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Volume 39 Number 4 November 2018

Tick-borne pathogens and diseases

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Cover image: Female Amblyomma triguttatum *(kangaroo tick) preparing to feed from a human. Photo credit: Peter Irwin.*

Dena Lyras

President of ASM

I will begin this Vertical Transmission by highlighting the links between the ASM National Executive and our State branches, and the steps we are taking to strengthen interactions between these important ASM groups. I recently spent a few days visiting with our South Australian/Northern Territory branch representatives. We discussed matters that are important to local members, and new initiatives that the national executive is implementing. I am visiting our Queensland branch in November and our Western Australian branch in December for similar discussions, and we continue to hold National-State tele-meetings four times a year. At this important time of change in our discipline ASM wants to provide members with the strongest possible support, and we welcome new ideas about how ASM can serve you better.

While in Adelaide I also met with members of the local organising committee for our national meeting in Adelaide in 2019. The conference venue is magnificent and excellent for our meeting, and the scientific program is equally excellent. The meeting will be held from 30 June to 3 July 2019 – please add the dates to your diary.

I am always impressed by the generosity of our members and how much time and effort they expend in promoting Microbiology. On 4 July I visited Science Alive! at the Adelaide showgrounds. The Microbiology stand, which was sponsored by ASM, was fantastic and drew tremendous public interest – children had great fun with some very imaginative activities. I include a few photos below of the Microbiology stand. I am delighted to have attended, it was a terrific event and I would like to personally thank all of our members who give their time to organise and participate in events such as this one.

Finally, I draw your attention to the AusMe (Australian Microbial Ecology) conference being held 11–13 February 2019 in Western Australia. AusMe is an ASM initiative, and the first meeting in 2017 was a wonderful success. The meeting next year is sure to be even better than the first one, so please do attend if you have an interest in any aspect of microbial ecology.

Please visit our website: www.theasm.org.au to access information regarding upcoming meetings and awards. Do look at all of our new awards, and encourage your colleagues to apply for them, or nominate others; most are due in late March. We have increased the number and range of our awards, and are committed to inclusion and promoting diversity; our awards embrace these ideals.

You may also like to follow, and contribute to ASM on Twitter, @AUSSOCMIC, or on Facebook to make sure you keep up with the latest news, trends and developments in Microbiology in Australia and around the world.

Tick-borne pathogens and diseases

Stephen R Graves

Welcome to this edition of *Microbiology Australia* in which we examine the second most dangerous ectoparasite for humans, the tick (the most dangerous being without doubt the mosquito!), for the pathogens it carries and the diseases it can cause.

Ticks of many different species, in all parts of the world, transmit many different infections to humans and various vertebrate animal species, including those that are of great importance to Man (e.g. cattle, sheep, goats, dogs and cats). Some tick-transmitted diseases, such as Lyme Disease, have been only recently recognised (late 1970s), while others, such as Rocky Mountain Spotted Fever in the Americas, have been known for over 100 years. Some have a very Australian focus, such as Q Fever and Flinders Island Spotted Fever, first recognised in Australia but now known to be virtually worldwide in their distribution.

Of the 11 articles included in this edition of *Microbiology Australia*, seven are about the Australian tick situation and four about overseas ticks (Europe, Asia and the USA) and cover both human and animal pathogens carried by ticks.

Overseas, the most important tick-transmitted infections are probably Lyme Disease (Abdallah *et al.*), tick-borne encephalitis (TBE) (Dobler) and rickettsiae (Robinson *et al.*). In Australia we appear to not have Lyme Disease *Borrelia spp* or TBE virus in our ticks. However, we do have protozoal infections (theileriosis) in cattle (Jenkins), bacterial infections, including *Rickettsia spp* and *Coxiella spp* in humans and animals (Irwin *et al.*, Oskam *et al.*) and possibly human viral infections also (O'Brien *et al.*). The field is still in its infancy and there remains a lot to be learned.

An understanding of the ticks present in Australia, both the endemic and exotic species, is crucial basic knowledge in tackling the problems of tick-transmitted illness (Barker and Barker).

There is also a plethora of non-infectious illness associated with tick bites (Beaman), which, in Australia, may well exceed the cases of infectious disease. This has been of recent concern by the parliament of the Commonwealth of Australia.

Diagnosing tick-transmitted infections requires both the treating doctor to think of the possibility of tick-bite in their patient and a microbiology diagnostic laboratory with the expertise to confirm or refute the tentative diagnosis. Newer lab techniques for diagnosing rickettsial infections in the USA (Kato) and Australian tick-transmitted microbes (Stenos and Graves) are reported.

As you browse through this edition of *Microbiology Australia*, I trust that you will come to appreciate that it is not only mosquitos that are the invertebrate curse of Mankind, but that the non-flying, biting tick is also a biological force with which to be reckoned!

Biography

Stephen R Graves is a medical microbiologist. He obtained his BSc (honours in Microbiology) from the University of WA, when Neville Stanley was the Professor of Microbiology. He then completed his PhD in the field of leptospirosis with Solly Faine at Monash University and later his medical degree at The University of Melbourne. After post-doctoral research on *Treponema pallidum* at the University of Minnesota with Russ Johnson, he became a Lecturer/Senior Lecturer in Microbiology at Monash University. He established the Australian Rickettsial Reference Laboratory in 1996 while Director of Microbiology at The Geelong Hospital, Geelong, Victoria. This not-for-profit boutique, diagnostic and research laboratory specialises in *Rickettsia spp* and other ectoparasite-transmitted microbes, including Q Fever (*Coxiella burnetii*). As the Approved Pathology Provider (APP) for the laboratory, and a Fellow of the Royal College of Pathologists of Australasia, his laboratory receives Medicare funding that is then applied to solving research questions. As one of the original Fellows of the Australian Society for Microbiology, he considers teaching and mentoring younger microbiologists to be his crucial role at this stage of his career.

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 Australia[1], 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 doxycycline[2].

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 rickettsial[3]. 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 Fever[11].

(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 Fever[17], 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*[23].
(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 fleas[26].
(4) **Scrub Typhus**, caused by *Orientia tsutsugamushi*, is seen in travellers from various regions of Asia and Oceania[26], 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 USA[28].
(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, L929, 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 way[30]. 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 rickettsial[31] 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 infections[33]. 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.

Could Australian ticks harbour emerging viral pathogens?



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Tick-borne viruses contribute significantly to the disease burden in Europe, Asia and the US. Historically, some of the most well-known viruses from this group include the human pathogens, tick-borne encephalitis virus and Crimean-Congo haemorrhagic fever virus. More recently multiple emerging tick-borne viruses have been associated with severe disease in humans with Bourbon virus and Heartland virus isolated from patients in the US and severe fever with thrombocytopenia syndrome virus reported from China, Japan, and South Korea. Such examples highlight the need for broader approaches to survey arthropod pathogens, to encompass not only known but novel pathogens circulating in Australian tick populations.

There are currently 70 recognised species of ticks in Australia, with 22 reported infesting on humans[1,2]. *Ixodes holocyclus* is the most significant tick in terms of human and animal health in Australia, causing a myriad of problems from toxin-induced paralysis, mostly in domestic animals[3], to allergic reactions in humans[4]. The feeding behaviour of *I. holocyclus* as a three-host tick, makes it a successful vector for Rickettsial pathogens that cause Queensland tick typhus, Flinders Island spotted fever, and Australian spotted fever[5]. Research into 'Debilitating Symptom Complexes Attributed to Ticks' has led to the discovery of several new bacterial species in *I. holocyclus* and other native Australian ticks from families containing known human pathogens[6,7]. Despite this, little is known of the viruses carried by Australian terrestrial ticks, with most isolations from seabird ticks collected on offshore islands[8–10].

Viruses isolated from Australian seabird ticks show similarities with tick-borne pathogens in other parts of the world (Table 1). The flavivirus Gadgets Gully virus (GGYV), first isolated from *Ixodes uriae* ticks in 1985, clusters with the mammalian tick-borne flavivirus group including human pathogens Powassan virus and Tick-borne encephalitis virus[9]. While GGYV has not been associated with disease in humans, a serological survey demonstrated evidence for infection in inhabitants of a research station on Macquarie Island[11]. Similarly, a second flavivirus, Saumarez Reef virus (SREV), was isolated from *Ixodes eudyptidis* and *Ornithodoros capensis* ticks taken from nests of sooty terns (*Onychoprion fuscatus*) and silver gulls (*Chroicocephalus novaehollandiae*) after reports of illness and tick bites by technicians servicing weather stations on Saumarez reef (~330 km off the Central Queensland coast)[10]. Although no serological evidence of SREV infection was gathered, it was noted that the closely

Table 1. Virus isolates from ticks collected in Australia and New Zealand.

Virus designation	Associated tick species	Region isolated	Virus genus	Closest relative (% amino acid[A])	Available sequence
Family: *Orthomyxoviridae*					
Upolu virus	*O. capensis*	Upolu cay (GBR)	Thogotovirus	Aransas bay virus (93% PB1)	Complete KC506156 – 61
Johnston Atoll virus	*O. capensis*	Johnson Atoll (NZ), Qld	Quarjavirus	Tjuloc virus (84% PB1)	Partial FJ861696 – 7
Family: *Phenuiviridae*					
Albatross Island virus	*I. eudyptidis*	Albatross Island (Tas.)	Phlebovirus	Heartland virus (67% RdRP)	Complete KM198925 – 7
Hunter Island group virus (HIGV)	*I. eudyptidis*	Albatross Island (Tas.)	Phlebovirus	Albatross Island virus (99% RdRP)	Complete KF848980 – 2
Precarious Point virus (PPV)	*I. uriae*	Macquarie Island	Phlebovirus	Murre virus (81% RdRP)	Complete HM566179 – 81
Catch-me-Cave virus (CMCV)	*I. uriae*	Macquarie Island	Phlebovirus	Precarious point virus (98% nucleoprotein, partial)	Partial EU274384
Family: *Nairoviridae*					
Finch Creek virus (FCV)	*I. uriae*	Macquarie Island	Orthonairovirus	Taggert virus (99% RdRP)	Partial EU267169
Taggert virus (TAGV)	*I. uriae*	Macquarie Island	Orthonairovirus	Avalon virus (80% RdRP)	Complete KU925491 – 3
Vinegar Hill virus (VINHV, CSIRO1499)	*A. robertsi*	Gatton (Qld)	Orthonairovirus	Dera Ghazi Khan orthonairovirus (97% RdRP)	Complete MF17881 – 3
Family: *Reoviridae*					
Nugget virus (NUGV)	*I. uriae*	Macquarie Island	Orbivirus	Great Island virus (serological data only)	None
Sandy Bay virus (SBV)	*I. uriae*	Macquarie Island	Orbivirus	Great Island virus (71% VP5)	Partial EU685329 – 33
Family: *Flaviviridae*					
Gadgets Gully virus (GGYV)	*I. uriae*	Macquarie Island	Flavivirus	Powassan virus (72% polyprotein)	Complete DQ235145
Samaurez Reef virus (SREV)	*I. eudyptidis, O. capensis*	Coral Sea Islands, Tasmania, Macquarie Island	Flavivirus	Tyuleniy virus (73% polyprotein)	Complete DQ235150
Unknown					
Lake Clarendon virus (CS704)	*A. robertsi*	Gatton (Qld)	Unknown	–	None
Little Diamond Island virus group (CSIRO 1759-1762)	*I. kohlsi*	Diamond Island (Tas.)	Unknown	–	None

[A]Amino acid similarity to closest relative by Blastx analysis. RdRP, RNA-dependent RNA polymerase; TAS, Tasmania; QLD, Queensland; GBR, Great Barrier Reef; PB1, polymerase basic subunit 1.

related Tyuleniy virus showed a 6% seroconversion rate in inhabitants of the Commodore Islands[12].

In 2002, a disease outbreak in shy albatross (*Thalassarche cauta*) on Albatross Island (129 km north-west of Burnie, Tasmania) led to the isolation of Hunter Island Group virus. Next-generation sequencing identified the virus as a phlebovirus related to human pathogens severe fever with thrombocytopenia syndrome virus and Heartland virus[13]. Following this, sequencing of Albatross Island virus (ABIV), an isolate from *I. eudyptidis* ticks from the same location in 1983, showed that the two viruses were the same species[14].

