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SPOTTED FEVER GROUP RICKETTSIAL INFECTION IN SOUTH-EASTERN AUSTRALIA: ISOLATION OF RICKETTSIAE

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Abstract—Flinders Island spotted fever (FISF), a spotted fever group (SFG) rickettsial disease first described in 1991, occurs in south-eastern Australia. The isolation of the aetiological agent is described for the first time having been obtained from the blood of two patients. An additional 22 cases are also reported. Of these patients four had positive initial serology, and 20 showed seroconversion (using *Rickettsia australis* as antigen). Acute phase blood specimens taken from seven patients caused neonatal mice to seroconvert to *R. australis* and a blood specimen from one of these patients (and one other) yielded rickettsiae. A field survey for possible reservoir and vector animals on Flinders Island, Tasmania and in Gippsland, Victoria (both in south-eastern Australia) yielded 217 vertebrates and 1445 invertebrate ectoparasites, mostly ticks. *Ixodes cornuatus* from humans and dogs in Gippsland produced seroconversion to SFG rickettsia when inoculated into mice but no invertebrate pools from Flinders Island produced seroconversion in mice. Haemolymph from an individual *I. cornuatus* removed from a human in Gippsland, yielded a SFG rickettsia on tissue culture. Sera from several species of native vertebrates, especially the bush rat, *Rattus fuscipes*, were positive for antibodies to SFG rickettsia.

Key words: Spotted fever group, rickettsia, isolation, south-eastern Australia, *Ixodes cornuatus*.

Résumé—La méningite des Iles Flinders (FISF), fièvre du groupe des rickettsiales, a été décrite pour la première fois en 1991 et sévit actuellement dans le sud-est de l'Australie. L'isolement de l'agent causal a été décrit et réalisé à partir du sang de deux patients. Depuis, 22 cas nouveaux ont été rapportés. Sur ces 24 cas, quatre étaient initialement seropositifs et 20 ont montré une seroconversion (en utilisant comme antigène *Rickettsia australis*). Des échantillons de sang prélevés à la phase aigüe chez sept malades ont induit une seroconversion à *R. australis* chez des souriceaux nouveau-nés et le prélévement sanguin de l'un de ces malades contenait des rickettsies. Une enquête portant sur les réservoirs et les vecteurs animaux possibles a été réalisée dans les Iles Flinders, en Tasmania, en Gippsland et en Victoria (ces deux dernières régions se situant dans le sud-est de l'Australie) rapporta 217 vertébrés et 1445 ectoparasites invertébrés (principalement des tiques) comme étant des réservoirs ou des vecteurs possibles. *Ixodes cornuatus* isolés d'humains et de chiens en Gippsland produisait une seroconversion à SFG rickettsia quand il était inoculé à des souris mais aucun groupe d'invertébré de Flinders Island ne produisait de seroconversion chez la souris. L'Hemolymphe d'*I. cornuatus* ayant piqué un humain entraîna la production de SFG rickettsia sur culture tissulaire. Des sérologies de plusieurs espèces de vertébrés originaires d'Australie (essentiellement le rat de bush, *Rattus fuscipes*) étaient positifs pour SFG rickettsia.

Mots-clefs: Fièvre de groupe, reckettsia, isolement, sud-est Australie.

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INTRODUCTION

Two spotted fever group (SFG) rickettsial diseases are recognized in Australia: Queensland Tick Typhus [1] (caused by *Rickettsia australis*), occurs in north-eastern coastal Australia, at least as far south as Sydney, New South Wales and the recently recognized Flinders Island spotted fever (FISF) [2], of south-eastern coastal Australia that occurs at least as far north as Eden, New South Wales. Patients with FISF have antibodies that react with *R. australis* [3]. These two diseases may be the same, as clinical symptoms and the serological response to infection are similar [4]. In this paper we report 24 cases of FISF and the isolation of SFG rickettsia for the first time from two of these patients. The isolates were obtained by inoculating patients' blood onto cell cultures and detecting rickettsiae with either Gimenez stain or immunofluorescence.

