Pathology (1998) **30**, pp. 147–152

BROADSHEET NUMBER 44: RICKETTSIAL DISEASES: THE AUSTRALIAN STORY SO FAR

STEPHEN R. GRAVES

The Australian Rickettsial Reference Laboratory, The Geelong Hospital, Geelong, Victoria, Australia

This Broadsheet is published on behalf of the Board of Education of The Royal College of Pathologists of Australasia.

INTRODUCTION

Rickettsiae are obligate intracellular bacteria that are responsible for a variety of infectious diseases, all of which are transmitted by invertebrate ectoparasite(s) to a vertebrate (mammalian) host, including humans. In the case of epidemic typhus, the human body louse (*Pediculus humanus*), infected with *Rickettsia prowazekii*, transmits rickettsiae to humans. In all other rickettsial diseases humans are an accidental host, where the invertebrate ectoparasite is normally associated with an animal species other than humans. Rickettsial diseases are difficult to diagnose and specialised laboratory techniques are required, mainly serology, to confirm a diagnosis. Treatment involves the use of appropriate antibiotics, but as the cell walls of rickettsiae are atypical, the cell wall-active antibiotics (eg; penicillins, cephalosporins) are not effective. Doxycycline is the drug of choice, with chloramphenicol an appropriate alternative.

Key words: Rickettsial disease, rickettsial infections, *Rickettsiae* sp., ectoparasites.

Abbreviations: FISF, Flinders Island Spotted Fever; QTT, Queensland tick typhus; SFG, spotted fever group; STG, scrub typhus group; TG, typhus group.

THE RICKETTSIAE

These bacteria are part of the α-subgroup of the class Proteobacteria.[1] Their closest relatives phylogenetically (based on 16S rRNA nucleotide sequence comparisons) are the *Ehrlichia, Bartonella* and *Brucella*. This group of bacteria also contains important plant and soil bacteria, such as *Agrobacterium* and *Rhizobium*. The group also contains the mitochondria of eucaryotic cells (ie; plant and animal cells[2]). It is now clear that mitochondria were originally procaryotic cells (ie; bacterial cells), free-living in their own right. There are extensive common DNA sequences in mitochondria and the α-subgroup Proteobacteria. At some stage in the long distant past a proto-mitochondrion-cum-bacterium established itself in the cytoplasm of a eucaryotic cell. Initially this was probably a parasitic relationship in which the intracellular bacterium fed on the contents of the host cell cytoplasm, utilising metabolites, molecular building blocks and perhaps energy molecules (eg; ATP). This is the stage of evolution of the currently extant rickettsiae. However, the proto-mitochondrion-cum-bacterium gradually co-evolved with its host cell, developing a symbiotic relationship and eventually becoming an obligate, intracellular organelle of the host cell—the modern-day mitochondrion.[3] A related branch of the α-proteobacteria evolved into the currently recognised rickettsiae.

The original rickettsiae would have started their intracellular life inside invertebrate cells, as invertebrate animals appeared on Earth long before vertebrates.[4,5] With the passage of time and the eventual appearance of vertebrates (firstly fish, amphibians and later reptiles, birds and mammals) the invertebrate animals (with their rickettsial symbionts) became ectoparasites on the vertebrate newcomers. This is presumably the stage when rickettsiae had to adapt themselves to the new vertebrate animals, as indeed the invertebrate ectoparasites were doing. The rickettsiae thus developed the ability to grow within invertebrate cells and vertebrate cells, and this enabled them to cycle between these two very different groups of animals. This is a remarkable biological adaptation but highly successful, as shown by widespread dispersal of rickettsiae into many ecological niches today.

Currently, rickettsial diseases are divided into three groups according to the type of ectoparasite that transmits the rickettsial infection. The groups are: 1, Scrub Typhus (mite transmitted); 2, the Spotted Fever Group (tick transmitted—with one exception); and 3, the Typhus Group (the *epidemic* type being louse transmitted and the *endemic* type being flea transmitted). Note, mites and ticks are arachnids, lice and fleas are insects. *Coxiella burnetti* (Q-fever) is a member of the γ-subgroup of the Proteobacteria and is no longer considered to be a rickettsia by many authorities. It will not be discussed in this paper.

