Australia has 4 rickettsial diseases: murine typhus, Queensland tick typhus, Flinders Island spotted fever, and scrub typhus. We describe 7 cases of a rickettsiosis with acute onset and symptoms of fever (100%), headache (71%), arthralgia (43%), myalgia (43%), maculopapular/petechial rash (43%), nausea (29%), pharyngitis (29%), lymphadenopathy (29%), and eschar (29%). Cases were most prevalent in autumn and from eastern Australia, including Queensland, Tasmania, and South Australia. One patient had a history of tick bite (Haemaphysalis novaeguineae). An isolate shared 99.2%, 99.8%, 99.9%, 99.9%, and 100% homology with the 17 kDa, ompA, gltA, 16S rRNA, and Sca4 genes, respectively, of Rickettsia honei. This Australian rickettsiosis has similar symptoms to Flinders Island spotted fever, and the strain is genetically related to R. honei. It has been designated the “marmionii” strain of R. honei, in honor of Australian physician and scientist Barrie Marmion.

Case Reports

Patient 1

A 37-year-old woman from Port Willunga, South Australia, sought treatment in February 2003, with a 2-
week history of headache, fever, and sweats. No rash or eschar was seen, and she had no recollection of arthropod exposure. She had traveled to Kangaroo Island 2–3 weeks before the onset of illness. Laboratory tests showed elevated levels of liver function test enzymes, mild leukopenia, and thrombocytopenia. Her health improved after receiving oral doxycycline for 5 days. Rickettsial serology later showed an increase in antibody titer. Both PCR and culture results were positive for an SFG rickettsia (Table 1).

Patient 2
A 9-year-old girl sought treatment at the Darnley Island Health Clinic, Torres Strait, Queensland, in February 2003. She was febrile (38.5°C) and reported headache, nausea, and abdominal pain. She had no eschar or rash. She was initially thought to have a viral illness; however, after 3 days she was still febrile (39.0°C), and the provisional diagnosis was changed to scrub typhus; a regimen of oral doxycycline, 100 mg per day, was begun. She was not seen by medical or nursing staff between day 3 and 8 of the illness, but was afebrile and well by day 8. Her SFG title increased, despite a 6-month delay in obtaining the convalescent-phase serum. Results of culture and PCR of the blood sample taken on day 8 were positive for an SFG rickettsia (Table 1).

Patient 3
A 27-year-old man sought treatment at the Darnley Island Health Clinic in March 2003. His temperature was 37.4°C, and he reported headache, arthralgia, and cough. He exhibited no eschar or rash. The provisional diagnosis was of viral upper respiratory tract infection. He was seen again on days 3 and 4 with persisting symptoms and a sore throat. On the latter visit his condition was diagnosed as tonsillitis, and treatment with penicillin V was begun. Blood tests for malaria and scrub typhus were initiated. He returned on day 29 with fever (37.6°C), cough, pharyngitis, and arthralgia. Results of serologic investigations for *Plasmodium falciparum* and rickettsia (taken on day 3) were negative. Antibiotics were not given because the illness was thought to be viral. His symptoms resolved within the following 2 weeks. Antirickettsial antimicrobial agents were not given at any stage during the illness. Day 3 serum and follow-up serum specimens obtained 6 months later were both negative for rickettsial antibodies; however, results of PCR and culture on the day 3 blood specimen were positive for SFG rickettsiae (Table 1).

Patient 4
A 10-year-old boy was brought to the Yam Island Health Clinic, Torres Strait, Queensland, in May 2003, five days into an illness with manifestations of fever (38.1°C), headache, and cough. Diagnostic tests for scrub typhus, malaria and leptospirosis were initiated but he was given no specific antimicrobial therapy. Two days later, he seemed improved, and a provisional diagnosis of viral upper respiratory tract infection was made. However, when he was seen on day 14, some symptoms remained (cough and headache), and treatment with amoxicillin was begun. He was well when examined on day 22. At no stage was he given antirickettsial therapy. His day 5 blood sample was negative for SFG/typhus group (TG) rickettsial antibodies, but results of PCR and culture were positive for a SFG rickettsia. Follow-up serum taken 14 months later was negative for rickettsial antibody (Table 1).

