Pathology (June 2005) **37(3)**, pp. 242–245

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MICROBIOLOGY

Not only 'Flinders Island' spotted fever

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Summary

Aim: To demonstrate that Flinders Island spotted fever (FISF), a spotted fever group rickettsial infection caused by *Rickettsia honei*, is found not only on Flinders Island (Bass Strait), Tasmania, but elsewhere in south-east Australia.

Methods: Cases of FISF were identified by rickettsial serology, culture and the detection of rickettsial DNA via PCR. Isolates and PCR products were sequenced to identify the aetiological agent as *R. honei*.

Results: Three new cases of FISF were detected outside of Flinders Island. One on Schouten Island, south of the Freycinet Peninsula, Tasmania, and two in south-eastern South Australia (McLaren Vale and Goolwa).

Conclusions: These cases show that FISF extends beyond Flinders Island and most likely has the same distribution across south-east Australia as its vector, the reptile tick *Aponomma hydrosauri*. FISF should be considered as a differential diagnosis in patients from south-eastern Australia presenting with fever, headache and rash following a tick bite.

Key words: Flinders Island spotted fever, spotted fever group *Rickettsia*, *Rickettsia honei*, *Aponomma hydrosauri*.

Received 29 November 2004, revised 17 January, accepted 21 January 2005

INTRODUCTION

Rickettsiae are obligate intracellular bacteria transmitted to humans by arthropod vectors.[1] They are found worldwide and consist of three main groups including the spotted fever group (SFG), typhus group (TG), and the scrub typhus group (STG) (*Orientia tsutsugamushi*). Examples from all three groups are found in Australia. Prior to the discovery of *Rickettsia honei*, the only SFG known to occur in Australia was *Rickettsia australis* the aetiological agent of Queensland tick typhus (QTT).

Since 1974, a spotted fever-like illness had been seen on Flinders Island, a small island in Bass Strait between mainland Australia and Tasmania. The disease, named Flinders Island spotted fever (FISF), was characterised by fever, headache, myalgia, transient arthralgia, maculopapular rash and cough in some patients. It had an abrupt onset and lasted approximately 19 days without antibiotic treatment, with most cases occurring during summer.[2] The illness was shown to be rickettsial, and probably SFG, through testing of acute and convalescent sera.[3]

The agent of FISF was first isolated from human blood specimens in 1990.[4] Later, the reptile-associated tick *Aponomma hydrosauri* was confirmed as its host.[5] This rickettsia was shown to be different from *R. australis* and other rickettsiae by DNA sequence comparison of several rickettsial genes. The name *R. honei* was proposed, after a pioneer in Australian rickettsiology Dr Frank Sandland Hone. In 1998, *R. honei* was formally named as a new species and the cause of FISF, with the human isolate RB designated as the type strain.[6] As *Ap. hydrosauri* is found on mainland Tasmania and other parts of Australia (Fig. 1A),[7] it was expected that further cases of FISF would appear within the geographical distribution of this tick.

CASE REPORTS

Case 1

A 22-year-old male was admitted to Royal Hobart Hospital, Tasmania, in January 2001 following a 10-day bushwalking and camping trip on Schouten Island, south of the Freycinet Peninsula (Fig. 1B) 7 days prior. He had a 5-day history of headache, fever, nausea and vomiting and a 2-day history of myalgia and a maculopapular rash. Another member of the party was hospitalised earlier with similar symptoms. The patient was unaware of being bitten by a tick but had found one on his tent floor.

On examination, the patient was febrile with a temperature of 38°C, with no lymphadenopathy or splenomegaly. The heart rate was 98 bpm and blood pressure 140/70. The maculopapular rash was wide spread on the trunk and limbs. The lesions were erythematous and non-blanching. There was an area of superficial ulceration on his left buttock, compatible with a tick bite, which was biopsied. Further tests revealed a neutrophil left shift and slightly raised levels in liver function tests. Blood was taken for rickettsial serology, culture and nucleic acid amplification by polymerase chain reaction (PCR). Convalescent serum was obtained on day 30 of his illness.