However, modern molecular biology provides a more phylogenetically accurate and scientifically correct classification of the rickettsiae.[1] According to 16S rRNA (and other gene) sequences the rickettsiae are most appropriately divided into two major groups (Table 1). The first contains the scrub typhus (ST) rickettsia, originally named *Rickettsia tsutsugamushi*, but now reclassified into a different genus, *Orientia tsutsugamushi*.[6] This new genus name reflects the geographic distribution of scrub typhus in

TABLE 1 The two major groups of rickettsia

1. Scrub typhus group (STG): *Orientia tsutsugamushi* (formerly known as *Rickettsia tsutsugamushi*).
2. *Rickettsia* sp.: all other rickettsial diseases

the orient, including east asia, southeast asia and northern Australia. The second group contains all the other rickettsiae and they retain the genus name *Rickettsia.*

The genus *Rickettsia* contains all other rickettsial species and is itself divided into two groups, the Typhus Group (TG) and the Spotted Fever Group (SFG).

The Typhus Group

This consists of the following species:

(i) *R. prowazekii.* This is the classic rickettsia, causing the classic rickettsial disease, epidemic louse-borne typhus. Rickettsiae are excreted in the feces of the infected human body louse. This species of rickettsia is also found in flying squirrels in eastern USA. This may be the primordial focus for this rickettsia, which may have then made its way to the "old world" in the early sixteenth century.[7] Epidemic typhus was first described in Europe in the 1520s, soon after Columbus' discovery of the "new" world in 1492.

(ii) *R. typhi* (formerly known as *R. mooseri*). This species of rickettsia is very closely related to *R. prowazekii*, both genetically and antigenically. It probably does not warrant separate species status,[8] but the disease in man (murine, or endemic, typhus) is clearly different from epidemic typhus. The natural hosts are rats and mice and the vectors are rodent fleas (*Xenopsylla cheopis; Nosopsyllus fasciatus; Leptopsylla segnis*). The rickettsia is excreted in the feces of the flea.

(iii) *R. felis.* This rickettsia is transmitted between cats and possums (in North America) by means of fleas. Human infection has been recorded. Some scholars believe that *R. felis* should be classified with the Spotted Fever Group rather than the Typhus Group.[9]

(iv) Other species not pathogenic for man, eg; *R. canada.*

The Spotted Fever Group

This group consists of many "species" (Table 2), all of which are closely related genetically and antigenically. They do not qualify for separate "species" status but historically distinct names have been used because the diseases occur in defined geographical locations: eg; *R. rickettsii*—Rocky Mountain Spotted Fever; *R. sibirica*—North Asian Tick Typhus; *R. australis* —Queensland Tick Typhus (QTT). Sometimes two different SFG diseases can overlap geographically, as occurs with *R. conorii*—Mediterranean Spotted Fever and *R. africae*—African Tick Bite Fever.

The SFG rickettsiae are transmitted via the bite of an infected tick. A large number of hard ticks are involved. In Australia *Ixodes* spp. are the only recognised vectors, including *I. holocyclus, I. tasmani* and *I. cornuatus.* The only SFG rickettsia that is not tick-transmitted is *R. akari* (rickettsial pox), this being mite-transmitted.