Field investigations in two locations in south-eastern Australia (Gippsland, Victoria and Flinders Island, in Bass Strait, Tasmania) were undertaken (see Fig. 1) to clarify the role of native vertebrate animals and their invertebrate ectoparasites in the ecology of the SFG rickettsiae, by detecting rickettsiae in invertebrates and antibodies to rickettsiae in vertebrate animals in this region.

MATERIAL AND METHODS

Patients

Sera from patients with suspected spotted fever were received by the Clinical Pathology Laboratory, Fairfield Infectious Diseases Hospital. The patients reported here were from the following locations: East Gippsland, Victoria, 3; mainland Tasmania, 4; Flinders Island, Tasmania 17. All patients had a febrile illness with an associated maculo-papular rash. The age, sex and other details of the patients are shown in Table 1.

Two patients from Flinders Island had rickettsiae grown from their blood. The first patient was RB, age 67 yr, who, in November 1990 suffered from headaches, myalgia and rigors for 24 h. On the third day of her illness she developed scattered macules on the trunk and limbs. She responded to doxycycline. In December 1990, RM, age 50 yr, developed a large erythematous papule, 15×7 mm which had developed within 2 days of the attachment of tick (*Aponomma hydrosauri*) to her groin. She became ill 9 days after the bite with a tender ingunial lymphadenopathy and within 2 days she developed fever, chills, myalgia and a maculopapular rash on the trunk and limbs. She showed a good response to doxycycline.

Human serology

Acute and convalescent human sera were tested with an indirect microimmunofluorescence test as previously described [3]. The antigen was *R. australis* grown in buffalo green monkey kidney (BGMK) cells. A similar test was used for mouse, guinea pig and rat sera, using fluorescein-labelled antisera specific for the animal species being tested. Serology for murine typhus (*R. typhi*) was performed using as group antigen *R. prowazeki* (Breinl strain) grown in BGMK. A serological test for scrub typhus was developed using as antigen *R. tsutsugamushi* (Gilliam and Kato strains) grown in BGMK. The murine typhus and scrub typhus microimmunofluorescence tests were similar to those for the SFG rickettsiae. In all cases a titre of $< 1/64$ was considered to be negative, a titre of 1/64 was considered to be equivocal and a titre $> 1/128$ to be positive.

Animal inoculation of human blood

Blood from 14 patients with suspected FISF was taken on presentation and prior to any antibiotic treatment. Whole blood (5 ml for guinea pigs and rats and 0.1 ml for neonatal mice) was inoculated intraperitoneally directly into the experimental animals. On average three litters of mice (*c.* 30 neonatal mice), four infant guinea pigs and two infant rats were inoculated with blood from each patient. Neonatal mice are known to be very susceptible to *R. australis* [5]. Animals were examined daily for 3 weeks for signs of illness. At 1 and 2 weeks after inoculation, a small number of animals in each group was killed and various organs removed aseptically for storage at −70°C for later attempts at rickettsial isolation. Organs stored were brain, lung, heart, kidney, liver and spleen. Serology on the remaining animals was performed between 1 and 2 months after inoculation and if positive for *R. australis* an attempt was made to isolate rickettsiae from the previously frozen animal

