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The precise classification of this illness will have to wait until an agent is isolated.

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References

1. Campbell R W, Domrow R. Rickettsioses in Australia: isolation of *Rickettsia tsutsugamushi* and *R australis* from naturally infected arthropods. *Trans R Soc Trop Med Hyg* 1974; 68: 397-402.
2. Roberts FHS. Australian ticks. Melbourne CSIRO, 1970: 35-36.
3. Burnet FM. The rickettsial diseases in Australia. *Med J Aust* 1942; 2: 129-134.
4. Hone FS. A series of cases closely resembling typhus fever. *Med J Aust* 1922; 1: 1-13.
5. Hone FS. A further series of cases closely resembling typhus fever. *Med J Aust* 1923; 1: 435-443.
6. Hone FS. Endemic typhus fever in Australia. *Med J Aust* 1927; 2: 213-226.
7. Maxcy KF, Havens LC. A series of cases giving a positive Weil-Felix reaction. *Am J Trop Med* 1923; 3: 495-507.
8. Mooser H, Castaneda MR, Zinsser H. Rats as carriers of Mexican typhus fever. *JAMA* 1931; 97: 231-232.
9. Dwyer JM, Atkinson N. Some observations on endemic typhus in South Australia. *Med J Aust* 1940; 2: 573-576.
10. Wheatland FT. A fever resembling a mild form of typhus fever. *Med J Aust* 1926; 1: 261-266.
11. Derrick EH, Pope JH. Murine typhus, mice, rats and fleas on the Darling Downs. *Med J Aust* 1960; 2: 924-928.
12. Saint EG, Drummond AF, Thorburn IO. Murine typhus in Western Australia. *Med J Aust* 1954; 2: 731-737.
13. Mathew RY. Endemic typhus in north Queensland. *Med J Aust* 1938; 2: 371-377.
14. Heaslip WG. An investigation of the condition known as coastal fever in north Queensland: its separation from scrub-typhus. *Med J Aust* 1940; 2: 555-564.
15. Brody J. A case of tick typhus in north Queensland. *Med J Aust* 1946; 1: 511-512.
16. Heaslip WG. Tsutsugamushi fever in North Queensland, Australia *Med J Aust* 1941; 1: 380-383.
17. Andrew R, Bonnin JM, Williams S. Tick typhus in north Queensland. *Med J Aust* 1946; 2: 253-258.
18. Funder JF, Jackson AV. North Queensland tick typhus: a comparative study of the rickettsia with that of murine typhus. *Med J Aust* 1946; 2: 258-263.
19. Plotz H, Smadel JE, Bennett BL, et al. North Queensland tick typhus: studies of the aetiological agent and its relation to other rickettsial diseases. *Med J Aust* 1946; 2: 263-268.
20. Streeten GEW, Cohen RS, Gutteridge NM, et al. Tick typhus in south Queensland: report of three cases. *Med J Aust* 1948; 1: 372-373.
21. Neilson GH. A case of Queensland tick typhus. *Med J Aust* 1955; 1: 763-764.
22. Knyvett AF, Sandars DF. North Queensland tick typhus: a case report defining a new endemic area. *Med J Aust* 1964; 2: 592-594.
23. Campbell RW, Abeywickrema P, Fenton C. Queensland tick typhus in Sydney: a new endemic focus. *Med J Aust* 1979; 1: 350-351.
24. Anonymous. Tick typhus. *Med J Aust* 1946; 2: 563.
25. Anonymous. Rocky Mountain spotted fever — United States, 1988 *MMWR* 1989; 38: 513-515.
26. Wilfert CM, MacCormack JN, Kleeman K, et al. Epidemiology of Rocky Mountain spotted fever as determined by active surveillance. *J Infect Dis* 1984; 150: 469-479.
27. Taylor JP, Tanner WB, Rawlings JA, et al. Serological evidence of sub-clinical Rocky Mountain spotted fever infections in Texas. *J Infect Dis* 1985; 151: 367-369.