From a phylogenetic viewpoint, the SFG rickettsiae can be subdivided further, based on nucleotide sequence divergence of key rickettsial genes (eg; citrate synthase gene). Currently the largest subgroup contains the main SFG disease-causing rickettsiae (see Table 2), such as *R. rickettsii* and *R. conorii.* Interestingly, the recently discovered *R. honei*, the cause of Flinders Island Spotted Fever (FISF), an Australian rickettsial disease, is located in this group.[10]

TABLE 2 The major sub-division of the genus: *Rickettsia*

1. Typhus group (TG)
   (a) *R. prowazekii* (epidemic typhus)
   (b) *R. typhi* (endemic, or murine, typhus)
   (c) *R. felis*
   (d) other species not pathogenic for humans, eg; *R. canada*
2. Spotted Fever group (SFG)
   (a) *R. rickettsii* (Rocky Mountain Spotted Fever)
   R. conorii (Mediterranean Spotted Fever)
   R. honei (Flinders Island Spotted Fever)
   R. africae (African Tick Bite Fever)
   R. japonica (Japanese Spotted Fever)
   R. sibirica (North Asian Tick Typhus)
   (b) *R. australis* (Queensland Tick Typhus)
   R. akari (rickettsial pox)
   (c) other pathogenic species, eg; *R. helvetica*
   (d) other species not pathogenic for humans, eg; *R. montana*

However, *R. australis*, the cause of QTT, is removed from the main SFG complex and linked to *R. akari.* It is not known if *R. akari* occurs in Australia.

THE RICKETTSIAL DISEASES, WITH EMPHASIS ON THE AUSTRALIAN SITUATION

Scrub Typhus

Orientia tsutsugamushi (until recently, known as *Rickettsia tsutsugamushi*) is a collection of serotypes and genotypes that show considerable antigenic heterogeneity.[6] The three classic serotypes are: Karp (from Papua New Guinea), Kato (from Japan) and Gilliam (from Burma).

However, there are many other serotypes detected in Japan, Korea, Thailand and other parts of east and southeast Asia. Scrub typhus is limited to these regions, but it is also found in northern (tropical) Australia, especially coastal Queensland, north of Mackay.[11-13]

If the same taxonomic logic was used for the genus *Orientia* as for the genus *Rickettsia*, each serotype of *Orientia* (eg; Karp) would be given its own species name, as the difference between "species" in the TG/SFG is no greater than the difference between "serotypes" in the STG. However, this is not done, so all scrub typhus is caused by the one species, *O. tsutsugamushi.*

Spotted fever group rickettsiae (Rickettsia *sp.*)

Only the two Australian diseases in this category will be mentioned.

1. Queensland Tick Typhus (R. australis) Recognised as a new illness amongst Australian soldiers training during World War II on the Atherton Tableland, the disease was originally called North Queensland Tick Typhus.[14, 15] Later it was recognised in locations further south, including Brisbane,[16] Sydney,[17] and the Gippsland region of Victoria.[18] Its most southerly range appears to be Wilson's Promontory in Victoria. It has not yet been recognised west of Melbourne. Cases are all on the eastern side of the Great Dividing Range,[19] as this is where rainfall is conducive to *Ixodes* spp. tick survival. These ectoparasites are able to feed on a number of native bush animals, especially rats. Dogs are also readily infested with *Ixodes* spp. ticks and

produce antibodies to *R. australis*.[20] *I. holocyclus* can cause tick-paralysis, but it is not related to their carrying of rickettsiae.

2. Flinders Island Spotted Fever (R. honei): The sole general practitioner on Flinders Island (in Bass Strait), Dr Robert Stewart, recognised this illness for many years before it was confirmed as a SFG rickettsial disease.[21, 22] The isolation of the causative agent by the author and his collaborators,[23] led to molecular studies on this organism that showed it to be different to *R. australis*.[10, 24] At the time of writing (October 1997) the invertebrate vector is unknown (not even if it is a tick) and the vertebrate reservoir is also unknown.

The disease is milder than QTT but is otherwise similar. The onset is sudden, with fever, headache and myalgia. A few days later a maculo-papular rash develops mainly on the trunk. An eschar is usually not detected (unlike QTT), and lymphadenopathy is less likely to occur than in QTT. Patients resolve their infection spontaneously in about one week and more quickly with appropriate antibiotics (doxycycline).

A similar SFG disease has been recognised in northern Tasmania, but no rickettsial isolate has yet been made[23, 25] and it is not possible to know if the condition is QTT, FISF, or another SFG disease.

