomic differences, suggesting local evolution from a relatively recent common source proto-SFG-rickettsia. However, there are some exceptions to this; *R. mongolotimonae* has been detected in both China and France. The reason for this is not clear but may represent transcontinental spreads via ectoparasites of migrating birds. The SFG rickettsia with the widest recognized distribution is *R. honei*. This rickettsia occurs in south-east Asia (Thailand), Australia (Flinders Island), and North America (south Texas). This exceptionally wide

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distribution is unusual. One possible explanation for this is a dispersal mechanism, probably via a migration avian species (with ectoparasites). Another possibility is that the strains of *R. honei* from these three locations are not the same, but appear so because relatively few genes (and hence only a small proportion of the total genome) have been sequenced and compared. This paper is an analysis of *R. honei* from these three diverse locations: Thailand, Texas, and Flinders Island.

The "Thailand" R. honei

Elisberg and coworkers (reported in Robertson and Wisseman[1]) isolated a SFG rickettsia (designated Thai TT-118) from a mixed pool of *Ixodes sp.* and *Rhipicephalus sp.* larval ticks taken from *Rattus rattus* trapped at Huai Mae Sanam, Cheingmai Province, Thailand, in November 1962.

It was shown to be serologically distinct from SFG rickettsiae from west Pakistan as well as other SFG rickettsiae known at that time.[1] These included *R. conorii*, *R. sibirica*, *R. parkeri,* and *R. rickettsii*. The comparisons involved toxin neutralization tests in mice. The new isolate was most closely related to strain JC-880 from Pakistan, a "typical member of the SFG of rickettsiae."[1] The authors described Thai TT-118 as follows: (1) grows in the nucleus and cytoplasm of infected cells; (2) is moderately pathogenic for guinea pigs and gerbils; and (3) differs from other SFG rickettsiae by the toxin neutralization test in mice.

In 1994 Sirisanthana and coworkers[2] reported the first three cases of human SFG infection in Thailand. The patients presented with fever, headache, lymphadenopathy, and petechial maculopapular rash. One patient had an eschar and encephalopathy. All patients were seen at the University hospital in Chiang Mai province, 700 km north of Bangkok, in 1990 or 1991.

Several serological tests were undertaken to establish the diagnosis. (1) Using Weil-Felix, all three sera were positive with OX-2 antigen (titer 320) and one was positive with OX-19 antigen also (titer 160). (2) For indirect immunofluorescence, several SFG-rickettsiae were used as antigens (*R. rickettsii*, *R. conorii*, *R. siberica*, *R. australis*, *R. akari*, and Thai TT-118). Two patient sera reacted strongly, with all strains and one patient showed a significant increase in titer on the second serum (taken 12 days later). (3) With indirect immunoperoxidase, all three patients had a reaction to TT-118 in both the IgG and IgM antibody classes. Finally, using enzyme linked immunosorbent assay, two patients had strong reactions to *R. conorii*.

Taken together these data from the four serological assays demonstrate that at least two (and probably three) of the patients had a SFG rickettsial infection. There were no reactions with Typhus Group or Scrub Typhus Group rickettsial antigens, although both these diseases are common in Thailand. All three patients responded to treatment with doxycycline. No attempts were made to isolate the agents. One possible explanation for these findings is human infection with TT-118.[2]

Kollars *et al.*[3] reported the detection of *R. honei* in the tick *Ixodes granulatus*. This was on the basis of amplification of the citrate synthase gene by polymerase chain reaction and detection by hetero duplex mobility assay. The tick was collected from *Rattus rattus* in the central province of Nakhon Ratchasima in 1974. They also detected a second SFG rickettsia in another tick (*R. thailandii* sp. nov.). *R. honei* and strain TT-118 are now considered to be the same rickettsia.[4]

In summary, Thailand (and presumably other contiguous nations of south-east Asia) contain SFG rickettsiae, one of which is *R. honei*. *Ixodes granulatus* is a tick host, although other species may also be involved. *Rattus rattus* is one of the vertebrate hosts. Man appears to be bitten by this tick as shown by three cases of SFG infection. However, isolation of *R. honei* (TT-118) from a patient has not yet been reported.