After treatment with oral doxycycline (100 mg twice daily) the patient became afebrile within 48 hours. He was discharged with a two-week course of doxycycline and

ISSN 0031-3025 printed/ISSN 1465-3931
DOI: 10.1080/00313020500099247

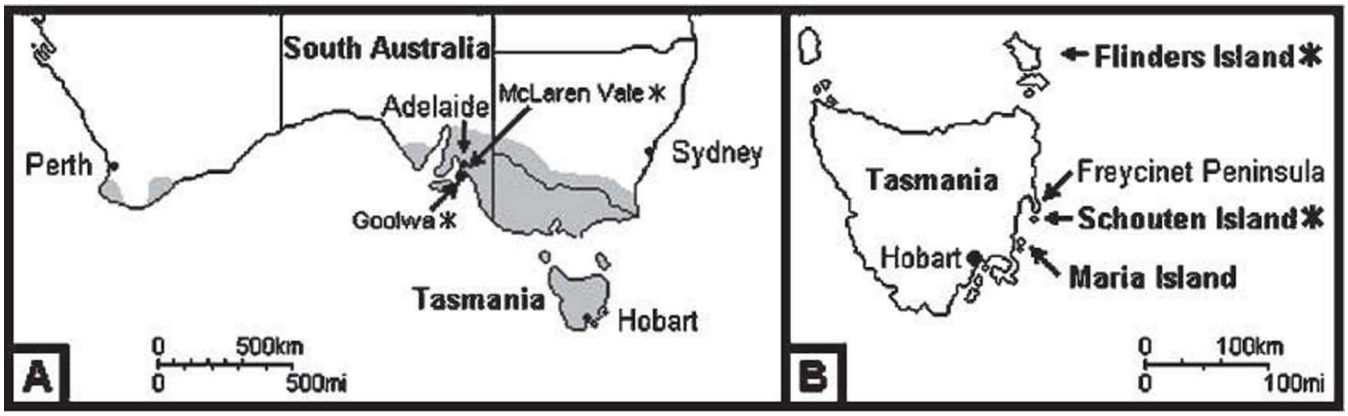

Fig. 1 (A) Map of southern Australia showing the distribution of the tick *Ap. hydrosauri*[7] (shaded area). (B) Detailed map of Tasmania. Cases of FISF are marked with an asterisk.

made a full recovery. His serum showed a seroconversion to SFG rickettsiae (Table 1). Histology of the skin biopsy, performed at the Royal Hobart Hospital, revealed lymphatic vasculitis with perivascular and interstitial eosinophil infiltrate and leukocytolysis, which was consistent with the lesion being an arthropod bite complicated by rickettsiosis.

Case 2

In December 2002, a 74-year-old male resident of Goolwa, on the south coast of South Australia, presented to the Flinders Medical Centre Emergency Department. The immediate surrounds of his house were on the edge of bushland where he had noticed numerous lizards. There was no history of animal contact or arthropod exposure. He had 5 days of high fevers (to 40°C), rigors, malaise, and severe prostration with mild confusion. A generalised non-pruritic maculopapular rash was noted, predominantly distributed on the trunk but with some lesions on the extremities including the palms and soles. No eschar was found on close inspection of the entire skin surface. Liver function tests revealed mild elevation of transaminases only. Empiric flucloxacillin and gentamicin was commenced on admission, with a single initial dose of ceftriaxone.

Following infectious diseases review 24 hours later, doxycycline 100 mg twice daily was substituted for these antibiotics and continued for 7 days in total. Within 48 hours the fevers had resolved and he was well enough for discharge. Upon review 6 weeks later, he was completely well. Histopathological examination of a skin biopsy showed a mild perivascular lymphocytic infiltrate but no specific abnormalities. Rickettsial serology showed a seroconversion by day 16 after onset of illness. Rickettsial cultures of blood and skin tissue were negative. Rickettsial PCR performed on skin biopsy tissue collected immediately prior to commencing doxycycline was positive, but buffy coat PCR was negative (Table 1). This case has been briefly reported by Dyer *et al.*[8]

Case 3

A 58-year-old male from McLaren Vale, south of Adelaide, presented to Flinders Medical Centre in May 2003 with a 7-day history of high fever (38.4°C on admission) followed 2 days later by a maculopapular rash on the trunk and limbs, which subsequently resolved within 72 hours. There was no eschar, history of tick bite or recent direct animal exposure. On the night of admission he awoke with dyspnoea, but there was no cough or sputum.