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Flinders Island spotted fever: a newly recognised endemic focus of tick typhus in Bass Strait

Part 2. Serological investigations

Stephen R Graves, Brian W Dwyer, Danny McColl and Joseph E McDade

ABSTRACT Twenty-six cases of a spotted-fever-like illness have been identified on Flinders Island, Tasmania, over a 17 year period. These patients and 335 healthy persons from the island were investigated serologically using the Weil–Felix agglutination test (*Proteus* sp. antigens OX2, OX19, OXK) and rickettsia-specific microimmunofluorescence. The antigens used in these latter tests comprised one member of the typhus group (*Rickettsia typhi*) and three members of the spotted fever group (*Rickettsia rickettsii*, *Rickettsia australis* and *Rickettsia conorii*). Patients with Flinders Island spotted fever showed a higher prevalence of positive reactions to the Weil–Felix tests (with OX2 and OX19 antigens) and a higher prevalence of positive results to rickettsia-specific serological tests (with the exception of antibodies to *Rickettsia typhi*) than did healthy persons; OX2 (36% v. <1%); OX19 (36% v. <1%); *Rickettsia rickettsii* (42% v. 1%); *Rickettsia australis* (46% v. 1%); *Rickettsia conorii* (42% v. 1%); *Rickettsia typhi* (4% v. 4%). In seven of the 26 patients (27%) seroconversion was demonstrated by means of Weil–Felix tests, confirming recent infection. In six of these patients seroconversion was also demonstrated in rickettsia-specific tests. Although these results support the clinical evidence that the illness on Flinders Island is caused by a rickettsia of the spotted fever group, the aetiological agent remains to be isolated.

(Med J Aust 1991; 154: 99-104)

In Australia, rickettsial diseases from all three rickettsial groups are known to occur: *(i)* scrub typhus, due to *Rickettsia tsutsugamushi* (the scrub typhus group) occurs in Queensland; *(ii)* murine typhus, due to *Rickettsia typhi* (the typhus group) occurs in most, if not all, States of Australia; and *(iii)* Queensland tick typhus, due to *Rickettsia australis* (the spotted fever group) occurs in Queensland and New South Wales.

Stewart has now described an illness occurring on Flinders Island with the clinical and epidemiological features of a spotted fever (see pages 94-99). Except for rickettsial pox (*Rickettsia akari*) all members of the spotted fever group of rickettsia are transmitted by ticks.[1] In Australia, the rickettsia causing Queensland tick typhus is transmitted by the tick *Ixodes holocyclus* and possibly also by *Ixodes tasmani*.[2-4] Hence the recognition of this endemic focus of probable rickettsial disease on Flinders Island is of interest. Because the aetiological agent has yet to be isolated and identified it remains unclear whether this disease is the same as Queensland tick typhus.

We have used two serological techniques to assist in the elucidation of the pathogen: *(i)* the classic (but non-specific)

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test for rickettsial disease, the Weil–Felix agglutination reaction, which makes use of three different *Proteus* sp. antigens (OX2, OX19, OXK) which cross-react with rickettsial antibodies in patterns typical of each major rickettsial group; and *(ii)* microimmunofluorescence tests in which known rickettsial species from defined groups react specifically with rickettsial antibodies.[5] In particular we have sought to demonstrate that patients with Flinders Island spotted fever have antibodies directed against rickettsia of the spotted fever group and, where possible, have attempted to demonstrate seroconversion.

The serological evidence presented here confirms the clinical and epidemiological evidence for a newly recognised focus of endemic rickettsial disease in south-eastern Australia. The species of rickettsia responsible remains to be defined.

Materials and Methods

Collection of sera

Dr Rob Stewart of Flinders Island collected sera from the 26 patients and 335 healthy persons on the island. As the population of Flinders Island is about 1000, approximately one-third of the island's population has been investigated serologically. The sera from cases were retrieved from frozen stocks stored at −20°C at the time of the acute illness as well as subsequent specimens taken months to years later. The other sera were solicited from the adult population over a period of 15 months until January 1989.

Weil–Felix agglutination

A microtitre technique was used in which the three *Proteus* sp. antigens (OX2, OX19 and OXK) (Wellcome, Australia) were diluted 1:4 before use. Final serum dilutions from 1:5 to 1:640 were used. Microtitre trays were incubated at 37°C for two hours, overnight at 4°C and then at room temperature for two hours. For OX2 we defined a titre of <1:40 as a negative result; 1:40 as marginally positive and >1:40 as positive. For OX19 and OXK we defined <1:80 as negative, 1:80 as marginally positive and >1:80 as positive. The limits for a negative result were determined by replicate assays of blood bank sera from central Victoria where this disease is unknown.