Typhus Group rickettsiae

1. Epidemic Typhus (R. prowazekii) This disease does not occur in Australia, although it did arrive via convicts and immigrants in the early days of colonisation.[26] Probably due to Australian environmental conditions (ie; warm weather, ready access to water for washing), lousy people soon lost their lice and did not spread them to other people.

Recent migrants to Australia from parts of the world where the disease still occurs (eg; Russia, Peru, Ethiopia) may carry latent infection with *R. prowazekii*. This situation develops when a patient recovers from primary infection (without antibiotics), but the immune response is insufficient to eradicate the intracellular rickettsiae. At some stage in the future, often many years later, when the patient's immune system is less effective, reactivation of the rickettsiae can occur, giving rise to a milder form of typhus. This is known as Brill–Zinsser disease and has been reported in Australia.[27]

2. Murine (endemic) typhus (R. typhi) An Australian, Dr Frank Hone, a general practitioner and chief quarantine officer for South Australia, gave the world's first description of this disease in 1922.[28] He recognised an outbreak amongst waterside workers as being a form of typhus, albeit not classical, epidemic, louse-borne typhus, Since the original Adelaide outbreaks, sporadic cases or small epidemics have been reported in Queensland[29–31] and Western Australia,[32, 33] although it is very likely the disease is Australia-wide.

Rats and mice are the natural hosts and humans can be infected by one of two routes. The infected rodent flea excretes *R. typhi* in its feces and this material is either scratched into the bite site, effectively inoculating *R. typhi* into the skin, or the infected flea faeces are inhaled into the patient's lungs, establishing infection via the pulmonary route. Symptoms include fever, headache, nausea and vomiting, rash and respiratory symptoms. There may be an eschar. Chest X-ray indicates a pneumonitis. Laboratory tests show raised liver function tests, decreased platelets and decreased serum albumin (due to growth of rickettsiae in endothelial cells and leaking of capillaries)

CLINICAL FEATURES, DIFFERENTIAL DIAGNOSIS AND TREATMENT

Epidemiological features and a travel history can be an important first step in diagnosing rickettsial disease.

Scrub typhus

Scrub typhus is the most serious of the rickettsial diseases in Australia. Patients must have a residential or travel history relating to east Asia (Japan, Korea, China), south-east Asia (Thailand, Malaysia, Indonesia, Papua New Guinea) or northern tropical Australia (north of the Tropic of Capricorn), especially the north Queensland coast, but also the Top End of the Northern Territory (eg; Litchfield Park[13, 34, 35]). It is not yet clear if the disease occurs in the Kimberley region of Western Australia, but it is likely, based on the environment and on one case to date.[36] Infection occurs following the bite of an infected "chigger", the larval form of the mite, *Leptotrombidium* spp. (*L. deliense* in Australia). The incubation period is one to three weeks. In untreated cases the fever lasts for about two weeks and subsides slowly and the mortality rate is about 20%.

Patients with scrub typhus are likely to be very unwell, with general organ failure due to hemodynamic collapse. Destruction of endothelial cells by the rickettsiae lead to widespread leaking capillaries. During the World War II campaign in Papua New Guinea, Australian soldiers with scrub typhus were given higher priority for evacuation than those with malaria.[37] An eschar and rash may be present.

Most doctors faced with an acutely septicemic patient will treat with a penicillin or cephalosporin antibiotic. Unfortunately *O. tsutsugamushi* does not respond well to these drugs, due to the unusual chemical structure of its cell wall. The antibiotic of choice is a tetracycline (usually given IV). Co-trimoxazole is also without value in treating rickettsial disease.