Laboratory tests revealed moderately elevated hepatic enzymes, elevated gamma glutamyl transferase (GGT) and mild neutrophilia with a left shift. Chest X-ray showed bilateral basal interstitial shadowing. Trans-thoracic echocardiogram was normal apart from a very small pericardial effusion. Following initial treatment with intravenous benzylpenicillin and erythromycin, he was switched to oral doxycycline 100 mg twice daily as treatment for presumed atypical pneumonia.

TABLE 1 Rickettsial serology PCR and culture results from three cases of Flinders Island spotted fever

		1st Serum		2nd Serum						
Case	Location	SFG titre	Days after onset of illness	SFG titre	Days after onset of illness	PCR specimen	PCR gene target	PCR result	Culture result	Microbial identity
1	Schouten Island, TAS	<128	5	>1024	30	EDTA blood	*glt*A	Negative	**Positive**	*R. honei*
						Rash biopsy	*glt*A	Negative	Negative	
2	Goolwa, SA	<128	5	>1024	16	EDTA blood	17 kDa	Negative	Negative	
						Rash biopsy	17 kDa	**Positive**	Negative	*R. honei*
3	McLaren Vale, SA	<128	7	<128	18	EDTA blood	17 kDa	Negative	**Positive**	*R. honei*

SFG, spotted fever group; *glt*A, citrate synthase gene; 17 kDa, gene coding the 17 kDa surface antigen.

He was afebrile within 48 hours of initiation of doxycycline therapy and was discharged. Convalescent rickettsial serology was negative, along with rickettsial PCR. Culture of blood obtained before starting doxycycline yielded a rickettsial isolate (Table 1). The patient subsequently moved interstate and was lost to further follow up. This case has been reported by Dyer *et al.*[8]

MATERIALS AND METHODS

Rickettsial serology was performed on patients' sera using SFG rickettsial species *R. honei*, *R. australis*, *R. akari*, *R. conorii*, *R. sibirica* and *R. rickettsii* as antigens in an indirect microimmunofluorescence assay (IFA).[9]

Either a buffy coat of blood or rash biopsy was subjected to rickettsial specific PCR, where either the citrate synthase gene (*gltA*) or 17 kDa gene were amplified (Table 1). DNA was extracted from white blood cells partially purified from EDTA blood using red blood cell lysis solution (Gentra, USA) or from 200 µL of homogenised biopsy material and was extracted in a class 2 biosafety cabinet using the QIAmp DNA Blood Mini Kit (Qiagen, Germany), following the manufacturer's instructions.

The citrate synthase gene was amplified using the primers Rp877p (5′-GGG GAC CTG CTC ACG GCG G-3′) and Rp1258n (5′-ATT GCA AAA AGT ACA GTG AAC A-3′) (Invitrogen, Australia) as previously described.[10] The 17 kDa gene was amplified using the primers MTO-1 (5′-GCT CTT GCA ACT CTA TGT T-3′) and MTO-2 (5′-CAT TGT TCG TCA GGT TGG CG-3′) (Invitrogen) as previously described,[11] with an annealing temperature of 51°C and a total of 45 cycles. All PCR reactions were set up in a class 2 biosafety cabinet using aerosol-resistant pipette tips (Westlab, Australia) with Uracil-*N*-glycosylase (Invitrogen). Each PCR run had a *R. typhi* positive control, a 'no template' control and was performed in a geographically isolated room in compliance with NPAAC guidelines. All products were visualised on a 1% Tris acetate EDTA (TAE) agarose gel (Amresco, USA) stained with ethidium bromide.

Highly experienced laboratory staff using recognised biosafety techniques performed tissue culture studies by resuspending patients' buffy coat cells or homogenised biopsy material in 1 mL phosphate buffered saline (PBS). This suspension was inoculated onto confluent L929 and Vero cell monolayers in 25 cm^2 tissue culture flasks (Nunc, USA) using RPMI 1640 medium supplemented with 5% heat-inactivated foetal bovine serum (Invitrogen). Cultures were incubated in 5% CO_2 at 35°C for up to 2 months with a change of media each fortnight. Cultures were examined microscopically weekly and stained by immunofluorescence (IF) monthly. If deemed positive for either cytopathic effect or by IF, DNA was extracted from the culture and the *glt*A or 17 kDa gene amplified via PCR as described above.