Patient 5
A 50-year-old man was admitted to Innisfail Hospital, Innisfail, Queensland, in June 2003. He reported a 7-day history of fever and rigors and a 4-day history of maculopapular rash. He also reported myalgia, arthralgia, conjunctivitis, swollen hands, dry cough, and constipation. An eschar was found on the right side of his neck. His temperature was 38.5°C and blood pressure 95/60 mm Hg. Serum chemistry showed elevated levels of total bilirubin (23; normal range 2–20 µmol/L), alkaline phosphatase (276; normal range 30–115 units/L), gamma-glutamyl transpeptidase (199; normal range 0–70 units/L), aspartate

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### Table 1. Rickettsial serology, PCR, and culture results from 7 cases of FISF*

| Patient | Location | 1st serum sample | 2nd serum sample | Microbial detection of FISF agent by |
|---------|----------|------------------|------------------|-------------------------------------|
|         |          | Day after disease onset | SFG titer | TG titer | Day after disease onset | SFG titer | TG titer | Sero-positivity | Seroconversion | PCR | Culture |
| 1       | Port Wilunga, SA | 14 | 128 | <128 | 190 | 256 | <128 | + | – | + | + |
| 2       | Darnley Is, QLD | 8 | 256 | <128 | 186 | 512 | <128 | + | – | + | + |
| 3       | Darnley Is, QLD | 3 | <128 | 179 | <128 | <128 | – | – | + | + |
| 4       | Yam Is, QLD | 5 | <128 | 128 | 515 | <128 | <128 | – | – | + | + |
| 5       | Innisfail, QLD | 7 | <128 | 128 | 17 | 4,096 | 2,048 | + | + | + | + |
| 6       | Launceston, TAS | 34 | 128 | 128 | 265 | 128 | 128 | – | – | +<sup>†</sup> | – |
| 7       | Iron Range, QLD | 5 | <128 | <128 | 18 | 1,024 | 128 | – | – | ND |

*FISF, Flanders Island spotted fever; SFG, spotted fever group; TG, typhus group; SA, South Australia; Is, island; QLD, Queensland; TAS, Tasmania; ND, not done.

<sup>†</sup>PCR positive at both 34 and 60 d after disease onset.

<sup>†</sup>Performed on serum and real-time PCR positive sample only.
transaminase (AST) (301; normal range 5–40 units/L), alanine transaminase (ALT) (129; normal range 5–40 units/L), and lactate dehydrogenase (LDH) (701, normal range 100–225 units/L). Further investigation showed proteinuria, moderate thrombocytopenia (59; normal range 150–400 × 10^9/L), mild neutrophilia with left shift (7.9; normal range 2.0–7.5 × 10^9/L), and lymphopenia (0.7; normal range 1.0–4.0 × 10^9/L). Examination of convalescent-phase serum showed seroconversion to SFG rickettsia. Results of rickettsial PCR and culture were positive for a member of the SFG (Table 1). He recovered after treatment with oral doxycycline (100 mg twice per day) for 5 days.

**Patient 6**

A 33-year-old man, an entomologist, at Iron Range, Cape York Peninsula in far north Queensland, removed a tick from the left ventrolateral side of his abdomen in late May 2002. Five days after removing the tick (day 5), an eschar appeared on his left lower abdomen. An SFG illness was suspected, and treatment with doxycycline, 100 mg twice per day, was begun. His doctor reexamined him on day 20, and his condition had improved. His myalgia had decreased, and the rash faded over 5 weeks.

Laboratory testing on day 10 showed lymphopenia (0.8; normal range 1.0–4.0 × 10^9/L) and mild thrombocytopenia (146; normal range 150–400 × 10^9/L). Liver function tests showed slightly elevated AST (48; normal range 5–40 units/L) and ALT (44, normal range 5–40 units/L) and mildly elevated LDH (325; normal range 100–225 units/L). Rickettsial serology was negative on day 10 but convalescent-phase serology on day 23 showed an SFG seroconversion. A real-time PCR on the day 10 serum specimen showed a positive result for the SFG/TG gltA gene, but the 17-kDa PCR result was negative (Table 1).