Spotted fever group rickettsiae

QTT requires the patient to have been bitten by a tick while on the east coast of Australia. Often the patient will recall this event, about one to two weeks previously. Most ticks remain attached for several days while taking a large blood meal (female ticks only). The tick bite site often develops into an eschar (due to rickettsial proliferation in endothelial cells and infarction of the dependent tissue). The eschar may be in a "hard to find" spot (eg; in the hair, natal cleft, under the scrotum, under the breast, etc.) and requires diligent searching to locate it. The patient presents with fever, headache, myalgia and within a few days a maculo-papular rash develops. The differential diagnosis,

on clinical grounds, is measles, chickenpox, secondary syphilis, meningococcal septicemia, unspecified viral exanthem, epidemic polyarthritis (although joint symptoms are not a major feature of QTT), dengue, leptospirosis and other rickettsial diseases.

Laboratory tests show a relatively normal blood film often with leucopenia and thrombocytopenia. Liver enzymes are mildly deranged. At this stage of the illness the specific serological tests for rickettsial infection are usually still negative. The untreated patient takes one to two weeks to defervesence. Treatment with doxycycline or chloramphenicol usually brings a significant improvement in 48 hours.

FISF is similar to QTT but milder. To date, cases have only been detected from this particular Bass Strait island.

Typhus group rickettsiae

Murine typhus is extremely difficult to diagnose clinically as it usually presents as a mild "viral" illness, with maculopapular rash and pneumonitis. The epidemiological clue is that the patient has a lifestyle that exposes him to rats or mice. The fleas of these animals excrete *R. typhi* in their feces. The feces (and rickettsiae) then contaminate the flea bite site (in which case an eschar may form) or are inhaled as an aerosol (eg; when a workman demolishes an old rat-infested building), leading to a pneumonic illness, without eschar. The incubation period is approximately one to two weeks, the duration of illness between four days and four weeks (depending on antibiotic treatment) and the untreated fatality rate is low (up to 4%). Hone's original description of this illness (in Adelaide in 1922) was astute in linking it to vermin in the workplace.[28] Treatment is with doxycycline.

LABORATORY DIAGNOSIS OF RICKETTSIAL DISEASES

Currently, only serology is routinely available to specifically identify a rickettsial infection. However, during an acute illness (when correct diagnosis is crucial), serology is usually negative. Hence, current laboratory diagnosis is unsatisfactory and new tests are required.

The time honoured Weil–Felix serological test, using three strains of *Proteus* sp. (OX 2, OX 19, OX K) is based on the presence of cross-reacting antibodies induced by some strains of *Rickettsia* to some strains of *Proteus*. This test is neither sensitive nor specific and should be discarded in favour of specific serological tests incorporating genuine rickettsial antigens.

The "gold standard" rickettsial serological test is the indirect immunofluorescence (IF) test, in which heat-killed whole rickettsiae are fixed onto glass slides as micro-dots and reacted with patients' sera.[38] The presence of any antigen–antibody reaction is detected by fluorescein-labelled anti-human immunoglobulin (usually anti IgM plus anti IgG). The patient's serum is first screened at a dilution of 1 in 64 (or 1 in 128) against the three rickettsial groups (typhus group, spotted fever group and scrub typhus group). Any positive serum is then re-tested in a series of doubling dilutions to get a titration end-point. The end-point is the inverse of the highest dilution of patient serum that causes immunofluorescence of the rickettsiae on the slide.

Although this test is the gold standard, and currently available in several laboratories around Australia, it suffers from many technical problems. These include:

1. The difficulty that inexperienced laboratory staff have in differentiating specific IF from non-specific IF and background staining. A number of positive and negative controls must be included on each slide to assist in the reading. A laboratory without staff experience in IF should not undertake this assay for fear of producing erroneous results.
2. Variability in batches of rickettsial antigen and the "quality" of the slides.
3. Variability in quality and titre of different fluorescein labelled anti-human conjugates.
4. The need to have positive sera from patients with all three rickettsial infection groups (which are not always readily available).
5. The subjectivity associated with determining an end-point in the titration of a positive serum.

These problems all relate to experience in doing the test, which can be best overcome by having one designated laboratory in each state carrying out all rickettsial serology. In this way experience can be acquired in a few laboratories at least. Sera that cause problems at state reference laboratory levels could then be reviewed at an Australian reference laboratory.