Rickettsial-specific micro-immunofluorescence

Tests for murine typhus *(R. typhi)* and Rocky Mountain spotted fever *(Rickettsia rickettsii)* were performed at the Centers for Disease Control, Atlanta using methods that have been previously described.[5] Titre definitions were: negative (<1:64); marginally positive (1:64); and positive (≥1:128).

We carried out tests for *Fièvre boutonneuse, (Rickettsia conorii)* using a commercial kit produced by bio Mérieux, (Charbonnières les Bains, France), following the manufacturer's instructions except that sera were screened at a 1:64 dilution instead of 1:40. If positive they were titrated to an end-point. Titre definitions were: negative (<1:64); marginally positive (1:64); and positive (≥1:128).

No test system was available for Queensland tick typhus so one was developed using *R. australis* (PHS strain) obtained from the Queensland Department of Health.

The agent was grown in Buffalo green monkey kidney (BGMK) cells. We used modified Eagle's minimum essential medium, with Earle's salts and 20 mmol hydroxyethylpiperazine ethanesulphonic acid (HEPES), containing 10% fetal calf serum (heat inactivated) without antibiotics. The viable *R. australis* was stored at −70°C in Sucrose PG Diluent[6] and thawed quickly to 37°C before inoculation into almost confluent BGMK cell monolayers. The rickettsia were left to invade the cells over half an hour to an hour at room temperature, after which the supernatant fluid was removed and fresh medium added. The plastic flasks were incubated at 36°C with the caps tight.

We monitored the progress of the infection once or twice weekly by removing all but 0.5 mL of medium and scraping some cells off the tissue culture flasks. Air-dried cells were acetone-fixed onto glass slides and stained by the methods of Giménez[7] (to detect small pink rickettsial rods in the cell cytoplasm) and Gram (to confirm the absence of contaminating bacteria). Between two and three weeks after inoculation the cell monolayer showed a mild cytopathogenic effect, consisting of rounded-up cells. No plaques appeared but, as the cells became progressively more infected with rickettsia, they became detached into the supernatant fluid.

These cells were collected by centrifugation of the supernatant (3000 *g* × 20 min). Infected cells that were attached to the plastic flask were removed by freezing and thawing. Both cell groups were pooled. Doubling dilutions of the pooled rickettsia-infected BGMK cells were spotted onto slides, air-dried and acetone-fixed for 10 minutes and examined by Giménez stain and microimmunofluorescence (with strongly positive stored human antisera to *R. australis*, obtained from patients with clinical Queensland tick typhus). The dilution that yielded several (3–10) rickettsia-infected BGMK cells per high-power (×40) field was selected as the dilution at which the routine microimmunofluorescence slides were made. These slides showed clear fluorescence within the cytoplasm of the infected cells, with some individual rickettsia fluorescing in the background, due to their release from ruptured BGMK cells.

Screening of sera was done at a 1:64 dilution and if positive, sera were titrated to an end-point. Titre definitions were: negative (<1:64); marginally positive (1:64); and positive (≥1:128). These definitions were established by examining 50 healthy human sera from central Victoria where no rickettsial diseases are known to occur. Of these, 49 were <1:64 and one was marginally positive at 1:64).

For the *R. australis* microimmunofluorescence test we placed 10 μL of human serum (at the appropriate dilution) onto one spot of *R. australis*. This was left to react for 30 minutes at 37°C in a moist atmosphere, washed three times with phosphate-buffered saline (pH 7.4), each time for five minutes, and air-dried. Then 10 μL of fluorescein-labelled antihuman gammaglobulin (bio Mérieux), at a dilution of 1:80, was added to each well and and left to react for 30 minutes at 37°C in a moist atmosphere. We determined the correct conjugate dilution for each new batch by using a known positive antiserum with varying dilutions of conjugate. We found 1:80 to be satisfactory for two different batches of conjugate. The slides were washed three times in phosphate-buffered saline (each time for five minutes), air-dried, and examined immediately for immunofluorescence.