Table 1. Spotted fever cases

Patient and location	Age	Sex	Date of illness	Serology (titre) *R. australis*†	Animal seroconversion to SFG rickettsia	Rickettsia recovered from tissue culture
Victoria (Gippsland)						
NM	30	M	January 1990	1/128	NA	NA
BN	40	F	May 1990	<1/64–1/512*	NA	NA
MM	49	M	October 1990	<1/64–1/2048*	NA	NA
Flinders Island						
PK	33	M	October 1989	<1/64–1/128*	None	NA
LK	34	F	November 1989	<1/64–1/512*	None	Negative
PA	67	M	November 1989	<1/64–1/128*	None	NA
MW	15	M	November 1989	<1/64–1/512*	NA	NA
EB	67	F	November 1989	<1/64–1/4096*	NA	NA
FR	64	F	December 1989	<1/64–1/1024*	First passage mice and guinea pigs. Not sustained in second, third and fourth passage mice.	NA
RW	2	M	December 1989	<1/64–1/512*	First passage mice. Not sustained in second and third passage mice.	Negative
BT	64	F	January 1990	<1/64–1/4096*	First passage mice. Not sustained in second passage mice.	NA
FW	70	M	October 1990	<1/64–1/1024*	None	NA
ND	61	F	October 1990	<1/64–1/256* (*R. typhi* 1/128)	None	NA
JMcK	38	M	November 1990	<1/64–1/256*	First passage mice. Not sustained in second passage mice.	Negative
GL	58	F	November 1990	<1/64–1/256*	First passage mice. Not sustained in second passage mice.	Negative
DR	71	M	November 1990	<1/64–1/1024*	First passage mice. Not sustained in second passage mice.	NA
RB	67	F	November 1990	<1/64–1/1024* (*R. typhi* 1/128)	First passage mice. Not sustained in second passage mice.	Rickettsia isolated
RM	50	F	December 1990	<1/64–1/512*	None	Rickettsia isolated
JX	38	M	December 1990	<1/64–1/256*	None	None
SK	24	M	December 1990	<1/64–1/512*	NA	NA
Tasmania						
WB	49	F	December 1987	1/128	NA	NA
SF	NR	M	February 1988	1/512	NA	NA
JO'C	35	M	October 1989	<1/64–1/2048* (*R. typhia* 1/64)	NA	NA
TMcL	NR	M	April 1990	1/128	NA	NA

*Seroconversion.
†Highest titre obtained.
NR: no record.
NA: not attempted.

organs. In these cases second and third animal passages were carried out using organs from animals killed at week 1 and 2.

Tissue culture of human blood

Blood from seven patients with suspected FISF was inoculated into BGMK monolayers, followed by centrifugation (100 *g* for 30 min). Whole blood, white blood cell-enriched preparations and triturated blood clots were used on different occasions. Excess cells were removed from the monolayer after 1–6 h incubation and the monolayer washed three times with Hank's balanced salt solution, followed by the addition of fresh medium (RPMI-1640, with 10% foetal calf serum, without antibiotics). Monolayers were cultivated in 5% CO_2 in air at 34.5°C, with two changes of medium in the first week and weekly changes thereafter, for a total of 1 month. At the conclusion of this period, cells were removed from the monolayer, fixed onto glass slides and stained for SFG rickettsia by an IF technique using high titre anti-*R. australis* guinea pig serum or convalescent serum from patients with FISF. The Giminez stain was also used.

Animal serology

Wild animal sera were tested using a competitive ELISA assay [6]. Laboratory mice and guinea pig sera were tested by immunofluorescence using as antigen *R. australis* grown in BGMK cells fixed onto glass slides.

Animal inoculation of invertebrate pools

Invertebrate pools (comprising the same species of ectoparasite obtained from the same species of vertebrate host, from the same region of south-east Australia) were disinfected by washing for 10 min in 1% tincture of iodine in 70% ethanol followed by rinsing in two changes of sterile distilled water each for 5 min. They were then ground up in a small volume of tissue culture medium and inoculated intraperitoneally into *c.* 10 neonatal mice (0.05–0.1 ml). Neonatal mice were used because they were known to be a sensitive host for *R. australis* [5]. If the mice died of overwhelming bacterial infection (usually within 48 h) the inoculation was repeated using more dilute preparations of the invertebrate mixture.

Field trips

Wild vertebrate animals were trapped at two sites in south-eastern Australia, both being areas where there had been cases of FISF; (i) Flinders Island, in Bass Strait, between the states of Victoria and Tasmania, and (ii) East Gippsland, a region within the state of Victoria, being the south eastern corner of the main Australian land mass (Fig. 1). Small mammals were caught in Elliot (box-type) or wire traps and anaesthetized by an intramuscular injection of a mixture of ketamine (10–20 mg/kg) and xylazine (2.5–5 mg/kg). All ectoparasites were removed (ticks, fleas, lice and mites) and a blood specimen taken by cardiac puncture (for serology). On recovery, the animals were returned to their site of capture and released. Ectoparasites were also recovered from domestic and farm animals (dogs, cats and cattle) and humans in the study areas. On-site identification of the vertebrate and invertebrate animals enable appropriate pools of ectoparasites to be made. The latter were then frozen in liquid nitrogen and not thawed until inoculated into experimental animals.