Another way to solve the problem of IF serology is to abandon it in favor of more user-friendly technology, technology that can be readily mastered by the average microbiology/serology diagnostic laboratory. To date only one other type of test is currently available in Australia. It is a solid-phase enzyme immunoassay (EIA) ("Dip-S-Ticks") produced by a US company (Integrated Diagnostics) and imported into Australia by PanBio Pty Ltd in Brisbane. The author has had only limited experience with this test, but it is easy to use and requires no special expertise (unlike IF). Its main drawback is its cost, which is greater than the Medical Benefit Schedule rebate for a serological test. It is unlikely that any Australian laboratory will take up a test that loses money, especially in the current climate of pathology cost containment. Furthermore, the "Dip-S-Ticks" kits have not yet been verified as being suitable for diagnosing Australian rickettsial infections. At least one Australian rickettsia (*R. australis*) is significantly different (genetically) from its northern hemisphere cousins. The Australian scrub typhus rickettsia (*O. tsutsugamushi*) is also significantly different.[39]

There is clearly a need for a simple, reliable, rickettsial serological test for use in Australia. PanBio Pty Ltd is currently developing EIA kits for rickettsial diseases.

Given that serological tests for rickettsial disease only become positive later in the patient's illness, there is an urgent need for diagnostic tests that are sensitive and specific during the acute phase of the disease. An antigen detection test on serum and other body fluids is one possible approach, but the author is not aware of any current research in this area. Another approach is the detection of rickettsial nucleic acid in the circulating leucocytes of sick patients. The amplification of certain nucleotide sequences of specific rickettsial genes by means of the polymerase chain reaction (PCR) has been shown to work well in overseas laboratories.[40–42] This technology is being developed (and tested against rickettsial culture as a

gold standard) at the Australian Rickettsial Reference Laboratory (ARRL). Routine PCR for rickettsial diagnosis should be available by 1998, but the issue of funding for this type of test is a more demanding problem.

One special diagnostic test that can be done (but is really in the category of "research") is culture for rickettsiae. Blood needs to be taken during acute illness and prior to starting the patient on antibiotics. Because rickettsiae are obligate intracellular bacteria, this procedure involves inoculation of patient's blood (or the buffy coat derived therefrom) onto monolayers of susceptible eucaryotic cells. Vero cells (monkey kidney) and L929 (mouse fiboblasts) are currently used in the ARRL. Tissue culture is best done without antibiotics, as the rickettsiae are susceptible to many antibiotics *in vitro*. Hence great care must be taken to avoid environmental bacteria contaminating the tissue culture. If the blood specimen taken from the patient is inadvertently contaminated with skin bacteria, the problem can sometimes be overcome by inoculating the blood into a mouse using the intraperitoneal route. The contaminating bacteria (usually of low virulence) are destroyed in the mouse's reticuloendothelial system, and the rickettsiae grow in the mouse's liver, spleen and other organs. After 10–14 days in the mouse, or earlier if the mouse becomes unwell, the animal is sacrificed and the liver and spleen removed aseptically. A liver/spleen homogenate is then passaged into another mouse or inoculated into tissue culture to grow rickettsiae to higher concentration. This method only works if the rickettsial strain is mouse virulent, and many are not. The dangers of mouse inoculation with highly virulent microorganisms requires a secure laboratory and mouse holding facility.

CONCLUSION

A number of rickettsial diseases are currently recognised in Australia. These include: Scrub Typhus in tropical Australia; Queensland Tick Typhus on the eastern seaboard; Murine Typhus in various locations (eg; southwestern WA and parts of Queensland); Flinders Island Spotted Fever; and an unknown disease in northern Tasmania.

These vector-borne infections, like other re-emerging diseases, are becoming increasingly recognised in other parts of the world as well as Australia. There are no reliable data on the prevalence and incidence of rickettsial diseases in Australia.