R. australis could also be grown in guinea pigs and neonatal mice and this proved useful on the occasions when tissue cultures became contaminated and the organism needed to be reisolated. After intraperitoneal inoculation, guinea pigs became febrile and developed an inflamed scrotum and diarrhoea, but did not succumb. The organism could be reisolated from the tunica vaginalis. Mice became lethargic and *R. australis* could be reisolated from the spleen and brain. The agent was lethal for newborn mice.

Results

Of the 26 clinical cases of this disease (Table 1) we had sera from only 11 patients in the acute phase of the illness or soon thereafter. Of these, 4/11 had a positive OX19 reaction (36%); 4/11 had a positive OX2 (36%); and none had a positive OXK. The values for OX19 and OX2 are much higher than those recorded for healthy persons on Flinders Island, where the prevalence was less than 1% for each of the three antigens (Table 2).

Sera from the 26 patients (taken at times ranging from less than one year to approximately 15 years after the time of illness) showed very substantial cross-reactions to the three different rickettsia of the spotted fever group. Against *R. rickettsii* 10/24 sera (42%) were reactive; against *R. australis* 12/26 (46%) were reactive; and against *R. conorii* 11/26 (42%) were reactive (Table 1). In the healthy population on Flinders Island, reactivity to each of the three spotted fever rickettsia was only about 1% (Table 2).

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TABLE 1: Rickettsial status of 26 patients with Flinders Island spotted fever

Case no.	Age, sex	Date of illness	Date sera taken (year)	Weil-Felix titre			Specific rickettsial titre*			
							Typhus group	Spotted fever group		
				OX2	OX19	OXK	*R. typhi*	*R. rickettsii*	*R. australis*	*R. conorii*
1	38, M	Jan 1973	1988	NR	NR	NR	±	+(256)	±	±
2	59, M	Dec 1975	1987	NR	NR	NR	±	–	–	–
3	14, M	Jan 1977	1988	NR	NR	NR	–	–	–	–
4	49, M	Sep 1978	1987	NR	NR	NR	–	±	–	–
5	43, F	Nov 1978	1987	NR	NR	NR	–	–	–	–
6	34, F	Oct 1979	1987	NR	NR	NR	–	–	±	±
7	58, F	Dec 1979	1979	+	–	–	–	+(1024)	+(4096)	+(4096)
8	38, F	Dec 1979	1987	NR	NR	NR	–	±	±	+(128)
9	52, M	Jan 1980	1987	NR	NR	NR	–	±	±	±
10	71, F	Oct 1980	1980	–	–	–	–	+(128)	+(128)	+(256)
11	27, M	Nov 1980	1987	NR	NR	NR	–	±	±	±
12	25, M	Jan 1981	1987	NR	NR	NR	–	+(256)	+(128)	+(128)
13	26, F	Feb 1981	1987	NR	NR	NR	–	–	+(128)	+(128)
14	53, M	Dec 1981	1982	–	+	–	–	+(1024)	+(1024)	+(1024)
15	68, M	Nov 1983	1987	NR	NR	NR	–	–	–	–
16	51, F	Dec 1983	1987	NR	NR	NR	–	±	+(256)	±
17	32, M	Jan 1984	1987	NR	NR	NR	–	±	±	–
18	58, M	Jan 1984	1984	–	±	–	–	+(128)	±	–
19	62, F	Dec 1985	1985	+	+	–	–	+(1024)	+(2048)	+(8192)
20	22, F	Feb 1986	1987	NR	NR	NR	–	+(1024)	+(512)	+(512)
21	75, M	Jan 1987	1987	–	+	–	+(1024)	+(128)	+(512)	+(1024)
22	26, F	Dec 1987	1988	–	+	–	–	–	–	–
23	9mo, F	Dec 1987	1988	–	–	–	–	–	–	–
24	62, F	Jan 1989	1989	–	±	–	NA	NA	+(1024)	+(1024)
25	38, M	Jan 1989	1989	+	±	–	–	+(1024)	+(4096)	+(4096)
26	2, M	Jan 1989	1989	+	–	–	NA	NA	+(128)	–
Total positive results: (%)				4/11 (36%)	4/11 (36%)	0/11 (0%)	1/24 (4%)	10/24 (42%)	12/26 (46%)	11/26 (42%)

*Numbers in brackets indicate highest possible dilution.
NR: not relevant, as serum not taken during acute phase of illness.
NA: not available.
+: positive test result.
±: marginally positive test result.
–: negative test result.