More recently, a novel orbivirus and two bunyaviruses were reported from *I. uriae* collected during a survey of ticks on penguins at Macquarie Island[15]. The two bunyaviruses, tentatively named Catch-me-Cave virus and Finch Creek virus, show similarities to previously isolated Precarious Point and Taggert viruses[9,16]. However, full genome sequencing is required to confirm whether these viruses are contemporary strains of known viruses or new species. During this study, GGYV was also re-isolated from *I. uriae* suggesting that it is probable that the viruses first described between 1975 and 1985 are still circulating in the tick and bird populations on Macquarie Island[15].

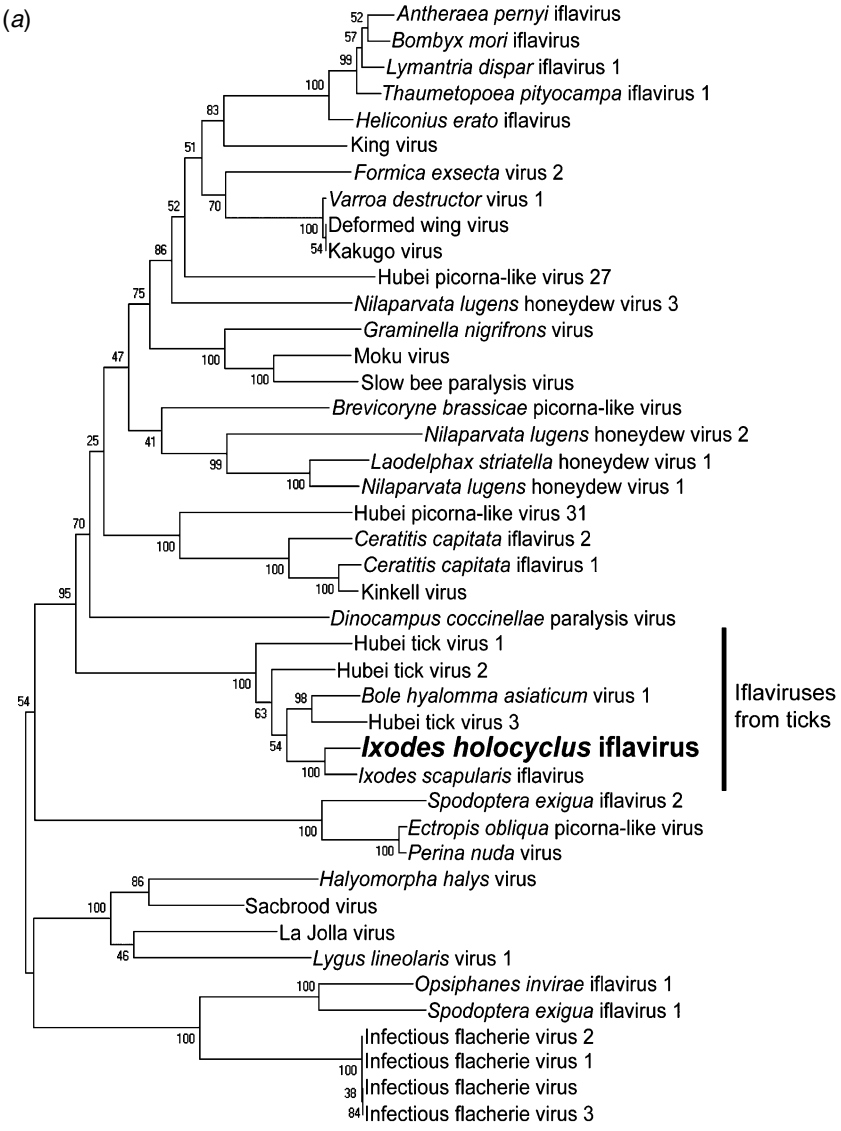
In contrast to the panel of viruses isolated from Australian seabird ticks, attempts to screen terrestrial tick populations have yielded only three viruses, all from *Argas robertsi*, a tick also found in Asia. Lake Clarendon virus (CSIRO704) was isolated from *A. robertsi* ticks collected at the Lake Clarendon cattle egret colony in Gatton, Queensland in 1980[17]. The virus was not identified and showed no relatedness to known arboviruses by serum-neutralisation and complement-fixation tests. Neutralising antibodies in cattle egret sera suggested the virus was able to infect the birds with no apparent disease[17]. To our knowledge, this isolate remains unidentified. A second virus was isolated from *A. robertsi* ticks at the same colony following reports of death in nestling chicks. This virus, originally designated CSIRO1499, was shown to have no serological similarity to Lake Clarendon virus. Experimental infections subsequently showed that the isolate was able to infect and cause mortality in birds[11]. More recently, the full genome sequence of this virus revealed it to be closely related to Dera Ghazi Khan virus (family *Nairoviridae*) and the name Vinegar Hill virus (VINHV) was proposed[8]. Serological surveys found antibodies to VINHV in 3.4% of avian samples and 1% of human serum samples tested[11]. Finally, a virus isolated from *A. robertsi* in the Northern Territory (NT15470) was suggested to be a member of the Dera Ghazi Khan genogroup, thought to be either a strain of Kao Shuan virus (KSV) or a close relative[18]. As no genome sequence is available for this isolate, it remains to be seen whether it is in fact KSV or another isolate of VINHV.

To our knowledge, there have been no virus isolations from an Australian terrestrial hard tick species. Next generation sequencing performed on *I. holocyclus* ticks collected in New South Wales and Queensland have allowed some insights into their potential virome[19]. Recently we published the genome of a novel iflavirus assembled from the transcriptome of *I. holocyclus* salivary glands (Figure 1*a*)[20]. Interestingly, related iflaviruses have also been identified in the *Ixodes scapularis* cell line (ISE6) and in ticks collected from China[21,22]. Several bunyavirus nucleocapsid sequences have also been identified in *I. holocyclus* transcriptome sequence data but whether these sequences belong to a virus or an integration in the host genome is yet to be elucidated (Figure 1*b*). This data indicates that the virome of Australian terrestrial ticks may mirror that of terrestrial ticks found in the northern hemisphere.

We have developed a broad-spectrum screening system that detects viral isolates in cell cultures inoculated with mosquito homogenates. The MAVRIC system (Monoclonal antibodies against viral RNA intermediates in cells) targets long (>30 bp) double-stranded RNA molecules, produced during replication of viruses, in a sequence-independent manner[23]. MAVRIC led to the isolation of at least 9 new viral species from 7 different families allowing the identification of numerous viruses previously not known to exist in Australian mosquito populations[24]. Based on the success of MAVRIC in mosquito screening, we aim to apply this system to screen Australian terrestrial ticks. One barrier is the lack of suitable cell lines derived from Australian tick species and their hosts. While the use of vertebrate cell cultures (generally BHK-21 and Vero cell lines) has proven successful for the isolation of mosquito and seabird tick viruses in Australia[9,25], alternative cell lines reflecting the common hosts of terrestrial ticks (i.e. marsupials), may need to be considered for tick virus isolation on the mainland. Furthermore, while mosquito cell culture has been well established, tick cell culture has proven more difficult requiring a complex mix of vitamins and minerals which must be formulated in-house[26].

Ixodes holocyclus iflavirus was unable to replicate in the *I. scapularis* cell line (ISE6), but appeared to replicate in the host tick raising the question of the suitability of cell lines derived from ticks of the northern hemisphere to isolate Australian tick-borne viruses[20,27]. Phylogenetic analyses have demonstrated that *I. holocyclus* and *I. uriae* are divergent from 'other' *Ixodes*

(*a*)



(*b*)

0.50

Sequence name	Length (nt)	Protein identity	Closest relative (% amino acid)	Sequencing technology
***Ixodes holocyclus* iflavirus (IhIV)**	9260	Complete polyprotein	*Ixodes scapularis* iflavirus (73%)	Illumina, 454
Phlebovirus-like 1	711	Nucleoprotein	Sandfly fever Sicilian virus (31%)	Illumina
Phlebovirus-like 1	1003	Non-structural protein	Saint Floris virus (32%)	Illumina
Phlebovirus-like 3	269	Nucleoprotein	Mukawa virus (36%)	Illumina
Rhabdovirus-like	337	RNA-dependent RNA polymerase	Chandipura virus (39%)	Illumina

Figure 1. (*a*) Evolutionary relationship of *Ixodes holocyclus* iflavirus within the family *Iflaviridae.* Mid-point rooted maximum likelihood phylogenetic tree was constructed based on an alignment of peptidase and RdRP proteins corresponding to position 2036–2944 of the IhIV polyprotein. (*b*) Summary of virus sequences identified in *I. holocyclus* by transcriptome sequencing[19].

species[28]. Preliminary analysis from our group has suggested that some of the vertebrate-infecting viruses of *I. uriae* and *I. eudyptidis* are able to replicate in the ISE6 cell line (Figure 2), however this remains to be demonstrated for viruses of terrestrial ticks. Bell-Sakyi and Attoui recently discussed the role of tick cell culture in virus discovery, particularly in relation to tick-specific viruses[29]. In this instance, the development of cell lines from Australian native ticks may be necessary.

Finally, the risk that Australian seabird-associated tick viruses pose to human health should be considered. A study undertaken to investigate the health risk posed to residents and tourists on the islands in the Great Barrier Reef and Coral Sea by seabird-

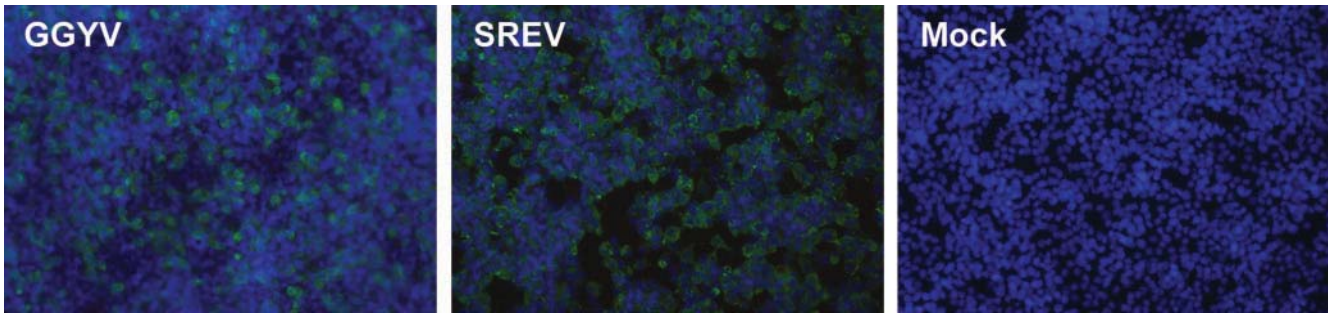


Figure 2. Anti-flavivirus E protein (green) staining in *Ixodes scapularis* (ISE6) cells infected with Australian tick-borne flaviviruses Gadgets Gully virus (GGYV) and Saumarez Reef virus (SREV).

associated arboviruses identified two isolates in *O. capensis* collected on Masthead Island, which appeared closely related to VINHV[30]. This may demonstrate the potential for incursion of seabird-associated viruses to the mainland and vice versa. Serological surveys undertaken after the initial isolation of ABIV identified a black noddy (*Anous minutus*) from Heron Island off the coast of Queensland with neutralising antibodies to the virus[30]. Finally, SREV was isolated from *O. capensis* ticks found on Saumarez reef off the coast of Queensland and 2000 km away in Tasmania[10].

While our knowledge of the viruses harboured by Australian ticks is still limited, the data thus far suggests that our tick viromes may mirror those seen in the northern hemisphere. Next generation sequencing of terrestrial ticks performed by our group and others will greatly contribute to the characterisation of tick-viruses in Australia. In this context, a recent deep sequencing study of Australian ticks by Eddie Holmes' group at the University of Sydney, has identified a plethora of novel viral sequences that will provide a useful reference for further studies (unpublished data available online: https://www.biorxiv.org/content/early/2018/08/07/386573). Complementary to next generation sequencing, a system for efficient isolation of newly discovered viruses will allow for complete characterisation. Finally, comprehensive characterisation of the current tick-borne virus isolates held in archive is required to avoid re-discovery of these viruses in future studies.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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Biographies

Caitlin O'Brien is a final year PhD student focusing on the optimisation of novel culture-based methods for virus discovery in Australian arthropods. Her most recent work includes the identification and characterisation of novel virus species in Australian mosquitoes and ticks. Her six years in research have led to the discovery of novel biological control mechanisms for pathogenic arboviruses, development of monoclonal antibodies to flaviviruses and insect specific viruses and the establishment of the ISE6 cell line for use in Australian tick virus work.