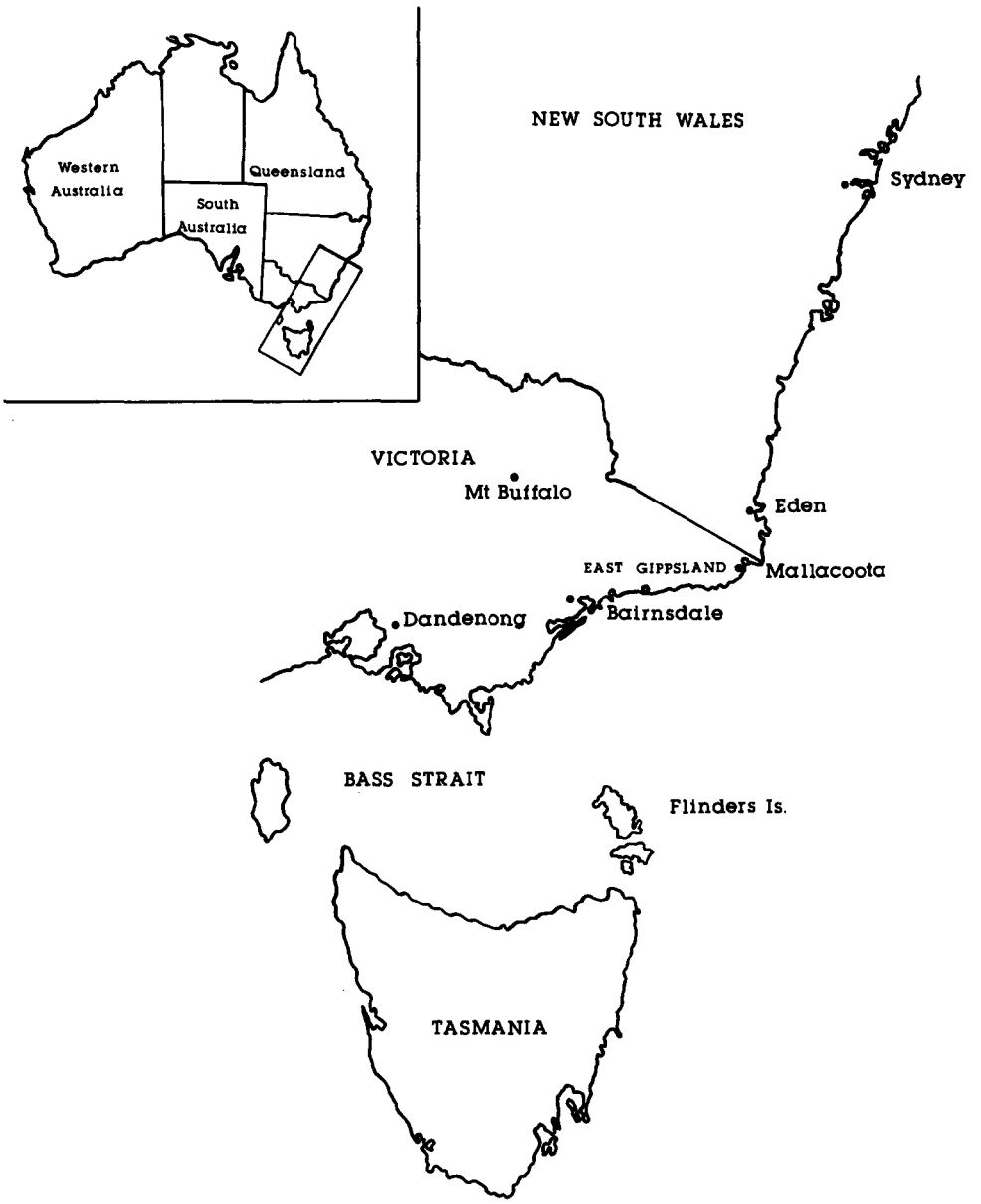

Fig. 1. South-eastern Australia (inset: Australia) showing locations mentioned in the text.