TABLE 2: Positive rickettsial results in 11 asymptomatic persons from 335 healthy persons on Flinders Island*

Person no.	Date serum taken	Weil-Felix titre			Specific rickettsial titre			
					Typhus group	Spotted fever group		
		OX2	OX19	OXK	*R. typhi*	*R. rickettsii*	*R. australis*	*R. conorii*
i	Oct 1987	–	–	–	+(128)	±	±	±
ii	Nov 1987	–	–	–	–	–	+(256)	+(256)
iii	Feb 1988	–	–	–	–	+(128)	±	±
iv	Mar 1988	–	–	–	+(128)	±	–	–
v	Apr 1988	–	–	–	–	–	+(256)	+(256)
vi	Apr 1988	–	–	+(320)	–	–	–	–
vii	May 1988	+(80)	+(320)	–	–	–	–	–
viii	May 1988	±	–	–	+(128)	–	–	–
ix	Jul 1988	–	–	–	–	–	+(128)	+(128)
x	Oct 1988	–	–	+(320)	–	–	–	–
xi	Dec 1988	–	+(160)	–	–	–	–	–
Total: (%)		1/335 (<1%)	2/335 (<1%)	2/335 (<1%)	3/76 (4%)	1/76 (4%)	3/335 (1%)	3/335 (1%)

*: numbers in bracket indicate highest positive dilution.
+: positive test result.
±: marginally positive test result.
–: negative test result.

Of the 26 patients, 24 were tested for antibodies to *R. typhi* but only one was seropositive (Table 1). This was the same as the prevalence of these antibodies in the normal population (4%) (Table 2). Thus Flinders Island spotted fever is not murine typhus.

We detected seroconversion to one of the Weil-Felix antigens in seven patients whose sera were sampled on more than one occasion (Table 3). Six of these patients also seroconverted to one of the rickettsial antigens.

Overall 335 healthy persons on Flinders Island donated blood for serological analysis, this being approximately one-third of the total population. Of these sera, 324 (97%) showed a negative reaction to *R. australis* and *R. conorii*. Of the original 335 sera, 76 were further tested for antibodies to *R. rickettsii* and *R. typhi* and 72 were

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TABLE 3: Seroconversion in seven cases of Flinders Island spotted fever

Case no.	Age, sex	Date of illness	Date sera taken (year)	Weil-Felix titre			Specific rickettsial titre			
							Typhus group	Spotted fever group		
				OX2	OX19	OXK	*R. typhi*	*R. rickettsii*	*R. australis*	*R. conorii*
7	58 F	Dec 1979	14/12/79	5	10	10	<64	512	<64	<64
			21/12/79	80*	10	20	<64	>512	>4096*	>4096*
18	58 M	Jan 1984	16/1/84	10	20	20	<64	<64	<64	<64
			6/2/84	20	80*	10	<64	256*	64	64
19	62 F	Dec 1985	30/12/85	10	10	20	<64	<64	<64	<64
			8/1/86	640*	160*	40	<64	>512*	2048*	8192*
21	75 M	Jan 1987	12/1/87	20	20	20	<64	<64	<64	<64
			21/1/87	20	640*	10	1024*	128*	512*	1024*
22	26 F	Dec 1987	14/12/87	5	80	40	<64	<64	<64	<64
			5/1/88	5	320*	20	<64	<64	<64	<64
24	62 F	Jan 1989	24/1/89	5	10	10	NA	NA	<64	<64
			14/2/89	5	80*	10	NA	NA	1024*	024*
25	39 M	Jan 1989	17/1/89	40	20	20	<64	<NA	64	128
			6/2/89	640*	80*	10	<64	>512	4096*	4096*

*Denotes seroconversion.

negative (Table 2). Thus only 11 of 335 sera (3%) had positive rickettsial status and these showed a variety of patterns. Seven showed positive rickettsia-specific reactions, probably representing past infection with either murine typhus (3/11) or Flinders Island spotted fever (4/11). None of these seven persons had positive Weil–Felix reactions.