Professor Roy Hall is a specialist in vector-borne virology at the University of Queensland. His research explores emerging arthropod-borne viruses with a focus on their pathogenesis and the development of novel vaccine and diagnostic platforms. The work of his group has led to the design and development of novel diagnostic assays and vaccine candidates and the discovery of several new mosquito- and tick-borne viruses.

Professor Ala Lew-Tabor is a molecular biologist and 'research focused' academic at the University of Queensland. Research highlights include the developing novel vaccines and molecular assays for ticks and tick-borne diseases, respectively. Her group produces translational outputs for cattle and pets including patented vaccines for commercial uptake (cattle tick and paralysis tick), and laboratory assays for government-based diagnostic facilities.

Tick-borne encephalitis and its global importance

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Tick-borne encephalitis (TBE) is the most important tick-transmitted human viral disease in Europe and Asia with up to 10 000 human cases annually. The etiologic agents of TBE are the three subtypes of tick-borne encephalitis virus (TBEV), a member of the genus Flavivirus in the family *Flaviviridae*. The Far-Eastern subtype and the Siberian subtype are both mainly transmitted by *Ixodes persulcatus*; the European subtype is mainly transmitted by *Ixodes ricinus*. Besides tick bite, TBEV can be transmitted by unpasteurised milk from goat, sheep and cattle during the viremic phase of infection by the oral route of infection (alimentary form of TBE). There is no treatment for TBE available, but there are effective and well tolerated vaccines against TBE, which are recommended for people living or travelling to endemic countries with a risk of infection.

Tick-borne encephalitis is the most important tick-borne viral disease in Europe and Asia, but regarding numbers of patients it is the most important tick-borne viral disease in the world[1]. The disease is endemic exclusively in Europe and Asia. It is caused by a group of viruses of the genus *Flavivirus* in the family *Flaviviridae*. Three subtypes, the European, the Siberian and the Far-Eastern subtype can be distinguished by molecular methods and they are transmitted by different vectors and cause a different clinical picture of disease[2] (Figure 1). An additional two other subtypes, the Baikalian (TBEV-Bkl) and the Himalayan subtype (TBEV-Him) have been described recently[3,4].

The five subtypes of TBEV have a different, but partially overlapping geographical distribution and biological transmission cycles involving different tick species and rodents[5]. The European subtype (TBEV-EU) is geographically distributed mainly in Europe[6]. However, some TBEV-EU strains have been isolated and characterised in Siberia (Lake Baikal region) and also in South Korea. In Europe the most important vector of TBEV is *I. ricinus*. Goats, sheep and cattle shed TBEV into the milk during the viremic phase of infection without showing signs of disease. Infection with TBEV by the alimentary route from drinking unpasteurised virus containing milk or dairy products is a common way of infection in some European countries and occasionally also occurs in countries with a highly industrialised agriculture, as recently reported in Germany and Austria[7,8].

The Siberian subtype is geographically distributed mainly in Russia east of the Ural Mountains, but it is also found in the Baltic countries and in localised places in Finland[9]. *I. persulcatus*, the Taiga tick, mainly transmits this subtype. The Far-Eastern subtype of TBEV is geographically distributed mainly in the far-eastern part of Russia and the northern parts of China. TBEV-FE is also found on the northern Japanese island of Hokkaido, where *I. ovatus* was identified as its vector[10]. The Baikalian subtype was detected at the Lake Baikal region in different *Myodes* spp. and in *I. persulcatus*[3]. The Himalayan subtype was identified two times from respiratory fluid of *Marmota himalayana* from the Qinghai-Tibet Plateau in China[4].

Earlier serological data suggest that only about 30% of the TBEV infections present with clinical symptoms, ranging from febrile ('flu-like') disease to meningitis, encephalitis and encephalomyelitis[1]. In milk-borne and diary product-borne infections the manifestation index seems to be much higher ranging in many outbreaks to up to 100% of exposed individuals[8].

The incubation period of TBE ranges from 5 to 14 days. In many of the human cases caused by the TBEV-EU a biphasic course is reported. In the first phase of the disease, symptoms of a general infection ('flu-like') are seen. Many patients report elevated temperatures, headache, muscle ache, fatigue and also gastrointestinal symptoms or symptoms of the respiratory tract. During this phase of disease, the TBE virus can be detected and isolated from the blood of the patients. The symptoms reflect the virus replication in the different organs of the body. The first phase lasts from four to seven days[11].

After a symptomless phase of 4–7 days, symptoms of general infection of the central nervous system (CNS) may follow in

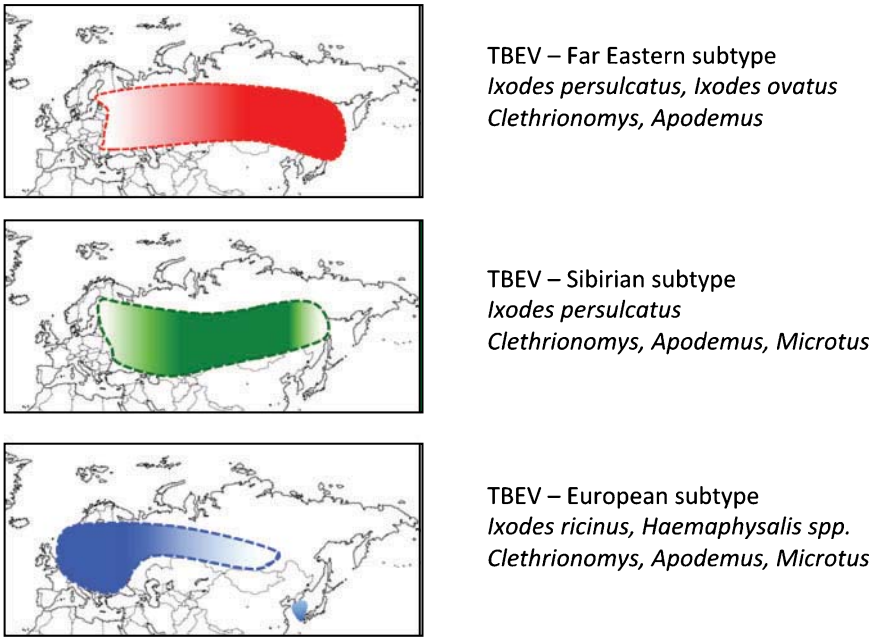

Figure 1. Geographical distribution of the three main TBE virus subtypes including main vectors and main vertebrate hosts.

~30% of patients. The CNS symptoms may range from headache and mild meningitis to severe encephalomyelitis with a fatal outcome. Generally three clinical forms of the CNS disease of TBE infection can be distinguished[11]:

- Meningitis: fever, headache, nuchal rigidity
- Encephalitis: change of consciousness, stupor, coma, epileptic attacks, disorientation, dysarthria
- Myelitis: flaccid paralysis of different muscles; mainly muscles of the upper musculo-skeletal system

The Siberian and the Far-Eastern forms of TBE infections follow a more severe clinical course. These infections mostly show a monophasic form with more severe encephalitic and myelitic features. The fatality rates range from 3% to 20%. In patients with the Siberian subtype virus infection a chronic form of TBE has been described. However, it is unclear, whether these more severe clinical courses in the Russian forms of TBE are due to differences in case-reporting, due to differences in medical interpretation or to genuine different pathogenicity of this TBE virus subtype[12].

Despite the manifestation of central nervous system disease, the isolation or detection of TBE virus in the cerebrospinal fluid (CSF) of the patient is difficult and only rarely successful. Within the brain the virus seems to spread from one cell to the next without being shed into the CSF. Therefore the diagnostic method of choice is the detection of specific antibodies. The detection of IgM and IgG antibodies against TBE virus together with typical clinical CNS symptoms and tick exposure is strong evidence for a diagnosis of acute TBE infection[11]. However, there are many serological cross-reactions with other flaviviruses. The detection of antibodies in a single serum, without further serological follow up and diagnostic information, may make the diagnosis difficult. Also IgM might be very low or even missing in the case of pre-existing antibodies against other flaviviruses (e.g. dengue virus, Zika virus) or after vaccination against other flaviviruses (yellow fever virus, Japanese encephalitis virus)[13]. Therefore, the diagnosis of TBE should be made after excluding other flavivirus antibodies, e.g. against yellow fever virus, dengue fever viruses, or Japanese encephalitis virus.

So far, there is no effective treatment for TBE[11]. Treatment may include symptomatic therapy to lower temperature, to relieve pain and especially in case of encephalitis to avoid complications from inflammatory brain damage. In severe clinical forms patients are put into an artificial coma. Rehabilitation medicine plays an important part in the post-acute treatment to control the neurological and psychiatric sequelae. The fatality rate of the European form of TBE ranges from 0.5% to 2%[10].

There are six inactivated and adjuvanted vaccines available against TBEV infection[14,15]. Two vaccines, Encepur (GSK) and FSME-Immun (Pfizer) are produced in Europe and contain European type TBEV. Three vaccines, TBE vaccine Moscow and EnceVir and Tick-E-Vac/Klesh-E-Vac are produced in Russia and contain Far Eastern TBEV strains. The name of the Chinese TBE vaccine is Sen Tai Bao; however, no further information on this vaccine is available. The two European vaccines need three doses for a basic immunisation. Two are given 1–3 months apart. Two weeks after the second dose a vaccine efficacy of more than 95% can be assumed. For both vaccines a third dose is recommended after

6–12 months after the start of immunisation. A fourth vaccine dose is recommended after three years. Depending on the patient's age, subsequent boosters are recommended after 3 or 5 years. For both vaccines, rapid immunisations schemes are available, which might induce immunity as early as three weeks. Both European vaccines can be used in special formulations in children >12 months of age. The Russian Tick-E-Vac/Klesh-E-Vac can also be used for children >12 months of age. Data indicate that the European vaccines also provide protection against infections with the Siberian and the Far Eastern subtype of TBEV. Due to the close genetic relatedness a similar assumption may be made also for the three Russian vaccines in relation to the European subtypes.

The two Russian TBE vaccines, TBE vaccine Moscow and EnceVir, are not licensed for children <3 years of age. They are administered in two doses 1–2 and 1–7 months apart. A third dose is recommended after 12 months. Further booster doses should be given after 3 years. TBE-E-Vac/Klesh-E-Vac is given in two doses 1–7 months apart. A third dose is recommended one year after the second dose.

In the northern hemisphere tick-borne encephalitis is the most important tick-borne virus infection. An estimated 10 000 human cases occur every year in Europe and Asia[16]. Incidence rates of TBE infection in endemic areas of Europe range from 0.1 to 20/100 000 inhabitants. The European countries with the highest incidence rates are the Baltic countries and Slovenia, followed by the Czech Republic, Slovakia, Poland and Austria (non-vaccinated population)[17]. However, the incidence rates may vary on a local district level. For example, in some German districts incidence rates of >10/100 000 per annum are recorded, while in the whole country the incidence rate is below 0.5/100 000 per annum (G. Dobler, personal observation).

Besides the imminent risk of infection in residents of endemic areas, TBE is becoming an important travel-related disease. According to estimates, the risk of exposure for acquiring a TBE infection was calculated at 1 case per 77 000 to 200 000 visitors[18]. Travel-related cases have been reported from Israel, The Netherlands, Australia, United States and England[19–22]. The Australian patient travelled by car from Moscow to Novosibirsk with ample contact in nature although it was unclear whether he was infected by a tick bite or by the alimentary route. He developed a generalised infection with drowsiness, fatigue and lower limb myalgia. After acute symptoms subsided the patient noticed a severe depressiveness and changes in his handwriting, which was explained as a cerebellar dysfunction of the right upper limb. However, the clinical course was complete recovery after one month[22].

Conflicts of interest

The author declares no conflicts of interest.

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Biography

Gerhard Dobler is a medical doctor and specialist in medical microbiology. He is head of the Department of Virology and Rickettsiology at the Bundeswehr Institute of Microbiology in Munich and associate professor at the Unit of Parasitology of the Institute of Zoology at the University of Hohenheim. He is head of the German reference laboratory for tick-borne encephalitis (TBE). His main areas of research are the molecular phylogeny, the eco-pathogenesis of TBE virus and the eco-epidemiology of TBE and other tick-borne diseases.

Ticks in Australia: endemics; exotics; which ticks bite humans?