The removed tick was subsequently identified as *Haemaphysalis novaeguineae*. DNA was extracted from the tick and PCR results performed targeting the rickettsial *rrs*, *ompA* and *ompB* genes. PCR products were sequenced, aligned, searched with BLAST (available from http://130.14.29.110/BLAST/), and submitted to GenBank (accession nos. AJ585043, AJ585044, and AJ585045 for the *rrs*, *ompA*, and *ompB* genes, respectively). Phylogenetic analysis of all 3 genes showed that the closest relatives were *R. honei* strain TT-118 (Thai tick typhus) and *R. honei* strain RB (FISF) (11).

**Methods**

**Rickettsial Serology**

Serologic testing was performed on human serum specimens using a goat anti-human IgM, IgG, and IgA fluorescein isothiocyanate–labeled secondary antibody (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA), by an indirect immunofluorescence assay (IFA) as described (7). Antigens used included *R. honei*, *R. australis*, *R. akari*, *R. conorii*, *R. sibirica*, and *R. rickettsii* from the SFG; and *R. typhi* and *R. prowazekii* from the TG. All titers >128 were considered positive.

**Rickettsia Isolation from Blood**

Rickettsial isolation was performed with Vero cell cultures as previously described (12). Cultures were observed microscopically weekly for a cytopathic effect and monthly by immunofluorescence. IFA-positive cultures had their DNA extracted and their rickettsial status confirmed by
PCR. Positive cultures were passaged onto confluent XTC-2 cell monolayers and grown at 28°C in Leibovitz L-15 media (Invitrogen, Melbourne, Victoria, Australia) supplemented with 5% heat-inactivated fetal bovine serum, 0.4% tryptose phosphate (Oxoid, Basingstoke, UK), and 200 mmol/L-glutamine (Invitrogen).

**Rickettsial PCR on Blood**

Rickettsial real-time PCR was performed on buffy coat (except for serum for case 7). DNA was extracted by using a DNA extraction kit (Gentra, Minneapolis, MN, USA) and the primers CS-F and CS-R and the probe CS-P (Table 2; Biosearch Technologies Inc., Novato, CA, USA) as previously described (13).

Confirmatory PCR was performed on the 17-kDa gene (orf17) by using the primers MTO-1 and MTO-2 (Table 2; Invitrogen) (14), with an annealing temperature of 51°C and a total of 45 cycles. PCR products were visualized by electrophoreses on a 1% Tris-acetate EDTA agarose gel (Amresco, Solon, CA, USA) stained with ethidium bromide. PCR-positive samples had their DNA cleansed using the QIAquick DNA clean up kit (QIAGEN, Düsseldorf, Germany) and were sequenced at Newcastle DNA (Newcastle University, Newcastle, New South Wales, Australia). Phylogenetic analysis of DNA sequences was performed with DNADIST and NEIGHBOR computer programs of the PHYLIP version 3.63 software package (available from http://evolution.genetics.washington.edu/phylip.html). Sequences were compared to those of the rickettsial strains considered to be valid species (19). Phylogenetic trees and bootstrap analyses were performed with 100 alignments by using the SEQBOOT and CONSENSE programs of PHYLIP.

**Rickettsial Molecular Characterization**

Rickettsial isolates had portions of their gltA, 16S rRNA, ompA, and Sca4 antigen genes amplified and sequenced to supplement the 17-kDa gene analysis done on buffy coat and cultures. The primer pairs CS-162-F with CS-731-SR and CS-398-SF with RpCS1258 (Table 2) were used to amplify the 5’ and 3’ ends of gltA, respectively (15).