Tissue culture of tick haemolymph

Each individual living tick was disinfected as before and each leg of the tick was cut aseptically. The ensuing small drop of haemolymph was collected in a fine-bore pipette. The material was mixed with brain heart infusion medium and stored at −70°C until

required, or immediately added to a BGMK cell monolayer, followed by centrifugation at 1000 *g* for 30 min. Cultures were incubated at 34.5°C in 5% CO_2 in air for 1 month. Medium (RPMI-1640 with 10% foetal calf serum, without antibiotics) was changed twice in the first week and weekly thereafter. Cells were then removed by scraping, fixed onto glass slides, and stained for SFG rickettsia by an IF method using high-titre anti-*R. australis* guinea pig serum or convalescent serum from patients with FISF. A Giminez stain was also used.

RESULTS

Human studies

All 24 patients showed evidence of infection with a spotted fever group rickettsia as indicated by the presence of antibodies to *R. australis*. No patient showed antibodies to *R. tsutsugamushi*. Cross-reactions between SFG and typhus group rickettsiae were observed in two of 24 patients. The cross-reactions were low (1/64 or 1/128) and distinctly less than the SFG associated titres. 20 patients showed a seroconversion to SFG rickettsia, while four showed a titre $>1/128$ in their initial specimens. Attempts to isolate rickettsiae from patients' blood were only made from patients on Flinders Island. Of the 15 attempts (from 14 different patients), blood from seven patients caused seroconversion in the first passage experimental animals (mice only in six patients and mice and guinea pigs in one patient). However, in no case did the first, second, or third passage animals become unwell, nor did the second or third passage animals seroconvert. Rickettsiae were not isolated from any animal, including those that seroconverted. Blood specimens from two patients (RB and RM), taken within 24 h of onset of illness, and inoculated directly into cell cultures, yielded rickettsiae. After 30 days of incubation, the cell monolayers, without any obvious cytopathogenic effect, were removed, fixed and stained. Large numbers of intracellular rickettsiae were observed in the cytoplasm of the BGMK cells, both with Giminez and immunofluorescence staining using polyclonal SFG antigens for both patients are consistent with SFG rickettsial infection [3, 7] and show similar serological characteristics to QTT patients. One patient's (RB) white cell enriched blood induced a seroconversion in mice.

Native animal studies

Ectoparasites were removed from a number of native animals. Ticks were classified to genus, and where possible, species level. Fleas, lice and mites were not identified further. Most vertebrates were fully identified. On Flinders Island a total of 125 vertebrate animals were examined for ectoparasites (including eight humans who voluntarily presented a tick they had removed from themselves). From these vertebrates, a total of 766 ectoparasites were collected, of which the great majority (90.9%) were ticks. Only two genera were detected, *Ixodes* (37%) and *Aponomma* (63%). The *Ixodes* ticks were found only on mammals whereas the *Aponomma* ticks were found predominantly (92%) on reptiles. The eight ticks detected on humans consisted of six *I. tasmani* and two *Aponomma* ticks. Relatively few fleas (4.7%), lice (3.7%) and mites (0.3%) were recovered from vertebrate animals and 0.5% of the ectoparasites were unidentified. The animal species most infested with ticks were (i) *Rattus lutreolus*, the swamp rat, which had 13 *I. hirstii* ticks per animal (average); (ii) *Macropus rufogriseus*, the Bennett's Wallaby, which had 7.6 *Ixodes* ticks (mainly *I. tasmani*) per animal (average); (iii) *Vombatus ursinus*, the wombat, which had 12.4 ticks per animal (average), including both *Aponomma* and *Ixodes* ticks; (iv) *Tiliqua*

nigrolutea, the blotched blue-tongue lizard, which had 18.4 *Aponomma* ticks per animal (average); and (v) *Notechis ater*, the tiger snake, which had 8 *Aponomma* ticks per animal (average). Fleas were found only on rodents (*Mus musculus*, the house mouse; *Rattus rattus*, the black rat; and *R. lutreolus*, the swamp rat). Lice were found mainly on the marsupials, *Macropus rufogriseus*, the Bennett's wallaby, and *Thylogale billardieri*, the pademelon, but in both cases there was less than one louse per animal. Only two mites were detected, both on *V. ursinus*, the common wombat.