Analysis of the distribution of seropositive and seronegative cases showed no evidence of special geographical clustering (Table 4). When the rickettsial seropositivity of cases was compared with respect to years after infection, there was an indication that antibodies to *R. australis* decayed with time. Whereas 9/12 cases (75%) recorded in the preceding two years were positive, only 3/5 cases (60%) between two and six years previously were positive, while 0/9 cases that had occurred seven or more years previously were positive.

Discussion

The results of these serological tests add strength to the clinical and epidemiological observations of Stewart and support his hypothesis that Flinders Island spotted fever is a true rickettsiosis of the spotted fever group. Nevertheless, some patients with clinical Flinders Island spotted fever have had negative serological test results for the spotted fever group rickettsia. It is conceded that misdiagnosis may explain the seronegative status of Cases 15, 22 and 23 (Table 1). In these three cases the illness was accompanied by a negative microimmunofluorescence test to typhus group antigen and all three spotted fever group antigens. However, in view of the well developed eschars that were present in Cases 22 and 23, the alternative of yet another undescribed rickettsia causing infection must be considered.

The waning of antibody to undetectable levels may apply to those whose illness dated back to the 1970s (Cases 2, 3, 4, 5 [Table 1]). Decline in detectable antibody levels is well described for *R. tsutsugamushi* and *R. conorii.*[8-10] If it is assumed that marginally positive titres represent genuine old cases, then positive spotted fever group results were obtained in 20/26 cases (77%). The remaining 6/26 cases (23%) may be another syndrome, clinically similar, but aetiologically distinct.

The Weil–Felix agglutinations reported here are now largely of historical importance. Several cases showed seroconversion only to Weil–Felix antigen OX19. Although this is a non-specific test that cannot be used with certainty to diagnose a rickettsial disease and may be positive in a variety of other illnesses, its development in Case 22, a patient with typical disease, including eschars, but negative rickettsial status, gives cause to reconsider its value. The Weil–Felix reaction becomes positive usually between the 8th and 12th day and disappears about the 4th or 5th week of illness.[11] The three antigens used are *Proteus* sp. bacteria antigenically defined as OX19, OXK and OX2. The antigens on these organisms are shared at least in part by pathogenic rickettsia. Seroconversion to OXK is considered to occur in approximately 50% of cases of scrub typhus *(R. tsutsugamushi)*[12] but not in spotted fever or typhus group rickettsial infections. A single reported case of tick typhus (due to *R. conorii)* did not have a positive OXK,[12] but this is extremely unusual. Seroconversion to OX19 and/or OX2 occurs in most (but not all) rickettsial infections of the spotted fever and typhus groups. It is not possible to distinguish between these two infections by the Weil–Felix reaction. In our patients, 36% had a positive OX19 reaction and 36% a positive OX2 reaction (Table 1) which is consistent with either a spotted fever or typhus group rickettsial infection, but not with scrub typhus.

Seroconversion detected by microimmunofluorescence to spotted fever group rickettsia is very good evidence of recent infection, because of the high specificity of these tests. Although the typhus group rickettsia *(Rickettsia prowazekii* and *R. typhi)* cause illness clinically distinct from the spotted fever group rickettsia, in sero-

TABLE 4: Geographical distribution of 15 seropositive* and 11 seronegative cases†

	Seropositive cases	Seronegative cases
Western side of Flinders Island (Whitemark, Happy Valley and Emita)	14‡	8§
Remainder of Flinders Island	1‖	3¶

*Cases seropositive for spotted fever rickettsia.
†$P = 0.16$ (not significant) by Fisher's exact probability test.
‡Cases 1,7,8,10,12,13,14,16,18,19,20,24,25,26.
§Cases 2,3,4,9,11,15,22,23.
‖Case 21.
¶Cases 5,6,17.

