

Laboratory diagnosis of human infections transmitted by ticks, fleas, mites and lice in Australia



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A wide range of human pathogens (viruses, bacteria, protozoa) are transmitted by ticks, fleas, mites and lice worldwide. Some of these infections occur in Australia¹, whereas others appear to be absent, although they may occur in returned travellers. The key to diagnosis is two-fold: recognition of the possibility of a vector-borne infection by the treating doctor and confirmation of the diagnosis in a diagnostic, microbiology laboratory. Laboratory diagnostic assays include culture (used rarely), nucleic acid amplification (used increasingly) and serology (used often).

In Australia the common vector-transmitted human infections (excluding those from mosquitos) are rickettsial infections, including Queensland Tick Typhus and Flinders Island Spotted Fever (from ticks), Murine Typhus and Cat Flea Typhus (from fleas) and Scrub Typhus (from mites). It is important for doctors to recognise these infections. While the patient may present with a ‘viral-like illness’, they actually have a bacterial infection that will respond quickly to treatment with an appropriate antibiotic, such as doxycycline².

Australian human infections transmitted by ticks, fleas, mites and lice

Viral infections

None recognised at present but it is very likely that they exist, just not yet discovered.

Bacterial infections

(1) The main bacterial infections are rickettsial³. These consist of two genera of bacteria (*Rickettsia* spp and *Orientia* spp), which have an obligate intracellular life cycle, and many of which live

in both invertebrate and vertebrate animals, moving between each with ease.

- (i) *Rickettsia australis* is found in at least two different Australian tick species (*Ixodes holocyclus*⁴ and *I. tasmani*) and various native mammals (e.g. bandicoots), causing Queensland Tick Typhus^{5,6} when they bite humans.
 - (ii) *Rickettsia honei*, found in the southern reptile tick *Bothriocroton hydrosauri*, causes Flinders Island Spotted Fever^{7–10}. A variant of this bacterium (*R. honei*, subspecies *marmionii*) found in the tick *Haemaphysalis novaguinea*, (and possibly others) causes Australian Spotted Fever¹¹.
 - (iii) *Rickettsia typhi*, found in the rodent flea, causes Murine Typhus^{12–14}, and enters humans when inoculated (by scratching the site of the flea bite) or inhaled (via dried flea faeces). The rickettsia is present in the flea faeces in very large amounts.
 - (iv) *Rickettsia felis*^{15,16} is found in the cat flea (which is also found on dogs). The rickettsia is present in the flea faeces.
 - (v) *Orientia tsutsugamushi*, found in Australia only in the tropical mite *Leptotrombidium deliense*, causes scrub typhus when the larval form of the mite (known as a ‘chigger’) bites humans.
- (2) Q Fever¹⁷, caused by the bacterium *Coxiella burnetii*¹⁸, can occur following the bite of an infected tick, although the most usual route of transmission is via inhalation of the dried products of parturition of an infected vertebrate animal, such as a cow, sheep or goat.
- (3) *Bartonella* spp. comprise two human-pathogenic species, *B. quintana*^{19,20} and *B. henselae*²¹, arise mainly from cat bites and scratches. While it is thought that some cases may be caused by the bite of an invertebrate vector, this has not yet been confirmed in Australia.

Protozoal infections

None recognised in Australia at present although there has been an enigmatic human infection with *Babesia microti*²².

Human infections transmitted by ticks, fleas, mites and lice seen in overseas travellers in Australia

- (1) **African Tick Bite Fever**, seen in persons returning from Africa, often having visited game parks and being bitten by an infected tick. The bacterium is *Rickettsia africae*²⁵.
- (2) **Mediterranean Spotted Fever**, seen mainly in persons returning from the Indian sub-continent. This tick-transmitted infection is caused by *Rickettsia conorii*^{24,25}.
- (3) **Murine Typhus**, caused by *Rickettsia typhi*, has been seen in travellers from Indonesia and elsewhere, following exposure to rodent fleas²⁶.
- (4) **Scrub Typhus**, caused by *Orientia tsutsugamusbi*, is seen in travellers from various regions of Asia and Oceania²⁶, following the bite of an infected mite and by *O. chuto*²⁷ in a case from Dubai. There is also evidence of scrub typhus being present in Africa and South America.
- (5) **Ehrlichiosis**, caused by *Ehrlichia chaffeensis*, is seen following the bite from an infected tick in the USA²⁸.
- (6) **Lyme Disease**, caused by the bite of an infected tick in Europe or north America, is caused by a Spirochaete bacterium, *Borrelia burgdorferi* (and related species)²⁹. This disease does not appear to be endemic in Australia and all confirmed cases in Australia to date have been in returned travellers.

Doctors in Australia should remember that malaria (a protozoal infection) and dengue (a viral infection) are more common infections in returned travellers than any of the above. They are both mosquito-transmitted infections and are still major problems.

Microbiology laboratory techniques used for the diagnosis of human infections transmitted by ticks, fleas, mites and lice

These can be divided into three groups:

- (1) culture
- (2) nucleic acid amplification (PCR)
- (3) serology

The Australian Rickettsial Reference Laboratory (ARRL), based at University Hospital Geelong, Geelong, Victoria, uses all these modalities, depending on the actual infection being considered by the treating doctor with possible confirmation by the laboratory.