PCR products were cleansed using the QIAquick DNA clean up kit (Qiagen, Germany) and *glt*A and 17 kDa products sequenced at Micromon DNA Sequencing (Monash University, Australia) or Newcastle DNA (Newcastle University, Australia), respectively.

RESULTS

Rickettsial serology on convalescent sera showed that only two of the three patients seroconverted (Table 1).

One specimen (Case 2) was positive for a rickettsial 17 kDa PCR (Table 1). This positive sample was amplified independently of the other samples. Subsequent sequence analysis confirmed the unique nature of the product when compared with the *R. typhi* positive control. When compared with GenBank sequences, the PCR product gave 100% homology with the 17 kDa gene (AF060704) of *R. honei* (Table 1).

From two specimens (Cases 1 and 3) a rickettsia was grown in cell culture (Table 1). A *glt*A PCR (Case 1) and a 17 kDa PCR (Case 3) confirmed that the sequences were 100% homologous with the *glt*A gene (AF018074) and 17 kDa gene (AF060704) of *R. honei*, respectively, confirming the infections as FISF (Table 1).

DISCUSSION

The three cases described, one from Schouten Island and two from south-east South Australia, are the first cases of FISF to be definitively proven outside of Flinders Island, but within Australia. Within Australia it is known that *R. honei* has the reptile tick *Ap. hydrosauri* as its main reservoir.[5] The vertebrate animals on which this tick is found include the Copperhead snake (*Austrelaps superbus*), Eastern Tiger snake (*Notechis scutatus*), Stumpy Tailed lizard (*Tiliqua rugosa*) and Blue Tongue lizard (*Tiliqua nigrolutea*). All three cases described here were in an area where these animals, and their ticks, are endemic. One of the patients (Case 2) reported that lizards were often seen around his house. All reported cases of FISF in Australia have been from coastal areas, despite its vector being distributed throughout south-eastern Australia (Fig. 1A).[7] This may be due to 85% of Australia's population living within 50 km of the coastline.[12] Consequently, we would expect to see few cases of FISF inland, corresponding to the distribution of *Ap. hydrosauri*.

Rickettsia honei has been detected outside of Australia and within ticks other than *Ap. Hydrosauri.*[13] These include a pool of *Ixodes* species, *Rhipicephalus* species and *Ix. granulatus,* both from Thailand, and *Amblyomma cajennense* from Texas. With this in mind, could *R. honei* have another reservoir other than *Ap. hydrosauri* and be found elsewhere in Australia?

In Adelaide in the 1920s, Frank Hone, in whose honour *R. honei* was named, described a 'typhus-like illness' and cases of 'endemic' (now called 'murine') typhus.[14–16] These were the first cases of murine typhus to be described in the world. The infection is caused by *R. typhi*. But is this the only rickettsial disease in this geographical location?

In 1940, further observations were made on endemic typhus and on a disease known as 'hills-typhus' in the Mount Lofty Ranges, east of Adelaide, South Australia.[17] Some blood of a 'hills-typhus' patient was inoculated into guinea pigs, with fatal consequences. There was also trapping of wild rats, none of which was found to harbour the 'hills-typhus' agent. This suggested that rats might not have been the vertebrate host for this rickettsial disease.

A rickettsial disease has been described previously from eastern Tasmania, originating from either the Freycinet Peninsula or Maria Island (Fig. 1B).[18] There have been other suspected cases, possibly originating from Schouten Island, with similar symptoms to those found in Case 1. One of these other cases was found to have rickettsial titres against both the SFG and TG of >1024 (personal communication, Dr Robert Stewart).

It appears that Schouten Island and probably mainland Tasmania are affected by FISF. South-eastern South Australia has also had sporadic cases of this disease. The distribution of FISF may well correspond to the range of the reptile tick *Ap. hydrosauri* (Fig. 1A). It is important to consider SFG rickettsial disease as a differential diagnosis in patients with a compatible illness in Tasmania and the

south-east region of Australia, not previously known to be endemic for rickettsioses.

ACKNOWLEDGEMENTS This research was funded by the Australian Rickettsial Reference Laboratory Foundation Ltd. We thank Ms Chelsea Nguyen for her serological expertise and Dr Mark Havlat (Royal Hobart Hospital) for his interpretation of the skin biopsy from Case 1. We would also like to thank Dr Robert Stewart from Flinders Island, Tasmania, for bringing other suspected cases of FISF from Schouten Island to our attention.

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