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At least 71 species of ticks occur in Australia; a further 33 or so species are endemic to its neighbours, New Guinea and New Zealand. The ticks of Australia and other parts of Australasia are phylogenetically distinct. Indeed, there are at least two lineages of ticks that are unique to Australasia: the genus *Bothriocroton* Klompen, Dobson & Barker, 2002; and the new genus *Archaeocroton* Barker & Burger, 2018. Two species of ticks that are endemic to Australia are notorious for feeding on humans: (i) *Ixodes holocyclus*, the eastern paralysis tick, in eastern Australia; and (ii) *Amblyomma triguttatum triguttatum*, the ornate kangaroo tick, in Western Australia, at one place in South Australia, and in parts of Queensland. Three of the other endemic species of ticks that feed on humans in Australia are also noteworthy: (i) *Bothriocroton hydrosauri*, the southern reptile tick, which is a vector of *Rickettsia honei* (Flinders Island spotted fever); (ii) *Haemaphysalis novaeguineae*, the New Guinea haemaphysalid; and (iii) *Ornithodoros capensis*, the seabird soft tick. Here, we present images of female *Ixodes holocyclus*, *Amblyomma t. triguttatum*, *Bothriocroton hydrosauri* and *Haemaphysalis novaeguineae* and our latest maps of the geographic distributions of *Ixodes holocyclus*, *Amblyomma t. triguttatum* and *Bothriocroton hydrosauri*. None of the five exotic species of ticks in Australia typically feed on humans.

The Australian tick fauna

At least 71 species of ticks are known in Australia: 57 hard ticks (family Ixodidae) and 14 soft ticks (family Argasidae)[1,2]. Five of these 71 species of ticks were brought to Australia by humans and thus might be called exotic: (i) *Argas persicus*, the poultry tick; (ii) *Otobius megnini*, the spinose ear tick, a recent introduction, probably in the ears of horses; (iii) *Haemaphysalis longicornis*, the bush tick, which occurs in much of east Asia; (iv) *Rhipicephalus sanguineus*, the brown dog tick, a worldwide species; and (v) *Rhipicephalus* (*Boophilus*) *australis*, the Australian cattle tick. Barker and Walker[3] has detailed species accounts for these five ticks.

Australia has a special place in the history of hard ticks (Ixodidae). Indeed, the hard ticks[4–6], soft ticks and nutalliellid ticks[1] may have first lived in Australia, or more accurately, that part of the super continent Gondwana that became Australia, as early as the Devonian era (362–409 million years ago). Accordingly, six of the eight subfamilies of ticks (Ixodida) are endemic to Australia: Argasinae,

Bothriocrotinae, Amblyomminae, Haemaphysalinae, Ixodinae and the Ornithodorinae. Furthermore, there are at least three lineages of ticks that are unique to Australasia: (i) the sub-family Bothriocrotinae Klompen, Murrell & Barker, 2002 from Australia and New Guinea; (ii) the Australasian *Ixodes* lineage[6]; and (iii) the genus *Archaeocroton* Barker & Burger, 2018, which was made for the tick of the tuatara, a singular lizard, from New Zealand[7].

Which ticks bite humans in Australia?

None of the five exotic species of ticks in Australia typically feed on humans. Two of the 66 species of ticks that are endemic to Australia are, however, notorious for feeding on humans or may often be found crawling on them: (i) *Ixodes holocyclus*, the eastern paralysis tick, in eastern Australia; and (ii) *Amblyomma t. triguttatum*, the ornate kangaroo tick, in Western Australia, at one place in South Australia, Innes National Park on Yorke Peninsula where it was recently introduced (refer to commentary and references[3]), and in parts of Queensland. These two ticks will be considered in detail in the present paper. Of the three other endemic species of ticks that may feed on humans in Australia: (i) *Bothriocroton hydrosauri*, the southern reptile tick, is a vector of *Rickettsia honei* (Flinders Island spotted fever); (ii) *Haemaphysalis novaeguineae*, the New Guinea haemaphysalid, which, although restricted to the far north of Australia and the Island of New Guinea is noteworthy because it is known to be a vector of *R. honei* strain marmionii[8,9], which caused a fatality in a patient admitted to Townsville General Hospital in late 2016[10]; and (iii) *Ornithodoros capensis*, the seabird soft tick, that will feed on humans and domestic poultry given the opportunity[3,11]. *O. capensis* is known to carry a large number of viruses although none of these have been confirmed to infect humans (see commentary[3]).

Ixodes holocyclus, the eastern paralysis tick

I. holocyclus (Figure 1) is known as the eastern paralysis tick since most cases of tick paralysis in eastern Australia in domestic animals, wildlife and humans are caused by this tick. *I. holocyclus* is also known as the scrub tick in Queensland, particularly in North and Far North Queensland. The name scrub tick echoes

Figure 1. *Ixodes holocyclus*, the eastern paralysis tick; *Amblyomma triguttatum triguttatum*, the ornate kangaroo tick; *Bothriocroton hydrosauri*, the southern reptile tick; and *Haemaphysalis novaeguineae*, the New Guinea haemaphysalid.

the apparent predilection of *I. holocyclus* for the edges of wet-forests ('scrub').

Geographic distribution and hosts: Ixodes holocyclus is strictly constrained to the east coast of Australia (Figure 2) despite the tick having been carried by domestic dogs, cattle, horses and people to many other parts of Australia that appear to be superficially suitable, such as Melbourne and Perth (SC Barker, E Teo and D Barker, unpublished data). *I. holocyclus* is considered to be catholic in its feeding habits. Indeed, *I. holocyclus* has been recorded from 34 species of mammals and seven species of birds[3], but whether it feeds successfully on all of these species is another question. Where *I. holocyclus* is abundant, it will be found on most of the species of mammals present, but the bandicoots *Isoodon macrourus* and *Perameles nasuta* have been considered the principal hosts in southeastern Queensland since at least 1975[12]. These bandicoots may carry many ticks. It seems that reasonable numbers of *I. macrourus* and *P. nasuta* are required for populations of *I. holocyclus* to persist from one tick season to another in southeastern Queensland[12] but this is probably not the case in other parts of the geographic range of *I. holocyclus* where there seem to be large numbers of ticks but few if any bandicoots (SC Barker and D Barker, unpublished data).

Figure 2. The geographic distribution of *Ixodes holocyclus*, the eastern paralysis tick.

Illnesses in humans associated with I. holocyclus*:* The toxins of this tick seem to be the most potent of all tick-toxins with at least 20 fatalities[13]: there have been comparable numbers of fatalities from red-back spiders ($n = 18$) and funnel-web spiders ($n = 13$)[13]. Thankfully, deaths from the bite of *I. holocyclus* are now rare due to the advent of intensive care-units in regional hospitals and expert medical treatment. The illnesses that *I. holocyclus* has been associated with include Australian multi-system disorder, post-infection fatigue, autoimmune disease, paralysis, allergies (particularly to the bites of larvae), Queensland Tick Typhus (*Rickettsia australis*), mammalian meat-allergy and tick anaphylaxis; Graves and Stenos[14] reviewed these illnesses. Barker[15] hypothesised that *I. holocyclus* may be a link in the transmission of Hendra virus from bats to horses to humans; this hypothesis has not yet been tested.

Amblyomma triguttatum triguttatum, the ornate kangaroo tick

Although known as a kangaroo tick, *A. t. triguttatum* (Figure 1) will feed on humans; it is one of four subspecies of *A. triguttatum* (see Barker *et al*.[1]).

Geographic distribution and hosts: The geographic distribution of *A. t. triguttatum* has two parts: eastern Australia and western Australia (Figure 3). *A. t. triguttatum* is primarily a tick of kangaroos (genus *Macropus*). *A. t. triguttatum* is also common on wild (feral) pigs in Australia[16]. Of a sample of 88 grey kangaroos, *M. giganteus*, in southeast Queensland, 84% were infested with *A. t. triguttatum;* 97% of all these ticks were found in the ears[16]. McCarthy[17] also found that *A. triguttatum* prefers to attach in the ears of kangaroos, sheep and cattle, sheep and cattle. No other species of ticks were found on these kangaroos[16].

Illnesses in humans associated with A. t. triguttatum: *A. t. triguttatum* is vector of the spotted fever organism, *Rickettsia gravesii*[18].

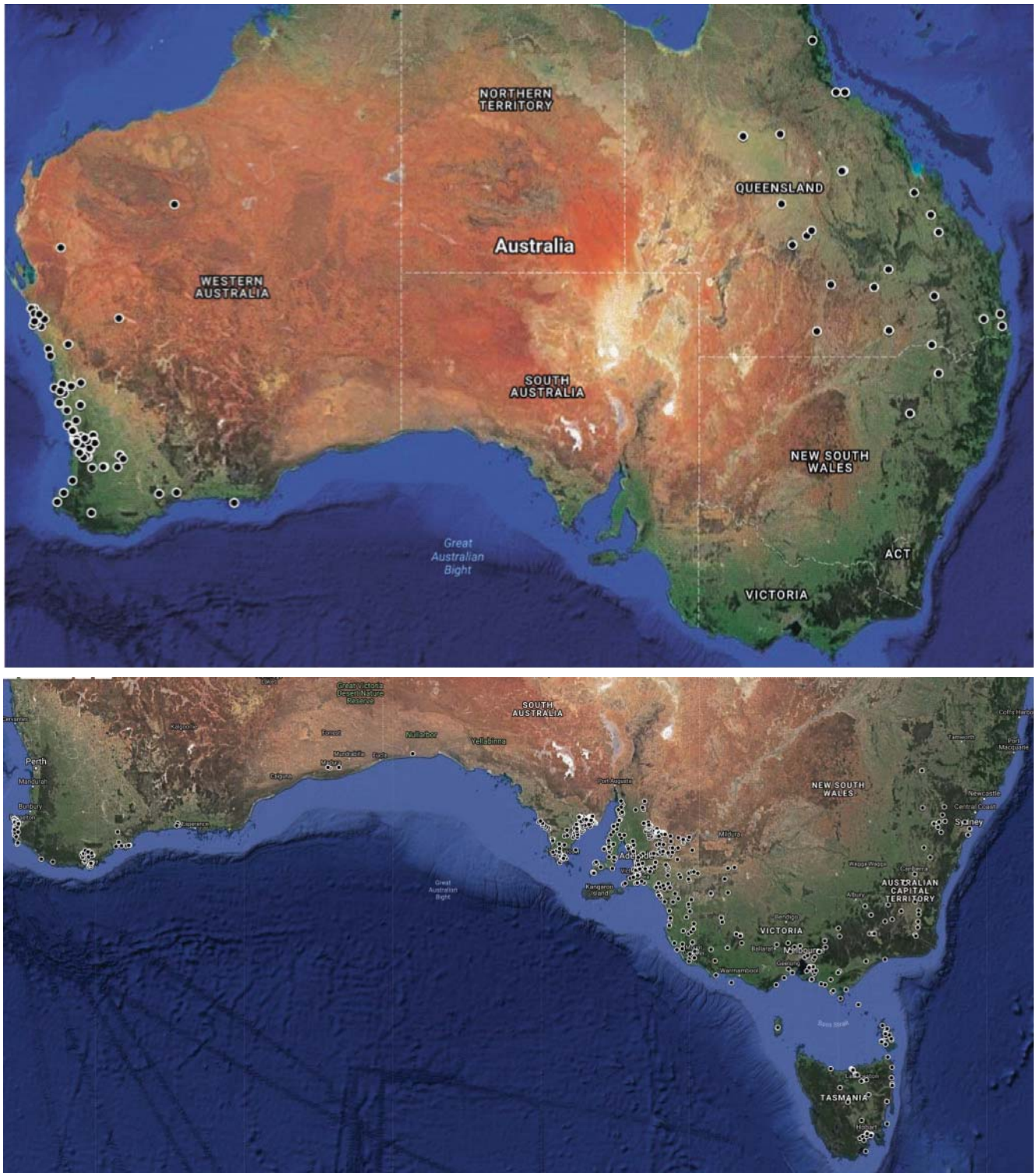

Figure 3. The geographic distributions of *Amblyomma triguttatum triguttatum*, the ornate kangaroo tick, and *Bothriocroton hydrosauri*, the southern reptile tick.