The "USA (Texas)" R. honei

Texas is a long way from Thailand, but in 1996 a SFG rickettsia was detected that was almost identical to *R. honei* (TT-118).[5] Three genes were sequenced (17 kDa, *gltA* (citrate synthase), and *ompA*) and showed 99.5–100% homology with *R. honei*. The rickettsial DNA was detected in the tick *Amblyomma cajennense* (the cayenne tick) taken from cattle. This tick is an indiscriminate feeder that will bite humans and has been shown to transmit *R. rickettsii* (Rocky Mountain spotted fever) in Brazil and Columbia.[6,7]

The *R. honei* DNA was detected in two different adult *A. cajennense* pools but not in larval or nymph pools. This may suggest that vertical transmission of the rickettsia was not occurring but this observation needs confirmation. Cases of human infection due to *R. honei* in Texas have not been published but anecdotal cases of tick-borne rickettsiosis were reported[5] close to the area from where the infected ticks originated. Isolation of *R. honei* from a patient is needed to support this hypothesis.

The "Australia" R. honei

The Australian connection to *R. honei* originated on Flinders Island, a small island off the south-east coast of Australia. It has a population of approximately 1,000 and only one doctor. A "spotted fever" illness had been seen for many years prior to it being recognized as rickettsial in 1991.[8,9] Stewart has been the only doctor on Flinders Island since 1974. During that time (1974–1991), 26 cases of fever and rash defied diagnosis. The usual features were a sudden onset of fever, headache, myalgia, slight cough and arthralgia without joint swelling. The rash appeared a few days later and was maculopapular on the trunk and limbs, each spot being about 5 mm in diameter. There was no vesiculation (unlike Queensland tick typhus due to *R. australis*). Approximately half the patients had an eschar with local lymphadenopathy and several recalled being bitten by a tick. Symptoms developed between 2 and 9 days after the bite. Most patients received no antibiotic treatment and the average duration of illness was 19 days (range 1 to 6 weeks). Although one third of patients were hospitalized there were no deaths or cases of re-infection. Cases occurred in spring and summer (predominantly in December and January). The incidence of infection was approximately 150/100,000/year. The main tick that bites man on Flinders Island is *Ixodes tasmani* and this was originally assumed to be the tick vector of the disease.

A serological study was undertaken of these 26 patients[9] (where serum was still available) and 335 normal persons from Flinders Island (approximately one third of the island population). Six patients had seroconversion to Spotted Fever Group rickettsiae (using *R. australis*, *R. rickettsii*, and *R. conorii* as antigens and 50% of the patients with historical sera had antibodies to SFG rickettsia, compared to 1% of the

background population. Seroprevalence to Typhus Group rickettsiae (*R. typhi*) was 4% in both patients and control populations. Weil-Felix OX2 and OX19 serology was also positive in patients. Hence it was concluded that Flinders Island spotted fever was a SFG rickettsial disease. Antibodies to this agent degrade quickly, with all Flinders Island spotted fever patients being seronegative seven or more years after infection.

Isolation of a SFG rickettsia from the blood of two patients (RB and RM) on Flinders Island was reported.[10] One patient had collected the tick that bit her 9 days prior to becoming ill. It was later identified as *Aponomma hydrosauri*, a reptile tick. During that summer season, eight people on Flinders Island collected the ticks that had bit them. Six were *I. tasmani* and two were *A. hydrosauri*. Both infected patients seroconverted to the SFG antigen being used in the micro-immunofluorescence assay at that time (*R. australis*).