The major issue for medical microbiologists and pathology laboratories is the timely diagnosis of rickettsial infections, which is currently less than satisfactory.

ACKNOWLEDGMENTS Funding for the Australian Rickettsial Reference Laboratory has been provided by The Geelong Hospital, The Geelong Regional Research Foundation and The Royal College of Pathologists of Australasia. The ARRL is most appreciative of the assistance provided by the CSIRO, Australian Animal Health Laboratory, Geelong, in allowing the mouse inoculation work to be conducted in its high security microbiological facility.

Address for correspondence: S.R.G., The Australian Rickettsial Reference Laboratory, The Geelong Hospital, Geelong, Vic 3220, Australia.

References

1. Stothard D, Fuerst P. Evolutionary analysis of the Spotted Fever and Typhus Groups of rickettsiae using 16S rRNA gene sequences. *System Appl Microbiol* 1995; 18: 52–61.
2. Weisburg W, Woese C, Dobson M, Weiss E. A common origin of rickettsiae and certain plant pathogens. *Science* 1985; 230: 556–8.
3. Margulis L. *Symbiosis in Cell Evolution*. San Francisco: W. H. Freeman, 1981.
4. Burnet F. The rickettsial diseases in Australia. *Med J Aust* 1942; 2(8): 129–34.
5. Marchette N. Ecological Relationships and Evolution of the Rickettsiae. Boca Raton: CRC Press, 1982.
6. Tamura A, Ohashi N, Urakami H, Miyamura S. Classification of *Rickettsia tsutsugamushi* in a new genus *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov. *Int J Syst Bacteriol* 1995; 45(3): 589–91.
7. Walker, D. (Personal communication).
8. Stothard D, Fuerst P. Evolutionary analysis of the spotted fever and typhus groups of rickettsiae using 16S rRNA gene sequences. *System Appl Microbiol* 1995; 18: 52–61.
9. Raoult D, Brouqui P. (Personal communication).
10. Stenos J, Ross B, Feng H-M, Crocquet-Valdes P, Walker D. Protein characterisation of Australian Spotted Fever Group rickettsiae and monoclonal antibody typing of *Rickettsia honei*. *J Clin Microbiol* 1997; 35: 261–3.
11. Carley J, Doherty R, Derrick E, Pope J, Emanuel M, Ross C. The investigation of fevers in north Queensland by mouse inoculation, with particular reference to scrub typhus. *Aust Ann Med* 1955; 4: 91–9.
12. Doherty R. A clinical study of scrub typhus in north Queensland. *Med J Aust* 1956; 2: 212–20.
13. Derrick E. The incidence and distribution of scrub typhus in north Queensland. *Aust Ann Med* 1961; 10: 256–67.
14. Brody J. A case of tick typhus in north Queensland. *Med J Aust* 1946; 1: 511–2
15. Andrews R, Bonnin J, Williams S. Tick typhus in north Queensland. *Med J Aust* 1946; 2: 253–8.
16. Streeten G, Cohen R, Gutteridge N, Wilmer N, Brown H, Smith D, *et al.* Tick typhus in south Queensland: report of three cases. *Med J Aust* 1948; 372–3.
17. Campbell R, Abeywickrema P, Fenton C. Queensland tick typhus in Sydney: a new endemic focus. *Med J Aust* 1979 (I); 350–1.
18. Dwyer B, Graves S, McDonald M, Yung A, Doherty R, McDonald J. Spotted fever in east Gippsland, Victoria: a previously unrecognised focus of rickettsial infection *Med J Aust* 1991; 154: 121–5.
19. Sexton D, Dwyer B, Kempt R, Graves S. Spotted fever group rickettsial infections in Australia. *Reviews Infect Dis* 1991; 13: 876–86.
20. Sexton D, Banks J, Graves S, Hughes K, Dwyer B. Prevalence of antibodies to spotted fever group rickettsiae in dogs from south eastern Australia. *Am J Trop Med Hyg* 1991; 45: 243–8.
21. Stewart R. Flinders Island Spotted Fever: a newly recognised endemic focus of tick typhus in Bass Strait. Part 1: Clinical and epidemiological features *Med J Aust* 1991; 154: 94–9.
22. Graves S, Dwyer B, McColl D, McDade J. Flinders Island Spotted Fever: a newly recognised endemic focus of tick typhus in Bass Strait. Part 2: Serological investigations. *Med J Aust* 1991; 154: 99–104.
23. Graves S, Stewart L, Stenos J, Stewart R, Schmidt E, Hudson S, *et al.* Spotted-Fever group rickettsial infection in south eastern Australia: isolation of rickettsiae. *Comp Immunol Microbiol Infect Dis* 1993; 16: 223–33.
24. Baird R, Stenos J, Stewart R, Hudson B, Lloyd M, Aiuto S, *et al.* Genetic variation in Australian Spotted Fever Group rickettsiae. *J Clin Microbiol* 1996; 34: 1526–30.
25. Chin R, Jennens I. Rickettsial spotted fever in Tasmania. *Med J Aust* 1995; 162: 669.
26. Cumpston J, McCallum F. The history of the intestinal infections (and typhus fever) in Australia, 1788–1923. Commonwealth of Australia, Department of Health, Service Publication No. 36, 1927.
27. Tonge J. Brill's disease (recrudescent epidemic typhus) in Australia. *Med J Aust* 1959; 919–21.
28. Hone F. A series of cases closely resembling typhus fever. *Med J Aust* 1922; 1(1): 1–13.
29. Wheatland F. A fever resembling a mild form of typhus fever. *Med J Aust* 1926; 1(10): 261–6.