So far this *Rickettsia* has been found only in *A. triguttatum* taken from feral pigs (*Sus scrofa*)[19] and humans[20] in Western Australia. Owen *et al.*[21] suggested that the geographic distribution of *R. gravesii* may coincide with that of *A. triguttatum* (possibly the subspecies *A. t. triguttatum* – inferred by us from the known geographic distribution of *A. t. triguttatum* (Figure 3)). *A. t. triguttatum* is also a vector of *Coxiella burnetii*, the aetiological agent of Q fever[14]. Pearce and Grove[22] described the local skin reactions of 175 soldiers who were bitten by *A. triguttatum* (probably *A. t. triguttatum*). Moorhouse[23] also reported local skin reactions, which he described as allergic dermatitis, in humans bitten by *A. triguttatum* (possibly the subspecies *A. t. triguttatum* – inferred by us from the known geographic distribution of *A. t. triguttatum* (Figure 3)).

Bothriocroton hydrosauri, the southern reptile tick

Although known as the southern reptile tick, *B. hydrosauri* (Figure 1) will, however, feed on humans.

Geographic distribution and hosts: The geographic distribution of *B. hydrosauri* (Figure 3) is very well known. In South Australia, at Bundey Bore Station, north-east of Adelaide, the distribution of *B. hydrosauri* has been mapped to a scale of metres[24,25]. *B. hydrosauri* was aptly named the southern reptile tick, since it may be found on all of the main types of reptiles in southern Australia: lizards, snakes and even a terrestrial turtle[3]. The main host of *B. hydrosauri*, in much of South Australia at least, is *Tiliqua rugosa* (sleepy lizard). Nonetheless, *B. hydrosauri* will, given the opportunity, attach to and feed on humans, cattle and horses.

Illnesses in humans associated with B. hydrosauri: *B. hydrosauri* is the arthropod-host of *R. honei* on Flinders Island, Tasmania[26] and mainland-Tasmania[27]. *R. honei* causes Flinders Island spotted fever in humans[9,28]. Flinders Island spotted fever is typically a relatively mild disease; no deaths have been reported[29] although a patient in Nepal had severe illness[30]. *R. honei* has been isolated from the blood of patients with chronic illness, including fatigue, from Melbourne, Victoria, and Adelaide, South Australia, but it is not known whether or not *R. honei* was causally related to the illness[31]. *R. honei* was not detected by PCR nor cell culture in the blood of *Tiliqua nigrolutea* (southern blue-tongue lizard), *Austrelaps superbus* (copperhead snakes) nor *Notechis scutatus* (tiger snake), but more than 60% of *B. hydrosauri* from those lizards and snakes were PCR-positive or cell culture-positive for *R. honei*[26]. So, *R. honei* is apparently sustained in populations of *B. hydrosauri* on Flinders Island by vertical, trans-ovarial transmission. That is, *R. honei* infects the eggs of *B. hydrosauri in situ* and thus the next generation of *B. hydrosauri* become infected with *R. honei*, without feeding on an infected vertebrate. So apparently, vertebrates are not needed for the survival of *R. honei* on Flinders Island, and probably elsewhere. Furthermore, horizontal transmission, that is transmission between the arthropod-host (tick) and the vertebrate-host (lizards and snakes), has not yet been demonstrated experimentally for *R. honei* although there are confirmed cases of infection with *R. honei* in people who had been bitten by ticks from Iron Range, Cape York Peninsula, Queensland (*H. novaeguineae*)[9]; and Nepal (species of tick unknown)[30].

Flinders Island spotted fever is now known from three continents: (i) Australia (Flinders Island, Tasmania; mainland Tasmania; and South Australia); (ii) Asia (Thailand; Orchid Island, Taiwan; Nepal); and (iii) North America (Texas)[30,32–34]. Confirmed tick-hosts of *R. honei* are: (i) *B. hydrosauri* from Flinders Island and mainland Tasmania; Cooma, New South Wales; and Bundy Bore Station, South Australia; (ii) *Haemaphysalis novaeguineae* (*R. honei* strain marmionii) from Iron Range, Queensland; (iii) *Ixodes granulatus* from *Rattus rattus* (black rat) from Thailand (adult ticks but not yet from larval or nymphal *I. granulatus*[35]); and (iv) *Amblyomma cajennense* from cattle from Texas, USA[26,27,32].

Where to next?

We still need *cytochrome c oxidase subunit I* (COX1) and Internal Transcribed Spacer (ITS2) rRNA nucleotide sequences for most of the Australian ticks. These sequences will help non-experts to identify quickly ticks, particularly larvae and nymphs. We also need to know where exactly *H. novaeguineae*, the New Guinea haemaphysalid, lives in northern Australia, and how abundant it is there.

Conflicts of interest

The authors declare no conflicts of interest.

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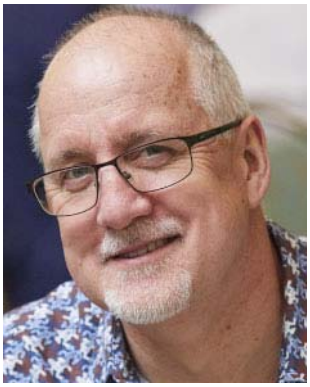
Bacterial tick-associated infections in Australia: current studies and future directions

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It may seem perplexing that there is any uncertainty in Australia about the existence of zoonotic tick-associated infections[1–3]. Outside this country, particularly in the northern hemisphere, tick-borne diseases such as human granulocytic anaplasmosis, babesiosis, Boutonneuse fever, ehrlichiosis, Lyme borreliosis, and tick-borne encephalitis, have well documented aetiologies, epidemiology, diagnostic methods, and treatments. Why is Australia different and what research is being conducted to address this issue? This article briefly addresses these questions and explains how high-throughput metagenomic analysis has started to shed light on bacterial microbiomes in Australian ticks, providing new data on the presence and distribution of potentially zoonotic microbial taxa.

Fundamental to understanding tick-borne infections in Australia is the recognition that the tick fauna of Australia is unique and distinct. Australia's long geological isolation since the breakup of Gondwana during the Mesozoic Era has allowed its animals to evolve separately from those in other parts of the world. Of the ~896 recognised tick species worldwide[4], 71 species are endemic to Australia; 66 of these occur only on the Australian continent and its islands, with a few species extant in Papua New Guinea[5]. The remaining five species were introduced to Australia with domestic animals (dogs, cattle and poultry) during the past 230 years, and of the 71 species, only about eight are well known to bite humans[6].

Infections caused by the five introduced tick species result in economically important diseases (e.g. canine and bovine babesiosis and anaplasmosis, bovine borreliosis, bovine theileriosis and avian spirochaetosis) that are restricted to domestic animal hosts and have been well studied internationally as well as in Australia. In contrast, the same cannot be said for endemic tick species, and herein lies the knowledge gap that underpins the debate around indigenous tick-borne infections of humans (and animals) in Australia.

Zoonotic tick-borne infections occur when humans encroach into natural environments where ticks, their microbial communities and wildlife reservoir hosts co-exist within well defined (and long-evolved) ecologies. These complex interactions, sometimes referred to as tick-borne pathogen guilds[7], have been the subject of research in other parts of the world for many years[8], and with increased intensity since the connection was first made between tick bites and an epidemic of arthritis, in Old Lyme, Connecticut, USA, in the late 1970s[9,10]. However, relatively little is known about host preferences and ecologies of Australian ticks, and even less is understood about the communities of organisms within these arthropods.

With the exception of rickettsial species and *Coxiella burnetii* (the causative agent of Q fever in humans and coxiellosis in animals), there has been a dearth of research into tick-associated microorganisms in Australia, especially viruses, and a hiatus of more than 20 years between studies[11,12] in the 1990s and the

start of investigations using metagenomic techniques[13]. Our studies have been designed to address two questions. (1) Which microbes are associated with Australian ticks? (2) Are any of these microbes known pathogens, or putative pathogens?

Answering these questions requires investigation of the tick microbiome, which comprises communities of microorganisms including viruses, bacteria and eukaryotes that can be explored in a rapid and cost-effective manner by next-generation sequencing (NGS)[14]. However, the presence of highly abundant bacterial endosymbionts such as '*Candidatus* Midichloria mitochondrii' (CMM) may challenge the effectiveness of this approach by masking less abundant bacteria, including pathogens. Application of specific CMM blocking primers to the Australian paralysis tick (*I. holocyclus*) ($n = 196$) removed from various hosts, including people in eastern Australia, decreased CMM sequences by 96%, and resulted in a significantly higher taxonomic diversity (an additional 103 genera detected)[13].

Metagenomic analysis reveals that Australian ticks, like their northern hemisphere counterparts, possess a rich and varied microbiome, with the tick species as the main factor influencing microbial composition (Figure 1). Novel '*Candidatus* Neoehrlichia' spp., *Anaplasma* spp. and *Ehrlichia* spp. were identified in ticks ($n = 460$) removed from people in Australia[15]. Phylogenetic characterisation of these new members of the Anaplasmataceae revealed two species; '*Candidatus* Neoehrlichia australis' and '*Candidatus* Neoehrlichia arcana' in 8.7% and 3.1% *I. holocyclus* ticks in New South Wales and Queensland, respectively[16]. Analysis of 16S rRNA and groEL gene sequences demonstrated that *Anaplasma bovis* genotype Y11 is a unique genetic variant, distinct from other *A. bovis* isolates worldwide, and the *Ehrlichia* sp. is most closely related to, but clearly distinct from, *E. ruminantium* (a bovine pathogen) and other ehrlichial species[17]. The zoonotic potential of these bacteria is unknown, however '*Candidatus* Neoehrlichia' is a sister genus to *Anaplasma* and *Ehrlichia*, and contains '*Candidatus* Neoehrlichia mikurensis', an emerging tick-borne zoonosis in Africa, Asia and Europe[16].

In recent years there has been increasing debate about whether Lyme borreliosis (LB) occurs in Australia[1–3]. The aetiological agents of LB in North America, Asia and Europe comprise spirochaetes belonging to the genus *Borrelia* that are transmitted, together with other tick-borne agents such as anaplasmosis and babesiosis, by hard ticks of the genus *Ixodes*, including *I. scapularis*, *I. pacificus*, and *I. ricinus*[18]. Whilst not being the only ticks capable of zoonotic disease transmission, none of these members of the *I. ricinus* group is known to have established in Australia, a finding supported by our recent survey of 4765 ticks parasitising companion animals nationwide[19].

In serological screening of 555 Australian dogs (which act as sentinels for LB in endemic regions), including foxhounds exposed to >160,000 adult *I. holocyclus* ticks for commercial antiserum

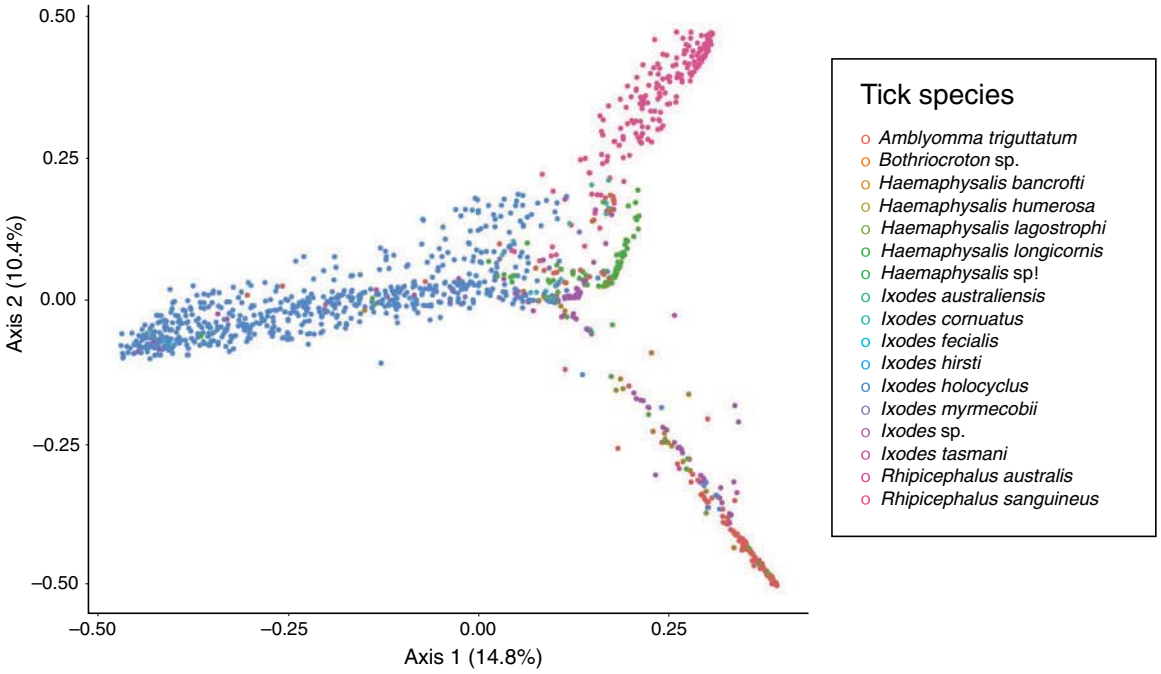

Figure 1. Multidimensional scaling plot (Bray-Curtis dissimilarity analysis) of tick 16S rRNA data from next-generation sequencing, showing that tick species is the main factor influencing microbial composition. Each point on the figure represents an individual tick sample ($n = 1276$) and tick species is represented by colour. Samples of the same tick species cluster together and therefore share a similar microbial diversity. This was further supported by ANOVA testing ($F_{16,1260}$ $P < 0.001$) where grouping by tick species was the only factor that resulted in a significant difference ($P < 0.05$) in the microbial diversity between samples (S. Egan, unpublished).

production, none was deemed positive for *B. burgdorferi sensu lato* infection[20]. However, recent studies have detected novel *Borrelia* DNA in 41% of *Bothriocroton concolor* ticks removed from echidnas[21], and phylogenetic analysis of '*Candidatus* Borrelia tachyglossi' indicate this antipodean *Borrelia* is closely related to, yet distinct from, the Reptile-associated and Relapsing Fever groups, and does not belong to the LB complex[22].