The 16S rRNA gene (rrs) was amplified by using the primer pairs rRNA1 with rRNA3 and rRNA2 with rRNA4 (Table 2) (17). The PCR contained 1 µmol of each respective primer, 200 µmol/L of each dNTP, 10× reaction buffer, 2 mmol/L MgCl2, 2 U Taq polymerase, and 4 µL of rickettsial DNA extract. The amplification was performed in a thermocycler (Rotor-Gene 3000, Corbett Research, Sydney, New South Wales, Australia) with an initial denaturation of 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for 1 min; with a final extension of 10 min. PCR products were visualized and sequenced as described above.

| Primer | Nucleotide sequence (5’→3’) | Gene | Reference |
|--------|-----------------------------|------|-----------|
| CS-F   | TCG CAA ATG TTC AGC GTA CTT T | gltA | 13        |
| CS-R   | TCG TGC ATT TCT TTC CAT TGT G | gltA | 13        |
| CS-P*  | TGC AAT AGC AAG AAC CGT AGG CTG GAT G | gltA | 13        |
| MTO-1  | GCT CTT GGA ACT CTA TGT T | orf17 | 14        |
| MTO-2  | CAT TGT TCG TCA GGT TGG CG | orf17 | 14        |
| CS-162-F | GCA GCT ATC GGT GAG GAT GTA ATC | gltA | 15        |
| CS-398-SF | 5’ATT ATG CCT GCG GCT GTC GG | gltA | 15        |
| CS-731-SR | AAG CAA AAG GGT TAG CTC C | gltA | 15        |
| RpCS1258p | ATT GCA AAA AGT ACA GTG GTA A | orf17 | 16        |
| rRNA1  | AGA GTT TGA TCC TGG CTC AG | rrs | 17        |
| rRNA2  | AAG GAG GTG ATC CAG CCG CA | rrs | 17        |
| rRNA3  | CCC TCA ATT CCT TGG AGT TT | rrs | 17        |
| rRNA4  | CAG CAG CCG CGG TAA TAC | rrs | 17        |
| Rrl190.70p | ATG GCC GAT ATT CCT CCA AAA | ompA | 16        |
| Rrl190.602n | AGT GCA GCA TTC GCT CCT | ompA | 16        |
| D1f    | ATG AGT AAA GAC GGT AAC CT | sca4 | 18        |
| D928r  | AAG CTA TTG CGT CAT CTC CG | sca4 | 18        |
| D767f  | CGA TGG TAG CAT TAA AAG CT | sca4 | 18        |
| D1390r | CTT GCT TTT CAG CAA TAT CAC | sca4 | 18        |
| D1210f | CCA AAT CTT CTT AAT ACA GC | sca4 | 18        |
| D1876r | TAG TTT GTT CTG CCA TAA TC | sca4 | 18        |
| D1738f | GTA TCT GAA TTA AGC AAT GCG | sca4 | 18        |
| D2482r | CTA TAA CAG GAT TAA CAG CG | sca4 | 18        |
| D2338f | GAT GCA GCG AGT GAG GCA GC | sca4 | 18        |
| D3069r | TCA GGC TTG TGG AGG AGG AAG | sca4 | 18        |

*5’ end labeled with 6-FAM; 3’ end labeled with BHQ-1.*
The ompA gene (ompA) was amplified by using the primers Rl190.70p and Rl190.602n (Table 2) by using the above protocol but with an annealing temperature of 48°C (16). The Sca4 antigen gene (sca4) was amplified by using the primer pairs D1f and D928r, D767f and D1390r, D1219f and D1876r, and D1738f and D2482r, following the specified protocol (18). The final segment of the gene was amplified with the primers D2338f and D3069r following the same protocol and an annealing temperature of 48°C (18).

**Results**

Seroconversion, defined as a 4-fold increase in antibody titer, occurred in only 2 of the 7 patients (patients 5 and 7), although positive titers were seen in 5 of 7 patients (Table 1). In 5 of 6 patients a rickettsia was isolated from blood (in EDTA-vacutainers; Table 1) in Vero cell culture, however, 4 of these 5 isolates did not persist in cell culture after their third passage. The remaining isolate, from patient 5, has been maintained in continuous culture in only the XTC-2 cell line.