A large number of invertebrate pools, consisting of the same species of invertebrate from the same species of vertebrate, were tested for their potential to induce antibodies to SFG rickettsia after inoculation into mice and guinea pigs. A total of 62 mice litters and 36 guinea pigs were inoculated and subsequently tested serologically. None of the invertebrate pools from Flinders Island stimulated antibodies to *R. australis*.

In Gippsland Victoria, 92 vertebrate animals were examined for ectoparasites, including six humans who were participants in the field trip. A total of 47 ticks was removed from these persons, all of whom acquired their ticks while setting animal traps in the bush. Most of the human ticks were *I. cornuatus* (83%). *I. holocyclus* was not detected, even though it is considered to be the most common tick to bite humans in eastern Australia [8]. Of the total ectoparasites collected, 77.8% were ticks, 8.5% fleas, 7.4% mites, 6.0% lice and 0.3% were unidentified. Three genera of ticks were detected, *Ixodes* (89.0%), *Aponomma* (7.6%) and *Haemaphysalis* (3.4%). The animal species most infested with ticks were (i) *R. lutreolus*, the swamp rat, which had 16 *I. trichosuri* per animal (average); (ii) *R. fuscipes*, the bush rat, which had 4.6 *I. trichosuri* and 2.3 *Ixodes* sp. ticks per animal (average); and (iii) *V. ursinus*, the wombat, which had 6.5 *A. auruginans* ticks per animal (average). Fleas, lice and mites were found mostly on *R. fuscipes*, which an average of 1.4, 1.2 and 1.4 respectively, per rat.

As was done with the Flinders Island invertebrate pools, the Gippsland pools were inoculated into a total of 47 mice litters. Two pools caused the mice to seroconvert to *R. australis*, indicating the presence of SFG rickettsiae in the pools; these were (i) *I. cornuatus* from humans and (ii) *I. cornuatus* from dogs. Seroconversion caused by these two invertebrate pools was confirmed by two separate inoculation experiments.

One *I. cornuatus* tick, removed from a human living in Mallacoota, in the East Gippsland region of Victoria, was sent to us. Haemolymph obtained from it subsequently yielded a spotted fever group rickettsia in tissue culture but it has not yet been characterized.

Although only 62 individual native animals were tested for antibodies to SFG rickettsiae, using a competitive ELISA assay [6] several were clearly positive (Table 2). On Flinders Island 8/37 (22%) were positive, being mainly *R. rattus*, the black rat (2/4); unidentified *Rattus* sp. (2/4); *Trichosurus vulpecula*, the brushtail possum (2/4) and *V. ursinus*, the wombat (1/1). In Gippsland, 21/25 (84%) of the animals were positive, but this was mainly due to a preponderance of *R. fuscipes*, the bush rat, in the sample, of which 17/19 (89%) were positive. In addition, *R. lutreolus*, the swamp rat (2/3), *M. musculus*, the house mouse (1/1) and *Antechinus swainsonii*, the dusky antechinus (1/1) were also positive.

It is apparent that several species of native animals of south-eastern Australia are exposed to SFG rickettsiae, but it is not known if illness occurs in these animals.