In summary, the microbiomes of Australian ticks comprise diverse genera with similarities to those of ticks in other parts of the world. To date, no known northern hemisphere bacterial pathogens have been discovered; however, phylogenetic analysis reveals multiple organisms that are related to but distinct from known pathogens overseas, and their zoonotic potential remains unknown. Investigation of disease causation by these organisms, if any, in order to meet Koch's postulates, is largely the direction of our future research.

Conflicts of interest

The authors declare no conflicts of interest.

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Tick-transmitted human infections in Asia





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Vector-borne pathogens of human significance cause a predicted 17% of infectious diseases worldwide, of which, ~23% are tick transmitted[1]. Although second to mosquitoes in terms of impact, ticks are thought to carry a greater diversity of pathogens than other arthropod vectors[2]. Asia is a key region for tick-borne pathogens, with tick species typically restricted to latitudes below 60–55°N[3] where the climate is warmer and wetter – from the steppe regions of Russia to the tropical rainforests of South East Asia.

There are approximately 896 species of tick (Ixodidae, Argasidae and Nuttalliellidae) worldwide[4]. In Asia the knowledge of key species is still limited, especially in the Southeast. Tick species that may transmit specific pathogens are highly dependent on distribution, with studies described below primarily identifying *Ixodes* spp, *Haemaphysalis* spp., *Hyalomma* spp. and *Dermacentor* spp. as important vectors for various pathogens.

Despite the prevalence of ticks and the clinical importance of the pathogens transmitted, very little information is available on the disease burden and distribution of tick-transmitted infections in Asia, particularly outside of Russia, China, Japan and Korea. This is most likely due to lack of research in ticks and tick-borne diseases (TBD) outside of the more developed northern Asian countries, and a lack of knowledge in the healthcare systems of LMICs (lower to middle income countries) as many TBD infections have similar clinical presentations and available diagnostics may be limited. Knowledge of TBDs is highly dependent on whether the diseases are notifiable within the country; in Russia for instance, seven TBDs are reportable providing incidence data, but little is known about other non-reportable infections[5]. In Russia approximately 0.5 million tick bites are reported each year, with an estimated ≤2% resulting in clinical infections, although this is likely to be much higher, particularly in rural regions[5]. In Japan, 12 TBDs are reportable, while in Korea, six diseases of potential tick origin are reportable[6,7]. TBD in Asia can be categorised into four distinct groups: rickettsias, other bacterial pathogens, protozoa and viruses.

Rickettsias

The rickettsias form the largest group of TBD in Asia. Although globally distributed, at least thirteen clinically important rickettsial species have been identified throughout Asia (east of the Caspian Sea) in either patients or ticks[8–11]. Currently a further 10 (including candidatus species) have been identified in ticks although their implications for public health is uncertain[8,12–14] (Table 1). Often, identification in patients is made by serological

Table 1. Tick-borne rickettsias identified in Asia. Identification either by serology (S), PCR (G) or isolation (I) from patients and/or ticks within Asia.

Known pathogenic rickettsias			Rickettsias of unknown pathogenicity		
Species	Human ID?	Tick ID?	Species	Human ID?	Tick ID?
R. aeschlimannii	–	G	*R. asiatica*	–	I
R. conorii indica	G/S	I	*R. bellii*	–	G
R. heilongjiangensis	G	G/I	*R. hoogstraalii*	–	G
R. helvetica	S	–	*R. tarasevichiae*	–	G
R. honei	G/S	G	Candidatus R. gannanii	–	G
R. japonica	G/I	G/I	Candidatus R. khammouanensis	–	G
R. massiliae	G	G	Candidatus R laoensis	–	G
R. monacensis	–	G	Candidatus R. mahosotii	–	G
R. raoultii	G	G/I	Candidatus R. principis	–	G
R. rickettsii	S	–	Candidatus R. tibetani	–	G
R. sibirica sibirica	G	G/I			
R. sibirica mongolitimonae	G	G			
R. slovaca	–	G			
R. tamurae	G/S	G/I			
Candidatus R. kellyi	G	–			

techniques, limiting identification to non-specific genus-level rather than species-level, which may obscure the clinically important species circulating in the region. Symptoms for infections are variable, with most causing fever, chills, headache, malaise and myalgia with a variable proportion developing a maculopapular rash. *R. sibirica* results in a lymphangitis-associated rickettsiosis[15].

Other bacterial pathogens

Closely related to the rickettsias are *Anaplasma* and *Ehrlichia*. *A. phagocytophilum* is the agent of Human Granulocytic Anaplasmosis (HGA)[16], while *E. chaffeensis* is the cause of Human Monocytic Ehrlichiosis (HME)[16,17]. Both share similar symptoms including fever, headache, leukocytopenia, with neurological symptoms more common in HME[18]. Borreliosis is becoming more important throughout the region, with *Borrelia afzelii* and *Bo. garinii* being the main species in Asia, although a *Bo. valaisiana*-related sp. has also been identified in patients[12,19,20]. Despite its dominance in the western hemisphere, *Bo. burgdorferi sensu stricto* has only been isolated from rodents in Asia[19,20]. Borreliosis may present with erythema migrans, fever, headaches and fatigue, and in a minority, cardiac and central and peripheral central nervous system abnormalities[19]. Although the following human pathogens (*Francisella* spp., *Bartonella* spp., *Brucella* spp. and *Coxiella* spp.) have been identified in ticks in Asia, the tick-human route of transmission for these four organisms is highly disputed or considered infrequent. Infection is more likely through other routes such as other vectors, direct contact with animals, food items or aerosols; nevertheless, ticks may still play a vital, yet indirect role in disease incidence. *Francisella tularensis* has been detected in ticks from Japan, China and Thailand[21,22], while *F. novicida*, has been isolated from a patient in Thailand[23]. At least 15 species of *Bartonella* are known in Asia, some of which have been identified in ticks[24]. There are reports of clinical *Bartonella* spp. infections in China, Thailand, Japan and Korea[24,25], although these may be due to transmission via fleas or mammalian contact. The greatest human incidences of brucellosis infections are reported from central Asia[26]. *Brucella melitensis* and *Br. abortus* (the most pathogenic species) have been identified in ticks and shown to be transmitted[27]. A number of tick species have been shown to harbour *Coxiella burnetii* (the etiologic agent of Q fever) in Malaysia, Laos and Thailand[12,22,28]. Transmission from ticks to mammalian hosts has been shown to occur experimentally but it remains to be seen if this is a viable route for human infections.

Protozoa

Although predominantly recognised as a TBD of veterinary importance, cases of human babesiosis have been identified throughout China (including the China-Myanmar boarder), Russia, Japan and Korea[29]. Infections are predominantly *Babesia microti*, although *Ba. divergens* and *Ba. venatorum* have also been identified. Clinical symptoms are similar to malaria infections and therefore often result in misdiagnosis and under-reporting of this pathogen. *Ixodes persulcatus* is considered the key tick species for transmission[29].

Viruses

Tick-borne encephalitis viruses (TBEV) have been identified in both ticks and patients across Asia[30], including serological evidence in rodents and humans in Vietnam[31]. Infection may result in central nervous system abnormalities. Of the Bunyavirales, outbreaks of Crimean-Congo haemorrhagic fever (CCHF) have been reported in China, the first of which was in Xinjiang Province in 1965[32] and in Pakistan and India[33]. Clinical symptoms include severe fever, haemorrhage, fatigue, myalgia, oliguria and disturbance of consciousness. Severe Fever with Thrombocytopenia Virus (SFTV) has been reported in China, Japan and South Korea and is transmitted by *Haemaphysalis longicornis* ticks. SFTV is characterised by fever, thrombocytopenia, leukocytopenia, increased serum liver enzyme levels, and organ failure[34]. Powassan virus is a rare, yet potentially fatal neurotropic virus seemingly restricted in Asia to Far Eastern Russia region. Symptoms vary between patients, making diagnosis difficult, but may rapidly develop into more severe symptoms including neurological defects[35]. Kyasanur Forest Virus (KFV, a flavivirus) is found in southern India, presenting with haemorrhagic and neurological symptoms and is thought to be transmitted predominantly by *Haemaphysalis spinigera*[36].

The zoonotic nature of TBDs, combined with a higher proportion of rural populations in Asia, heightens the risk of exposure to TBDs and places a significant weight on scarce public health resources. Surveillance of ticks for potential human pathogens across Asia is needed to alert for clinical problems[12]. Improved diagnostics, evidence for appropriate management and public and policy engagement are very much in need, supported by validated survey and surveillance research to better understand the distribution and epidemiology of these potentially life-threatening diseases.

Disclaimers

JCH is a military service member or federal/contracted employee of the United States government. The views expressed in this article reflect the results of research conducted by the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defence, nor the United States Government.

Conflicts of interest

The authors declare no conflicts of interest.

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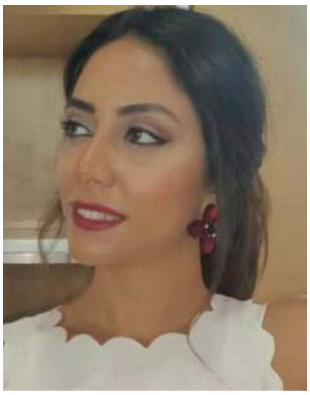
A concise overview on tick-borne human infections in Europe: a focus on Lyme borreliosis and tick-borne *Rickettsia* spp.



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Ticks are blood-feeding external parasites of mammals. Almost all ticks belong to one of two major families, the *Ixodidae* or hard ticks, and the *Argasidae* or soft ticks. Ticks are responsible of transmitting many diseases called 'tick-borne diseases'. *Borrelia* and *Rickettsia* spp., are the most important tick-transmitted bacterial pathogens circulating in Europe. In this review we will focus on the two tick-borne diseases caused by these bacterial pathogens, their vector, epidemiology, clinical diagnosis and symptoms.

Ticks are blood-feeding external parasites of mammals, birds and reptiles throughout the world. They belong to the order Ixodida. Almost all ticks belong to one of two major families, the *Ixodidae* or hard ticks, which are difficult to crush, and the *Argasidae* or soft ticks. The *Ixodidae* contain over 700 species of hard ticks with a scutum or hard shield, which is missing in the *Argasidae* family. The *Argasidae* contain about 200 species[1]; Table 1 shows the most important tick-borne diseases and their vectors.

Tick-borne diseases have been described throughout human history. A papyrus scroll dating back to the 16th century B.C. referred to what could be 'tick fever'[2]. At the end of the nineteenth century, Theobald Smith and Frederick Kilbourne were the first to demonstrate that ticks were responsible for transmitting diseases. Their experiment in cattle allowed them to conclude that the absence of ticks lead to the absence of Texas fever[3]. In this review we will focus on *Borrelia* and *Rickettsia* spp., the most important tick-transmitted bacterial pathogens circulating in Europe.