Patients 1–6 had rickettsial DNA detected in their buffy coat DNA extracts by real-time PCR. Patient 7 had rickettsial DNA detected in his serum by using real-time PCR. Of the 7 cases, all but 1 (patient 7), were PCR positive for the 17-kDa gene and all 5 positive rickettsial cultures were also PCR positive for the same gene (Table 1). The 17-kDa PCR sequences for the buffy coats of cases 1–6 and cultures of patients 1–5 were found to be 100% homologous to one another and to the Japanese *Haemaphysalis* tick sequences Hf151 and Hl550 (GenBank accession nos. AB114816 and AB114807, respectively). A 399-bp sequence also exhibited 99.2% homology with *R. honei* strains RB and TT-118 (GenBank accession nos. AF060704 and AF060706, respectively) as shown in the phylogenetic tree (Figure).

Analysis of a 1082 bp gltA sequence from the KB strain exhibited 99.7 and 99.8% homology with *R. honei* strains RB and TT-118, respectively (GenBank accession nos. AF018074 and U59726, respectively) (Figure). An 1142 bp rrs sequence exhibited 100% homology with the Australian *Haemaphysalis novaeguineae* tick sequence AL2003 (II) (GenBank accession no. AJ585043) and a 1,388-bp sequence exhibited 99.6% and 99.9% homology with the *R. honei* strains RB and TT-118, respectively (GenBank accession nos. U17645 and L36220, respectively) (Figure). A 511-bp sequence of ompA exhibited 100% homology with the *H. novaeguineae* sequence AL2003 (II) (GenBank accession no. AJ585044) and a 513-bp sequence had 99.8% homology with the *R. honei* strains RB and TT-118 (GenBank accession nos. AF018075 and U43809, respectively). Only 1 nucleotide substitution was found in a 2,961-bp sequence of the Sca4 gene (100%
homology) with R. honei strain RB (GenBank accession no. AF163004) (Figure).

These R. honei strain “marmionii” sequences have been submitted to GenBank with the accession nos. AY37683 for the 17-kDa gene, AY37684 for the gltA gene, AY37685 for the 16S rRNA gene, DQ309095 for the Sca4 gene, and DQ309096 for the ompA gene.

**Discussion**

These 7 cases of FISF are an example of many newly emerging rickettsial diseases (21). Its symptoms are consistent with a relatively mild rickettsial SFG disease. The most frequent acute symptoms observed were fever (100%), headache (71%), arthralgia (43%), myalgia (43%), cough (43%), rash (maculopapular/petechial) (43%), nausea (29%), pharyngitis (29%), and lymphadenopathy (29%). In only 2 patients was an eschar evident. The rash did not appear on the palms or soles, unlike previously reported FISF cases (6,12). One patient (patient 7), had a history of a H. novaeguineae tick bite, which may imply an incubation period of 5 days. The cases in this report occurred between February and June (late summer and autumn), in contrast to previously described cases of FISF and QTT, which have their peak onsets in summer and late winter, respectively (5,6).

The biphasic illnesses seen in patients 3 and 6 were unusual for SFG rickettsial diseases. Because no specimens were taken during the initial phase of either patient’s illness, that this phase was rickettsial in nature cannot be confirmed. Patient 6’s illness may have been rickettsial in nature because of the appropriate incubation time after a fishing trip in an area endemic for ticks. His illness had the longest duration of all the reported cases, with rickettsiae still detectable 27 days after the onset of the second febrile illness. This is possibly the first report of an SFG rickettsia being associated with a chronic infection in a human. Relapsing rickettsial diseases are known to exist, such as Brill disease, a recurrent form of epidemic typhus (22). Rickettsiae persisting in human and animal organs after illness have been reported with scrub typhus and SFG rickettsia (23,24). An Australian case of recurrent rickettsial illness was diagnosed serologically as QTT (25).

The isolation of rickettsiae from patient 2 after antimicrobial drug therapy and while she was clinically well is unusual. The presence of rickettsiae may be due to the bacteriostatic nature of the patient’s treatment, which allowed a small number of rickettsiae to survive before being eliminated by her immune system. This phenomenon may also have been the beginning of a chronic infection, as described above in patients 3 and 6.