30. Derrick E, Pope J. Murine typhus, mice, rats and fleas on the Darling Downs *Med J Aust* 1960; 24: 924–8.
31. Graves S, Banks J, Dwyer B, King G. A case of murine typhus in Queensland. *Med J Aust* 1992; 156: 650–1.
32. Saint E, Drummond A, Thorburn I. Murine typhus in Western Australia. *Med J Aust* 1954; 19: 731–7.
33. O'Connor L, Kelly H, Lubich J, Lindsey J, McCormish M. A cluster of murine typhus cases in Western Australia. *Med J Aust* 1996; 165: 24–6.
34. Currie B, O'Connor L, Dwyer B. A new focus of scrub typhus in tropical Australia. *Am J Trop Med Hyg* 1993; 49: 425–29.
35. Currie B, Lo D, Marks P, Lum G, Whelan P, Krause V. Fatal scrub typhus from Litchfield Park, Northern Territory. *Comm Dis Intel* 1996; 20: 420–1.
36. Quinlan M, Chappell T, Golledge C. Scrub typus in Western Australia. *Comm Dis Intel* 1993; 17: 570–1.
37. Walker A. *Clinical Problems of War.* Chapter 9 Typhus fever. Australian War Memorial. 1952.
38. Philip R, Casper E, Ormsbee R, Peacock M, Burgdorfer W. Microimmunofluorescence test for the serological study of Rocky Mountain Spotted fever and typhus. *J Clin Microbiol* 1976; 3: 51–61.
39. Dasch G. (Personal communication).
40. Carl M, Tibbs C, Dobson M, Paparello S, Dasch G. Diagnosis of acute typhus infection using the polymerase chain reaction. *J Infect Dis* 1990; 161: 791–3.
41. Murai K, Okayama A, Horinouchi H, Oshikawa T, Tachibana N, Tsubouchi H. Eradication of *Rickettsia tsutsugamushi* from patients' blood by chemotherapy as assessed by the polymerase chain reaction. *Am J Trop Med Hyg* 1995; 52: 325–7.
42. Furuya Y, Katayama T, Yoshida Y, Kaiho I. Specific amplification of *Rickettsia japonica* DNA from clinical specimens by PCR. *J Clin Microbiol* 1995; 33: 487–9.