Table 2. Serology of native Australian animals from Gippsland, Victoria and Flinders Island, for spotted fever group rickettsia, using *R. australis* in a competitive ELISA assay

	Serology for SFG rickettsia*	
Animal Species	Flinders Island	Gippsland, Victoria
(a) Placental mammals		
(i) *R. rattus* (black rat)	2/4	
(ii) *R. fuscipes* (bush rat)		17/19
(iii) *R. lutreolus* (swamp rat)		2/3
(iv) *Rattus* sp. (not identified)	2/4	
(v) *M. musculus* (house mouse)	1/4	1/1
(b) Marsupial mammals		
(i) *Macropus rufogriseus* (Bennett's wallaby)	0/6	
(ii) *Thylogale billardieri* (pademelon)	0/12	
(iii) *Trichosaurus vulpecula* (brush-tail possum)	2/4	0/1
(iv) *Pseudocheirus peregrinus* (ring-tail possum)	0/1	
(v) *V. ursinus* (common wombat)	1/1	
(vi) *Antechinus swainsonii* (dusky antechinus)		1/1
(vii) *Antechinus minimus* (swamp antechinus)	0/1	
Total	8/37 (22%)	21/25 (84%)

*denominator = number of animals tested; numerator = number of animals positive.

DISCUSSION

This paper follows those of Stewart [2], Graves *et al.* [3] and Dwyer *et al.* [7] who described spotted fever group (SFG) rickettsial disease on Flinders Island, Tasmania and in Gippsland, Victoria. Their descriptions were based on clinical presentation and serological reaction (including seroconversion) to *Rickettsia australis*, the only currently recognized member of the SFG rickettsia in Australia, and the aetiological agent of Queensland tick typhus (QTT). We now report for the first time the isolation of rickettsiae from the blood of two patients with Flinders Island spotted fever (FISF). Whether this newly recognized focus of infection is QTT or a different and new rickettsial disease, will be determined by the degree of similarity between the two extant *R. australis* strains (PHS) [1] and (JC) [9] and the two newly isolated rickettsial strains from patients (RC and RM) with FISF.

Three major groups of rickettsial disease occur in Australia: (i) scrub typhus group, (ii) typhus group (murine typhus); and (iii) spotted fever group (QTT and FISF) and their differentiation may be difficult [10]. Laboratory assistance, including serology may be required. Although serological cross-reactions between SFG rickettsiae and typhus group (TG) rickettsiae may occur [11] these were not a major problem in the current study.

The SFG rickettsial infection was the last to be recognized as a separate entity, and named North Queensland Tick Typhus (now called Queensland Tick Typhus, although it occurs at least as far south as Sydney) [12]. The first case was reported in 1946 [13] and soon after Andrew *et al.* [1] described twelve cases in soldiers. Two strains (PHS and FIK)

were isolated and were later named *R. australis* [14, 15]. A further isolate (JC) was made by Pope in 1955 [9]. The JC and PHS strains are identical with respect to the 17 kDa antigen gene [23], but no comparison has been made with the FIK strain as it is no longer in existence. Thus, only two human isolates are currently available for study (PHS and JC). The JC strain is also known as "Cutlack" or W58.

Attempts by Fenner [16] to isolate the rickettsia of QTT from 116 native animals and their ectoparasites were not successful, although serological evidence of exposure (by complement-fixation test) was present in the bandicoot, possum, rat-kangaroo and uromys. He stated that *I. holocyclus* was the presumed vector of the human disease. A rickettsia-like organism (agent R799) was isolated from a water rat in Brisbane [17] but was serologically unrelated to SFG, typhus group or scrub typhus group rickettsia. In 1974 *R. australis* was isolated from the tick *I. holocyclus* (obtained from a dog and by flagging) and *I. tasmani* (obtained from *R. fuscipes*, the bush rat [18], but these strains have also been lost. Hence, no rickettsiae have been isolated in Australia during the past 17 yr.

Renewed interest in rickettsial infections in Australia has been reinforced by the recognition that these diseases are widespread and represent diseases of public health importance. On Flinders Island (population *c.* 1000) the annual incidence is almost 1%. Between October 1989 and December 1990 (a period of 15 months), 17 new cases of FISF, clinically diagnosed and serologically confirmed, were recognized. The incidence in other parts of Australia is unknown, but probably under reported.