(1) Culture

Most of these specialised bacteria are obligate intracellular bacteria and must be grown in cell cultures (not agar) using cell lines that support the growth of the bacterium being sought in the patient specimen. Routine lines include mammalian (VERO, I929, DH82), amphibian (XTC2) and tick (IE6). Growth is often slow and can take many weeks, during which time the cultures need to be monitored, looking for a cytopathogenic effect (CPE) on the monolayer, or detection by nucleic amplification or antigen detection. The cell culture medium above the cell monolayer must be changed fortnightly and all work is done aseptically without antibiotics as the bacteria being sought may be susceptible to the antibiotic used. Cultures are kept for a total of six weeks. All the *Rickettsia spp*, *Orientia spp* and *C. burnetii* are isolated in this way³⁰. PC-3 (BSL-3) laboratory conditions are needed to amplify these bacteria but not

for the initial detection in a diagnostic modality. Culture is not useful in patient management as it is far too slow.

(2) Nucleic amplification

Real-time polymerase chain reactions (qPCR) are used routinely for detecting rickettsial³¹ and *C. burnetii*³² DNA in patient samples. These assays are performed on either blood in EDTA (containing the circulating leucocytes in which the bacteria are located), serum or on a biopsy of an eschar (invertebrate bite site), a rash or an operative specimen (e.g. a cardiac valve from a patient with Q fever endocarditis).

Once microbial DNA has been detected, a specific microbial gene may be amplified by conventional PCR and the product sequenced to compare it with known species of microbes. 100% homology is not always obtained as there may be polymorphisms (genetic variants) seen in some genes. Sometimes these variations are so great as to define the bacterium as a new species. Most commonly, conventional amplification cannot occur as the microbial DNA is in limited amounts and without an isolate the amplification and subsequent sequencing may be impossible to perform.

(3) Serology

This is the main diagnostic modality for this group of infections³³. However interpretation is fraught with difficulties:

- (i) The patient serum sample may have been taken very early in the illness before the patient's immune system has had time to produce antibodies. The serology will yield a false-negative result. A 2nd serum taken a few days/weeks later is extremely valuable diagnostically as it may now be positive for antibodies to the microbe causing the infection. This is a 'sero-conversion' and good evidence of recent infection by the microbe to which the patient has sero-converted. Alternatively, a significant rise (usually a 4-fold rise) in antibody concentration (titre) between the 1st and 2nd sera is also good evidence of recent infection.
- (ii) Just because a patient has antibodies in their serum to a microbe tested for in the laboratory, it does not prove that their current illness is due to infection with that microbe. Antibodies can last for years in the patient's blood and while their presence is a marker of exposure to the microbe, it is not necessarily recent exposure. It may have been from years earlier.
- (iii) A positive serology result may be a false-positive due to cross-reactivity between the patient's antibodies and a related (or even unrelated) microbial antigen being used in the laboratory assay. Using the appropriate serum screening dilution by the laboratory is crucial to prevent reporting cross-reactions as genuine positive serology results.

The ARRL uses a 1/128 serum screening dilution for rickettsial serology, a 1/25 dilution for Q fever serology, 1/12 for Bartonella IgM and 1/64 for Bartonella IgG and 1/64 for Babesia serology screening.

These serum dilutions are based on the serology kit manufacturers' recommendations and the characteristics of the local Australian population being tested, as obtained following extensive use of

the assays. Generally speaking, the higher the antibody titre, the more likely it is to be a genuine positive. The laboratory interpretation of the result is always important to consider.

There are many laboratory modalities for detecting antibodies (in the patient's serum) reacting with microbial antigens (in the laboratory). Enzyme immunoassay (EIA) is commonly used: complement fixation (CFT) was widely used in the past but not much now, etc. The ARRL uses mainly microimmunofluorescence (MIF) as MIF has the best sensitivity (ability to detect a genuine case) and specificity (ability to not incorrectly detect a non-case) for most of the infections transmitted by ticks, fleas, mites and lice. The laboratory should always be part of an external quality assurance program to ensure that its results are consistent with its peers. Incorrect results can lead to erroneous conclusions and inadequate management of the patient.

Conflicts of interest

The Australian Rickettsial Reference Laboratory is a human pathology (microbiology) diagnostic laboratory and Dr Stephen Graves is the Approved Pathology Provider (APP) who receives income from Medicare on a fee-for-service basis for diagnostic testing on referred patient specimens. Dr John Stenos receives income as an employee of the laboratory.

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Biographies

Dr John Stenos undertook his post-doctoral studies into rickettsiae in the USA and then returned to Australia to take up appointment as the senior scientist in charge of the Australian Rickettsial Reference Laboratory, based at University Hospital Geelong, Victoria, where he has remained for the past 20 years. He is now the Research Director of the laboratory and responsible for the 'WHO Collaborating Centre for Reference and Research on Rickettsioses'.

The biography for **Dr Stephen R Graves** is on page 181.