Lyme borreliosis (LB)

Causative agent, reservoir and vector

LB is an infectious disease caused by the extracellular bacterium *Borrelia burgdorferi* sensu lato (sl). This microorganism was determined to be the causative agent of LB by W. Burdogorfer in the early 1980s[4]. It is a spirochaete belonging to the order Spirochaetales, helically shaped, motile, 20–30 µm in length and 0.2–0.3 µm in width[4]. Three human pathogens can be distinguished within *B. burgdorferi* sl: *B. burgdorferi* sensu stricto (ss), *Borrelia afzelii* and *Borrelia garinii*. All of them are present in Europe[5].

Regarding the vector, *Ixodes* ticks transmit all species belonging to *B. burgdorferi* sl[6]. Eighty per cent of tick bites transmitting LB are caused by nymphal ticks. The most important reservoirs of *B. burgdorferi* sl in Europe are rodents, insectivores, hares and

Table 1. Most important tick-borne diseases and their vectors in Europe.

Disease	Specific disease names	Agent	Vector
Anaplasmosis		*Anaplasma phagocytophilum*	*Ixodes* spp.
Lyme borreliosis		*Borrelia burgdorferi* *Borrelia afzelii* *Borrelia garinii*	*Ixodes* spp.
Tularemia		*Francisella tularensis*	*Dermacentor* spp. *Ixodes* spp.
Rickettsioses	Mediterranean spotted fever Lymphangitis-associated disease Scalp and enlarged neck lymphadenitis	*R. conorii* *R. helvetica* *R. monacensis* *R. sibirica mongolitimonae* *R. massiliae* *R. aeschlimannii* *R. slovaca* *R. raoultii* *R. hoogstraalii*	*Rh. sanguineus* *I. ricinus* *I. ricinus* *Hyalomma* spp./*Rhipicephalus pusillus* *Rh. sanguineus* *Hy. marginatum* *Dermacentor marginatus/ Dermacentor reticulatus* *Dermacentor marginatus/ Dermacentor reticulatus* *Haemaphysalis sulcata*
Babesiosis		*B. divergens* *B. venatorum* *B. microti*	*I. ricinus*
Tick-borne encephalitis		Tick-borne encephalitis virus	*I. ricinus* *I.scapularis* *I. persulactus*
Tick-borne relapsing fever		*Borrelia* spp. (*Borrelia miyamotoi*)	*Ixodes* spp.

several bird species[7]. Birds are more likely to carry *B. garinii*, so this microorganism may be carried over very long distances, especially in the case of migratory sea birds[8].

Epidemiology

LB is one of the most prevalent vector-borne diseases in Europe[9]. However, precise epidemiological data are not available for all European countries because the disease is notifiable in only a few countries[10,11]. The highest average incidence rates among the reporting countries were found in Belarus, Belgium, Croatia, Norway, the Russian Federation and Serbia (<5/100 000), Bulgaria, Finland, Hungary, Poland and Slovakia (<16/100 000), the Czech Republic, Estonia, and Lithuania (<36/100 000) and Slovenia (<130/100 000) (ecdc.europa.eu).

There are clear differences in LB incidence rates and clinical presentations across Europe[12]. Incidence rates in European countries vary from less than less than one per 100 000 inhabitants to about 350 per 100 000, with a mean annual number of notified cases in Europe exceeding 65 400[13]. Furthermore, the incidence rates of LB across Europe are influenced by geographical, environmental and climatic factors[14,15]. Studies on the future potential distribution of *I. ricinus* notably showed that this tick may emerge in European areas in which they are currently lacking, thus leading to an increased risk to human health[16].

Clinical symptoms and diagnosis

During LB, symptoms may vary depending on the stage of the disease. Early LB may be divided into early localised (1–4 weeks after the tick-bite) and early disseminated (3–10 weeks after the tick-bite) diseases, while late disseminated LB develops months to years later. In early LB, various clinical manifestations may be identified, notably the pathognomonic erythema migrans.

Erythema migrans (EM) is the most specific and frequent finding in patients with LB. European studies showed that it is present in 40 to 77% of LB patients[17]. Primary erythema migrans is a round or oval, expanding erythematous skin lesion that develops at the site of the infecting tick-bite[18]. Three to 30 days after the tick bite the skin lesion becomes apparent (most commonly 7–14 days). It is not associated with significant pruritis[19]. If the skin lesion disappears within a few days, it is not considered as an EM. Other

Table 2. Lyme borreliosis (LB) symptoms.

LB stage	Symptom name	Description	Epidemiology
Early disseminated	Early neurologic disease	Isolated meningitis Encephalopathy Radiculopathy Cranial neuropathy Mononeuropathy Multiplex lymphocytic meningitis Encephalomyelitis	Frequent in Europe
	Cardiac manifestation	Chest pain Palpitations Rhythm disorder	5% of LB cases
Late disseminated	Lyme arthritis	Long-lasting objective joint swelling (synovitis)	More frequent in the US than Europe
	Acrodermatitis chronica atrophicans	Red or bluish-red lesions	Rarely reported in the US Well recognised in Europe
	Borrelial Lymphocytoma	Bluish-red tumour-like skin infiltrate	–

secondary ring-shaped lesions may develop in certain cases and are named multiple EM. Less common LB symptoms are cited in Table 2.

The diagnosis of LB is difficult due to the unspecific nature of the majority of clinical symptoms, and makes laboratory support crucial. The culture of *Borrelia* spp. is time consuming and labor intensive, and is thus not used for the routine diagnosis[20]. In addition, in Europe, the microbiological diagnosis of LB must consider the heterogeneity of the agents depending on countries.

Serology is usually the routine method used to support clinical diagnosis[19,20]. However, serology suffers limitations. In particular, the antibody response in early LB may be weak or absent, especially in EM and early LNB[21]. Western blotting is used currently as a confirmatory assay in the serodiagnosis of LB, but is usually only employed following a positive screening assay. In addition, many biomolecular tests were developed in order to supplement western blot such as sensitive and specific Lyme Multiplex PCR-dot blot assay (LM-PCR assay) and nested polymerase chain reaction (nPCR)[21] applicable to blood and urine samples. However, PCR-based methods are not standardised.

Rickettsioses

Causative agents and vectors

Rickettsioses are worldwide zoonosis caused by obligate intracellular bacteria from the genera *Rickettsia* and *Orientia*. These bacteria belong to the alpha-*Proteobacteria* (Figure 1) and are transmitted by arthropods, mainly ticks, but also fleas, lice and mites[22]. These zoonoses are among the oldest known vector-borne

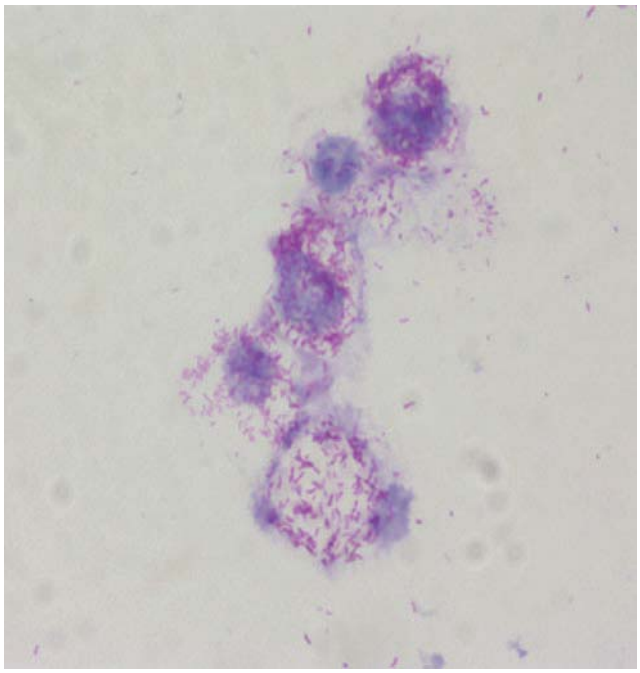

Figure 1. *Rickettsia conorii* cultivated on Vero cells.

diseases. In Europe, only *Rickettsia* spp. are etiological agents of rickettsioses[23]. Tick-borne rickettsioses (TBR) are the main rickettsial infections in Europe and will be developed in the next section.

Epidemiology

The prevalence of tick-borne rickettsioses depends on several parameters and most of them are directly related to the tick vectors: (i) the abundance of the tick itself, which is influenced by many factors, including climatic and ecological conditions; (ii) their affinity for humans; and (iii) the prevalence of

rickettsia-infected ticks. In Europe, several *Rickettsia* species are responsible for tick-borne rickettsioses[24]. *Rickettsia conorii*, the main etiological agent of Mediterranean spotted fever (MSF), is the most important in terms of numbers of cases[24]. Its distribution is restricted to the Mediterranean area where it occurs in Spring and Summer. Other pathogenic *Rickettsia* species in Europe include *R. aeschlimannii, R. helvetica, R. hoogstraalii, R. massiliae, R. monacensis, R. raoultii, R. sibirica mongolitimonae,* and *R. slovaca* (Table 1, Figure 1).

Clinical symptoms and diagnosis

Typically, the clinical symptoms of tick-borne rickettsioses develop 6 to10 days after a tick-bite. Human rickettsioses are characterised by various combinations of symptoms, the most common being a triad consisting of a generalised maculopapular rash, an eschar at the inoculation site (Figure 2) and flu-like symptoms including high grade fever, myalgia, malaise, headache and nausea[25]. However, depending on species and the underlying patient's status, these major clinical signs vary greatly.

The diagnosis of rickettsial infections usually relies on epidemio-clinical data, and may be confirmed by laboratory testing[6]. In addition, the arthropods collected at the bite site or eschar may be useful for the diagnosis.

Serology is the most commonly used and available method worldwide due to its quick turnaround time, need for minimal sample preparation and to the serological cross-reactions observed among *Rickettsia* species that enable limiting the number of tested antigens[6]. Molecular testing is also well adapted for the diagnosis of tick-borne rickettsioses[26]. Molecular techniques overcome the drawback of seroconversion time, needed with serological testing[27]. PCR (either real-time or conventional) can be performed on whole blood, buffy coat or eschar material (crust, swabs, or biopsies)[6,28].

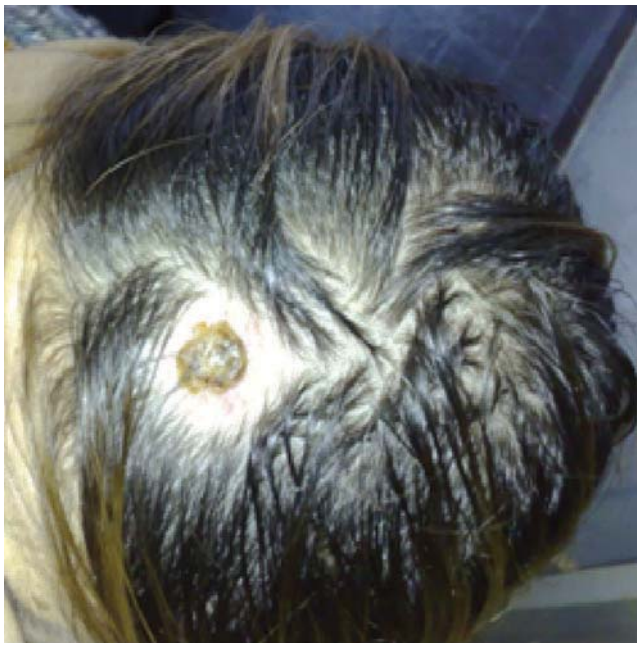

Figure 2. Inoculation eschar to the scalp of a patient with *R. slovaca* infection (SENLAT).

Rickettsial culture is also time consuming and should be performed in a BSL3 laboratory[29]. It is thus reserved to highly specialised laboratories. Matrix-assisted laser desorption/ionisation-time of flight Mass Spectrometry (MALDI-TOF MS) is a promising technique enabling both tick speciation and determining infection with *Rickettsiaceae*[30].

Conclusion

In Europe, the number of vector-borne disease is increasing in some regions. Ticks are notably expanding their range with climate changes. In addition, increased human travel and animal transport result in the epidemiology of tick-borne disease to be in a continuous dynamic change, thus leading to the emergence and/or spread of numerous tick-borne pathogens in Europe. Preventive measures that minimise tick-bite risk are one of the best ways to avoid contracting these diseases. Standardised diagnostic tools are crucial for treating and combating vector-borne diseases, especially when clinical symptoms are not specific. Finally, an increased interest should be given to tick-borne disease to avoid the small bite causing a big problem.