Apart from patients 5 and 7, antibody levels of paired serum specimens (Table 1) did not show a marked rise in titer. Because the second serum sample from 4 of the case-patients was received in excess of 6 months after illness, the antibody levels may have subsided, explaining the apparent lack of seroconversion in patients 3 and 4. Because most rickettsioses are diagnosed through serologic tests, some cases of rickettsial disease are likely being missed due to a lack of seroconversion, as we have observed with these cases of FISF. This demonstrates the usefulness of PCR for diagnosing acute rickettsial diseases. Cases of rickettsioses without seroconversion or positive serology titers have been previously described with “R. sibirica mongolotimonae” (26). Despite the initial isolation of R. honei strain “marmionii” in Vero and L929 cells at 35°C, no isolate could be continuously grown in these cell lines. This may be due partially to temperature-dependent growth kinetics, similar to those of R. felis (27)

The 7 described cases were distributed widely throughout eastern Australia. Cases have appeared on the eastern seaboard of Australia (including the Torres Strait), Tasmania, and in South Australia. Cases are yet to be reported in Victoria, New South Wales, the Northern Territory, or Western Australia. The discovery of FISF cases in the Torres Strait suggests its possible presence in Papua New Guinea. In comparison, QTT is found only down the eastern seaboard and not south or west of Wilson’s Promontory in Victoria. Traditionally, FISF has only been found in the southeastern states, including Tasmania and South Australia (6,12,28).

At present, R. honei has been found on 2 other continents, with potential reservoirs in *Ixodes* and *Rhipicephalus* ticks in Asia and in *Amblyomma cajennense* in North America (29). The only known vector/reservoir of R. honei in Australia is *Bothriocroton hydrosauri* (10). R. honei strain “marmionii” has not been found in any *B. hydrosauri* ticks, although *H. novaeguineae* may be a vector/reservoir, as a *H. novaeguineae* tick was removed from patient 7 before the onset of illness. Rickettsial *rrs* and *ompA* gene sequences within the tick demonstrated 100% homology with *R. honei* strain “marmionii” (11). *H. novaeguineae* is known to bite numerous animals including humans and is found in both northern Australia and Papua New Guinea (30). The vectors and reservoirs of *R. honei* strain “marmionii” in southern Australia are not known.

When compared phylogenetically to other rickettsiae, *R. honei* strain “marmionii” has the closest homology with Australian *R. honei* strain RB, which had been isolated from a febrile patient on Flinders Island. When the *gltA*, *rrs*, *ompA*, *orf17*, and *sca4* genes are compared between *R. honei* strains RB and “marmionii,” they are 99.7%, 99.6%, 99.6%, 99.0%, and 100% homologous, respectively. Homologies of 99.8% and 99.9% are seen with the *gltA* and *rrs* genes, respectively, when *R. honei* strains TT-118 and “marmionii” are compared. An 811-bp *ompB* gene...
sequence from the *H. novaesoulei* tick removed from patient 7 also showed 100% homology with *R. honei* (11). This supports its description as an SFG rickettsia but not a new species by using previously proposed criteria (19). Further analysis is needed to further define the taxonomic position of *R. honei* strain “marmionii.”

The 7 cases of an illness similar to FISF demonstrate that new emerging rickettsioses are present in Australia. These described cases encompass a geographic distribution larger than those of FISF and QTT. The only known tick host of *R. honei* strain “marmionii” is *H. novaesoulei*, a tick not previously recognized as a trans- mitter of human pathogens. Genetically, the etiologic agent of these 7 cases is closely related to *R. honei*. We propose to name the agent *Rickettsia honei* strain “marmionii,” in honor of the Australian physician and scientist Barrie P. Marmion, for his research into Q fever, another important rickettsial disease.

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Dr Unsworth is a postdoctoral research associate at Texas A&M University, College Station, Texas, USA. His interests include the epidemiology of Australian rickettsiae and Q fever pathogenesis.

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Address for correspondence: John Stenos, Australian Rickettsial Reference Laboratory, Geelong Hospital, PO Box 281 Geelong, Victoria, Australia 3220; email: johns@barwonhealth.org.au