Clinically, FISF differs slightly from QTT, with a significantly lower incidence of tick-bite and eschar and regional lymphadenopathy [4]. The only epidemiological evidence for FISF being a separate rickettsial disease is its lack of geographic continuity with Queensland tick typhus. No cases of the latter have been reported south of Sydney, New South Wales and no cases of FISF have been detected yet on the south coast of New South Wales, apart from one case in Eden, on the New South Wales-Victorian border [7]. This latter case could be linked geographically with the Victorian Gippsland cases. Until the rickettsia of QTT and FISF are better characterized it may be better to consider them as separate SFG rickettsial diseases, with the understanding that this conclusion may need revision.

I. cornuatus is described by Roberts [19] as being very closely related to *I. holocyclus*. It occurs in south coastal New South Wales, eastern Victoria (as far west as Dandenong and Mount Buffalo) and in Tasmania (Fig. 1). Its vertebrate hosts include dog, cat, wombat, koala, kangaroo, cattle and humans. In our investigations *I. cornuatus* was found on dog, cat, wombat, cattle, swamp rat, bush rat and humans in Gippsland, Victoria but not on Flinders Island. The northern range of *I cornuatus* overlaps with the southern range of *I. holocyclus*. This latter species occurs along the east coast of Australia from North Queensland to Bairnsdale, Victoria [20]. It does not occur on Flinders Island or Tasmania. Both *I. holocyclus* and *I. cornuatus* have been associated with tick paralysis.

Our serological studies on native animals from Gippsland and Flinders Island (Table 2) suggest that many species of animals are bitten by ticks carrying the SFG rickettsia. Although numbers of individual animals were small, it seems likely that the bush rat (*R. fuscipes*) was often involved, as 17/19 specimens in East Gippsland had antibodies to SFG rickettsia. *I. cornuatus* was commonly found on this vertebrate species in Gippsland, thus supporting the role of *R. fuscipes* in the natural rickettsial cycle.

It therefore appears that in East Gippsland, Victoria, the rickettsia responsible for FISF uses *I. cornuatus* as an invertebrate vector and *R. fuscipes* as a vertebrate reservoir. On

Flinders Island the situation is less clear. Rickettsia have not yet been isolated from any tick and none of the tick pools caused experimental animals to seroconvert. Although seroconversion of mice is a sensitive method for detecting the presence of rickettsiae, it is not as sensitive as the development of plaques in tissue culture after centrifugation onto the monolayer of material containing *R. australis* [21]. Furthermore, rickettsiae may have been present but did not replicate in the mice and consequently did not stimulate any antibody response. Further investigations of invertebrate vectors are needed. *I. cornuatus* was not detected during the Flinders Island field trips. Serological studies on native animals (Table 2) suggest that the *Rattus* sp., mice, possums and wombats are involved. The tick most commonly found on humans on Flinders Island was *I. tasmani* but *Aponomma hydrosauri* (normally found on the blotched blue-tongue lizard) was occasionally detected on humans. In fact, a FISF patient from Flinders Island and from whom a rickettsia was isolated, was bitten by *Aponomma hydrosauri* 9 days before becoming ill and 12 days before developing a spotted rash. She kept the tick for identification. There is no certainty that this patient was infected via this particular tick, as some other agent or some other mode of transmission may have been responsible.

Domestic and farm dogs may play a role in the natural cycle of rickettsial transmission. *I. cornuatus* from dogs in Gippsland, Victoria, contained SFG rickettsia, as shown by mice seroconversion. Domestic dogs had been previously examined serologically and overall 11.2% of dogs from south-eastern coastal Australia were seropositive. In Gippsland, 2/18 (11%) of dogs and on Flinders Island 2/13 (15%) of dogs were seropositive [22]. Dogs are known to become heavily infested with ticks and tick-paralysis in dogs is a common problem in south-eastern Australia.

Note added in proof

Baird *et al.* [23] have now shown that the FISF rickettsial isolates are significantly different from *R. australis* (PHS and JC strains) with respect to the 17 kDa antigen gene and rRNA genes. They suggest that the FISF isolates may represent a new rickettsial species.

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