A field study on Flinders Island concentrated on native animals and their ectoparasites as suspected components of the natural cycle of SFG infection in humans.[10] Of 125 vertebrate animals caught and examined, five were most infested with ticks. (1) *Rattus lutreolus*, the swamp rat, yielded 13 *I. hirstii* ticks per individual animal (av). (2) *Macropus rufogriseus*, the Bennetts' wallaby, yielded 7.6 *I. tasmani* ticks per individual animal (av). (3) *Vombatus ursinus*, the wombat, yield 12.4 ticks per individual animal (av) including both *Aponomma sp.* and *Ixodes sp.* (4) *Tiliqua nigrolutea*, the blotched blue-tongue lizard, yielded 18.4 *A. hydrosauri* per individual animal (av). (5) *Notechtis ater*, the tiger snake, yielded 8 *A. hydrosauri* per individual animal (av).

Pools of ticks from the mammals (but not reptiles) were inoculated into mice, but no mice seroconverted to SFG rickettsia or became unwell. At that time (1990) only mammals were being seriously considered as vertebrate hosts of SFG rickettsiae, as is the situation elsewhere in the world. Later fieldwork concentrated on reptiles and the reptile tick *A. hydrosauri.*[11] Of 19 reptiles examined in 2001, 12 blue-tongue lizards (*T. nigrolutea*), 4 copperheads snakes (*Austrelaps superbus*), and 3 tiger snakes (*N. ater humphreysi*) had their ticks removed and analyzed for SFG rickettsiae.

All 46 reptile ticks were *A. hydrosauri*. No *Ixodes* sp. were detected on reptiles. Of these ticks 29 (63%) were positive for SFG rickettsia by PCR amplification of the citrate synthase gene and 7 (15%) yield a rickettsia by culture of hemolymph in VERO cells. Two engorged female ticks laid eggs in the laboratory and both egg pools were positive for SFG rickettsia by PCR. Sequence comparisons of the 381 bp amplified product showed 100% homology with *R. honei*.

It appears likely that vertical transmission of *R. honei* occurs in *A. hydrosauri*. Electron microscopy of tick reproductive organs showed intracellular structures consistent with rickettsial morphology.[11]

Only limited study of the reptile host has been undertaken to date. Of 17 blood specimens obtained from the blue-tongued lizards, copperhead snakes and tiger snakes, none yield SFG DNA by PCR. Of 14 blood specimens subjected to culture, none yielded a rickettsia.

A study of four genes in *R. honei* (16S rRNA, rompA, citrate synthase and 17 kDa) showed this rickettsia to be closely aligned to the "mainstream" SFG rickettsiae.[4] It was most closely related to TT-118 (Thai tick typhus) and very distantly to *R. australis* and *R. akari*. The authors calculated the divergence of *R. honei* and *R. australis* as

occurring approximately 65 million years ago. The name (honei) was proposed in honour of Frank Hone, an Australian public health doctor who first described murine typhus in Adelaide, Australia (in 1922) and differentiated the illness from epidemic typhus, the only form of typhus then known.

SUMMARY

R. honei has been described in three diverse locations and associated with three different species of ticks: in Thailand, *Ixodes granulatus*; in the USA (Texas), *Amblyomma cajennense*; and in Australia (Flinders Island), *Aponomma hydrosauri.* This newly described spotted fever group rickettsia is the most widespread SFG rickettsia yet described. Could it have a world-wide distribution? Its mechanism of dispersal is not known, but a clue may be obtained from Flinders Island, Australia, where reptiles, that are the natural hosts of *A. hydrosauri*, share burrows with mutton birds (*Puffinus tenuirostris*) that migrate annually from the northern hemisphere. An ectoparasite associated with the migrating bird may also feed on local reptiles and thus introduce *R. honei* to the reptile ticks. This hypothesis needs to be investigated.

R. honei is closely related phylogenetically to the main body of SFG rickettsiae. It is not closely related to the endemic Australian SFG rickettsia, *R. australis,* and may have further geographical niches yet to be discovered.

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