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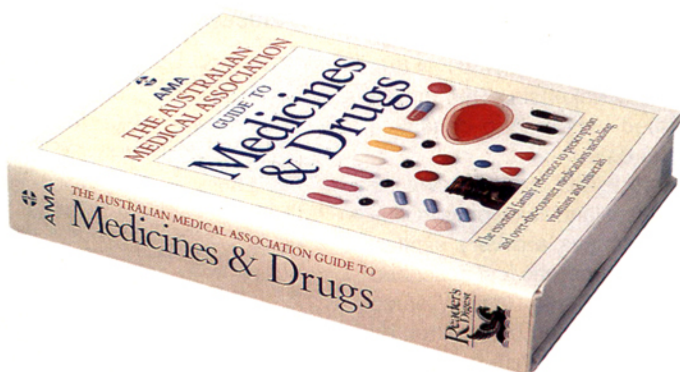

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logical terms there is some overlap by immunofluorescence,[5] and toxin neutralisation,[13] In general, the antibody titre against the infecting rickettsia is considerably higher than against heterologous rickettsia. Of our 26 patients, 20 had antibodies against spotted fever group rickettsia and in most of these cases the level of antibodies against each of the three individual members of the group was similar. Only three patients had antibodies against *R. typhi* and two of these were marginally positive titres only. In case 21, however, a high titre against both *R. typhi* and the spotted fever group rickettsia was noted. This elderly man was, in his youth, a British soldier in India and may well have been infected with typhus or have been immunised against typhus. Subsequent infection with Flinders Island spotted fever, later in his life (1987) may have generated a heterologous anamnestic antibody response. Cross-absorption of his serum with heterologous antigens showed that the most likely infecting agent was a spotted fever group rickettsia.

The titres obtained with the three spotted fever group rickettsia are not strictly comparable. Titrations against *R. rickettsii* were performed at the Centers for Disease Control, Atlanta, whereas the other two were carried out in our laboratory. Hence it is not surprising that there was some variation in titres for individual sera against the antigens.

The low prevalence (1%) of antibodies of spotted fever group rickettsia amongst the 335 healthy persons on Flinders Island (Table 3) was surprising and suggests either that asymptomatic infection is very rare or that decay of antibody over time is very rapid, or, indeed, both of the above. The low prevalence (1%) of positive Weil–Felix reactions amongst healthy persons (Table 3) also contrasts dramatically with the cases (Table 1). Unfortunately, for most of the early cases (Cases 1-17), no acute phase serum was still available. By way of comparison, 10% of persons in Western Sicily had antibodies to *R. conorii*[14] and most of these infections were apparently subclinical. This is quite different from our findings on Flinders Island. The prevalence of antibodies to *R. typhi* was the same for controls and cases (4%) suggesting that murine typhus does occur on Flinders Island, but that it is not the same disease as Flinders Island spotted fever. Murine typhus has been reported in South Australia,[15] Western Australia,[16] Queensland[17] and New South Wales (NSW Department of Health, personal communication), so it is not surprising that it may be present on Flinders Island. There was no epidemiological evidence suggesting that Flinders Island spotted fever was murine typhus.

Flinders Island spotted fever appears to be a definite clinical entity, in which a high proportion of cases has specific spotted fever group antibodies. Should the latter be a necessary condition for defining a case of Flinders Island spotted fever? At this stage of our knowledge, without the aetiological agent having been isolated and identified, we have declined to specify rickettsial seropositivity as being a prerequisite for diagnosing this disease. The clinical entity may well comprise more than one infection, at least one of which is due to a spotted fever rickettsia. This matter can be clarified when the causative agent(s) is (are) determined.

Could Flinders Island spotted fever be the same as Queensland tick typhus? Clinically, the two diseases are very similar. Queensland tick typhus was first described in 1946 by Andrew, Bonnin and Williams amongst soldiers who had been bitten by ticks *(Ixodes holocyclus)* while training on the Atherton Tableland of North Queensland.[3] *R. australis* was isolated from two soldiers by means of inoculation of the patients' blood into mice and guinea pigs. Of their 12 patients, nine (75%) had a diagnostic titre with OX19 and three (25%) had a diagnostic titre with OX2. These results are similar to our own 11 cases, where Weil–Felix tests were performed. In neither series was a positive OXK reaction obtained. This suggests that we are dealing with the same disease.

Cases of Queensland tick typhus have occurred along the east coast of Australia from north Queensland to Sydney (see Part I of this paper for references), but none south of Sydney, neither published cases nor cases reported to the New South Wales Department of Health (from 1967 to 1988). Do cases occur along the south coast of NSW but go unreported? Or is this area free of Queensland tick typhus? The ticks *I. holocyclus* and *Ixodes tasmani* occur in this area.[18] Why would the disease occur on Flinders Island, but not on the south coast of New South Wales? Recently we have recognised cases of a spotted-fever-like illness, very similar to Flinders Island spotted fever, on the east coast of Victoria (Gippsland region), which we report in a companion publication (see pages 121-125). It appears to us that a tick-borne rickettsial disease, known as Queensland tick typhus in Northern Australia, as Flinders Island spotted fever on Flinders Island, and as an unnamed illness in Gippsland, Victoria, is probably the same disease. Recovery of the aetiological agent and comparison with *R. australis* will resolve this question.

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References

1. Marchette NJ. Ecological relationships and evaluation of the *Rickettsiae.* Boca Raton: CRC Press, 1982.
2. Campbell RW, Domrow R. Rickettsiosis in Australia: isolation of *Rickettsia tsutsugamushi* and *Rickettsia australis* from naturally infected arthropods. *Trans R Soc Trop Med Hyg* 1974; 68: 397-402.
3. Andrew R, Bonnin MJ, Williams S. Tick typhus in north Queensland. *Med J Aust* 1946; 2: 253-258.
4. Campbell RW, Abeywickrema P, Fenton C. Queensland tick typhus in Sydney: a new endemic focus. *Med J Aust* 1979; 1: 350-351.
5. Philip RN, Casper EA, Ormsbee RA, et al. Microimmunofluorescence test for the serological study of Rocky Mountain spotty fever and typhus. *J Clin Microbiol* 1976; 3: 51-61.
6. Elisberg BL, Bozeman FM. The rickettsia. In: Lennette E H, Schmidt N J, eds. Viral, rickettsial and chlamydial infections, 5th ed. Washington DC: American Public Health Association Inc, 1979: 1061-1108.
7. Hendrickson DA. Reagents and stains. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, eds. Manual of clinical microbiology, 4th ed. Washington DC: American Society of Microbiologists, 1985: 1093-1107.
8. Saunders JP, Brown GW, Shirai A, Huxsoll DL. The longevity of antibody to *Rickettsia tsutsugamushi* in patients with confirmed scrub typhus. *Trans R Soc Trop Med Hyg* 1980; 74: 253-257.
9. Bourgeois AL, Olsen JG, Fang RCY, et al. Humoral and cellular responses in scrub typhus patients reflecting primary infection and reinfection with *Rickettsia tsutsugamushi. Am J Trop Med Hyg* 1982; 34: 532-540.
10. Mansueto S, Vitale G, Bentivegna M, et al. Persistence of antibodies to *Rickettsia conorii* after an acute attack of boutonneuse fever. *J Infect Dis* 1985; 151: 377.
11. Bull LB. Serological examinations. *Med J Aust* 1923; 1: 443-445.
12. McDonald JC, MacLean JD. Imported rickettsial diseases: clinical and epidemiologic features. *Am J Med* 1988; 85: 799-805.
13. Bell EJ, Stoenner HG. Immunologic relationships among the spotted fever group of rickettsias determined by toxin neutralization tests in mice with convalescent animal serums. *J Immunol* 1960; 84: 171-182.
14. Mansueto S, Vitale G, Miceli MD, et al. Seroepidemiological survey of asymptomatic cases of boutonneuse fever in Western Sicily. *Trans R Soc Trop Med Hyg* 1984; 78: 16-18.
15. Hone FS. Endemic typhus fever in Australia. *Med J Aust* 1927; 2: 213-226.
16. Saint EG, Drummond AF, Thorburn IO. Murine typhus in Western Australia. *Med J Aust* 1954; 2: 731-737.
17. Derrick EH, Pope JH. Murine typhus, mice, rats and fleas on the Darling Downs. *Med J Aust* 1960; 2: 924-928.
18. Roberts FHS. A systemic study of the Australian species of the genus *Ixodes. Aust J Zool* 1960; 8: 392-485.

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