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Biographies

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Non-infectious illness after tick bite

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Tick bites are common and may have non-infectious complications. Reactions range from local reactions to systemic syndromes, tick paralysis, mammalian meat allergy and tick anaphylaxis. Management revolves around prevention with vector avoidance and immediate removal of the tick if bitten. Treatment of bite reactions is usually symptomatic only with anti-histamines or corticosteroids. Adrenaline may be indicated for severe cases.

Ticks are ubiquitous arthropods which incidentally bite humans during outside activities (i.e. exposure to burrows and caves in regards to Argasid (soft) ticks, and exposure to vegetation for Ixodid (hard) ticks)[1]. Seventy species of ticks have been recorded in Australia[2]. Common Argasid ticks that bite humans include *Argas* and *Ornithodoros* species, whereas Ixodid ticks include *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma* and *Ixodes* species[1]. Human-biting ticks in Australia include *A. triguttatum*[3] and Ixodes genus ticks (predominantly *I. holocyclus and I. cornuatus* but include *I. fecialis, I. tasmani, I. australiensis)*[2]. Two biting seasons have been described in south-eastern Australia, the predominant one peaking in October/November with a secondary peak in April[4].

Accurate data about the prevalence of post tick-bite illness are hard to find, but as many as 10% of tick-bite victims may experience illness overseas[5]. This includes local reactions (57.6% of total reactions in Polish patients)[6], systemic syndromes, tick paralysis and anaphylaxis.

Studies of tick saliva

Tick saliva is injected during a bite and contains a complex mix of chemicals. These neutralise host protective mechanisms such as pain, haemostasis, inflammation (which can reduce transmitted infections) and immune reactions[7]. Transcriptome analysis has characterised the sialotranscriptome of specific ticks[8], which changes depending on life stage and feeding status.

Of the human biting ticks, *Ixodes* spp. saliva contains proteins encoded by a metalloproteinase family of genes that inhibit wound healing and facilitate prolonged feeding via anti-haemostatic agents[7].

Boophilus (previously *Rhipicephalus*) bites differentially induce acute phase proteins in infested cows (increased haptoglobin in sensitive and serum amyloid A in resistant strains)[9]. Cows have varying genetic susceptibilities to *Boophilus* tick bite that may be mediated by induction of inflammation (via leukocyte adhesion modulated by ICAM-1, VCAM-1 and P-selectin)[10]. Downregulation of host immunity via regulatory dendritic cells in murine bone marrow[11] and bovine leucocyte recruitment (eosinophils, basophils) have been reported[12]. Cows resistant to tick bite express more E-selectin[12] and downregulate genes encoding production of volatile compounds that attract tick larvae[13].

Local reactions

These can have an erythaematous, nodular, pustular or plaque-like appearance[14]. Local reactions are minimised by immediate removal of the tick[14] with symptomatic treatment (i.e. anti-histamines or corticosteroids).

Gauci divided allergic reactions to *I. holocyclus* into six classes using skin-prick tests and radioimmunoassay (RIA). All systemic hypersensitivity (class 3) and atypical reactions (class 4) were IgE-mediated. 73% of the large local reactions (class 2) and only 12.5% of the small local reactions (class 1) were associated with IgE specific for tick allergens. Heavy exposure to tick-bite was associated with positive RIA values. There was an association between atopic status and tick allergy[15].

Biopsies of tick bites in humans demonstrate deep perivascular and interstitial infiltrates of lymphocytes, neutrophils and eosinophils. Late biopsies show vascular eosinophilic hyaline thrombi which can mimic Type 1 cryoglobulinaemia[16]. Retention of tick mouth parts may drive this inflammatory reaction[17]. Other local reactions include foreign body granuloma, tick bite alopecia (may be scarring or non-scarring[14]), intermediate cell histiocytosis and cutaneous lymphoid hyperplasia[18]. Chronic papular urticaria due to *A. reflexus* has been reported[19].

The local immune response to early tick bite lesions in humans (predominance of macrophages and dendritic cells with elevated mRNA for macrophage and neutrophil chemoattractants as well as IL-1β and IL-5) differs from those with longer tick attachment times (increased lymphocytes and decreased macrophages and neutrophils)[20]. Antibodies directed against components of tick saliva can be detected in humans and used to determine the epidemiology of specific tick activity in certain regions[21].

Systemic syndromes

These include headache (10.8%), fever (5.4%), lymphadenitis (5.9%) and arthralgia (4.3%)[6]. No *in-vivo* physiological studies in humans with systemic symptoms induced by tick bite exist, but systemic toxicosis was demonstrated in an animal model[22]. After *Ornithodoros* ticks fed on rats, hyperaemia of oral mucosa and ocular mucosa, pilo-erection, tachypnoea, ocular and nasal discharge was observed in association with local haemorrhagic lesions. Increased bleeding times, eosinophilia and basophilia, raised creatinine kinase (total and MB) and LDH were noted. Myocardial myocyte degeneration and necrosis was also documented.

In-vitro studies of blood collected from humans previously bitten by ticks, when stimulated with *Ixodes* antigens, was shown to induce basophilia[23].

Symptomatic treatment with anti-histamines or corticosteroids are usually sufficient for this syndrome.

Tick paralysis (TP)

Tick paralysis is caused by several neurotoxins that vary according to tick species and (therefore) region of the world[24]. The best characterised is a 5 kDa protein contained in the saliva of gravid females that interferes with acetyl choline release[25]. Bancroft described the first human case of tick toxicosis in Australia in 1888[14]. TP can be induced by 69 tick species worldwide but Ixodes ticks (*I. holocyclus* or *I. cornuatus*) are usually implicated in Australia[26] and *Dermacentor* (*D. andersoni* and *D. variabilis)* in North America[24]. Widespread reports of TP have subsequently come from Spain, Turkey, Egypt, Ethiopia, Thailand, and Argentina[24]. Cases acquired in Australia but presenting elsewhere have been reported[24], and may delay the diagnosis. Aside from humans, dogs and cats are the most commonly affected animals but sheep, cattle, goats, pigs and horses may also be involved.

Tick attachment sites are predominantly on the head in the US but vary in different regions. Ectopic sites (such as intra-aural[26]) are often associated with delayed diagnosis. Most US cases occur in young girls (possibly due to long hair obscuring the attached tick) but adults are also affected. A flu-like prodrome followed by development of weakness, ascending symmetrical paralysis, ataxia, dilated pupils, slurred speech and depressed deep tendon reflexes is described. Laboured breathing, bradycardia and asystole may develop requiring supportive care. Myocarditis, diplopia and facial palsy may also occur. The duration of illness is very short in American cases after tick removal but is often longer in Australian cases[26]. The differential diagnosis includes Guillain–Barre syndrome, spinal cord lesions, myaesthaenia gravis, botulism, poliomyelitis, organophosphate or heavy metal poisoning and diphtheria. Rapid recognition enables prompt tick removal and avoids inappropriate therapy such as plasmaphoresis[26].

Treatment requires immediate removal of the tick, which may be associated with temporary worsening of the paralysis. In order to not facilitate envenomation, the tick must be killed before removal, which is most readily achieved by freezing with ether-containing agents (i.e. Wart-Off, Tick-Off)[15]. The tick may be removed with narrow forceps applied as close to the skin as possible (which is the most common method used in the USA)[26].

Tick anaphylaxis (TA)

This may due to a direct IgE–mediated reaction against components of tick saliva, or an indirect IgE reaction against galactose-α-1,3-galactose (α-gal, a saccharide found in all non-primate mammalian cells, but not in humans[15]) injected by the tick. It was first reported in Australia in 1940[27] and has since been recognised overseas after *Ixodes*[15] *Rhipicephalus*[28] and *A. reflexus* (in 8%)[29] tick bites.

Management includes prevention with vector avoidance (i.e. application of diethyltoluamide (DEET) to skin, permethrin impregnation of clothes, tucking trousers into socks and daily tick checks), immediate removal of the tick, anti-histamines and corticosteroids and adrenaline for severe cases.

Mammalian meat allergy (MMA)

Red meat allergy triggered by tick bite was first recognised in Sydney in 2007[15] when 25 cases related to *I. holocyclus* bites were reported. Subsequent cases were recognised in eastern Australia and Costa Rica, South-east USA, France, Spain, Germany, Switzerland, Sweden, Italy, Korea, Japan, and China[20]. Aside from *I. holocyclus* and *I. cornuatus*, ticks triggering these events have included *A. americanum*, *I. ricinus* and *H. longicornis*. The author's laboratory recently diagnosed a case of MMA that

was acquired in the Kimberley region, demonstrating that this condition is also found west of the Nullabor Plain. Another subsequent case in WA, possibly related to *I. australiensis* has confirmed this observation[15].

In 2009, delayed anaphylaxis triggered by consumption of mammalian meat was found to be associated with the presence of α-gal-specific IgE antibodies[15] and it was noted that >80% of these patients had a history of tick bite. Subsequently α-gal IgE antibodies were prospectively shown to develop in response to tick bite. α-gal has now been definitively identified in the gastrointestinal tract of *I. ricinus*[15] completing the pathogenetic puzzle. These reactions have been described after eating beef, lamb and pork[15]. Anaphylaxis has also occurred after eating kangaroo meat, but the patient's tick bite status was not known[30]. As well as meat, cetuximab (a mouse-human chimeric antibody)[15], gelatine[15] or milk products can also trigger MMA.

Clinical manifestations, including a delay of 3–6 hours after oral exposure, can range from gastrointestinal upset to angioedema and frank anaphylaxis[15]. Skin prick testing (SPT) typically gives weak reactions (<5 mm) to commercial preparations of mammalian meats but stronger reactions with fresh meat extracts. Patients always have elevated specific IgE levels (>1.0 IU/mL) to the relevant meat, cow's milk, cat and dog reagents as well as to α -gal. SPT and specific IgE levels are always negative to poultry or fish reagents. Management of MMA revolves around avoidance of meat and tick exposures with ready availability of adrenaline (i.e. Epi-Pen) for severe reactions[15].

Australian Multisystem Disorder (AMD)/ 'Debilitating Symptom Complexes Attributed to Ticks' (DSCATT)

Recently a number of Australians have become convinced that a protean illness, which may or may not be associated with tick bite, is a manifestation of locally acquired Lyme Disease (cited in Boyle *et al*.[31]). Enquiries by the Chief Health Officer (cited in Boyle *et al*.[31]) and both houses of Parliament (cited in Boyle *et al*.[31]) were unable to identify convincing proof of this concept. I have proposed that a non-controversial name for the syndrome, 'Australian Multisystem Disorder', should be adopted[32]. The Australian Senate has counter-proposed with the title 'Debilitating Symptom Complexes Attributed to Ticks'[33].

Appropriate management of this syndrome relies on development of adequate research funding to identify the aetiology and efficacious protocols.

Conclusion

Non-infective complications of tick bites are common and may have potentially fatal consequences. Prevention of tick bites is crucial and prompt removal of ticks will limit their adverse effects.

Conflicts of interest

The author declares no conflicts of interest.

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Biography

Professor Beaman graduated from the University of Western Australia and trained in Clinical Microbiology and Infectious Diseases at Sir Charles Gairdner Hospital. He completed a Post Doctoral Fellowship at Stanford University under Professor Remington and then established the first Infectious Diseases Department in Western Australia at Fremantle Hospital. He joined Western Diagnostic Pathology in 2002, where he was Medical Director and Deputy CEO until recently. He is currently an Infectious Diseases specialist at Joondalup Health Campus in Perth.

Bovine theileriosis in Australia: a decade of disease

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Theileriosis refers to the clinical disease caused by organisms from the genus *Theileria*, tick-borne haemoprotozoans infecting a diverse range of mammalian hosts. In Australia, *Theileria* spp. have been identified in both domestic and wildlife species but the bovine parasite, *Theileria orientalis*, has received the most attention due to the emergence and spread of clinical disease over the past 12 years, particularly in cattle herds on the east coast. At an estimated $20 million per annum, the burden to cattle production is significant but despite over a decade of disease, there are still no effective chemotherapeutic treatments or vaccines available in Australia. Recent insights from genome sequencing studies reveal species level diversity within *T. orientalis*, which may help direct efforts at disease control.

Clinical presentation

Theileria orientalis is an apicomplexan parasite that requires both a bovine and a tick host in order to complete its lifecycle (Figure 1).

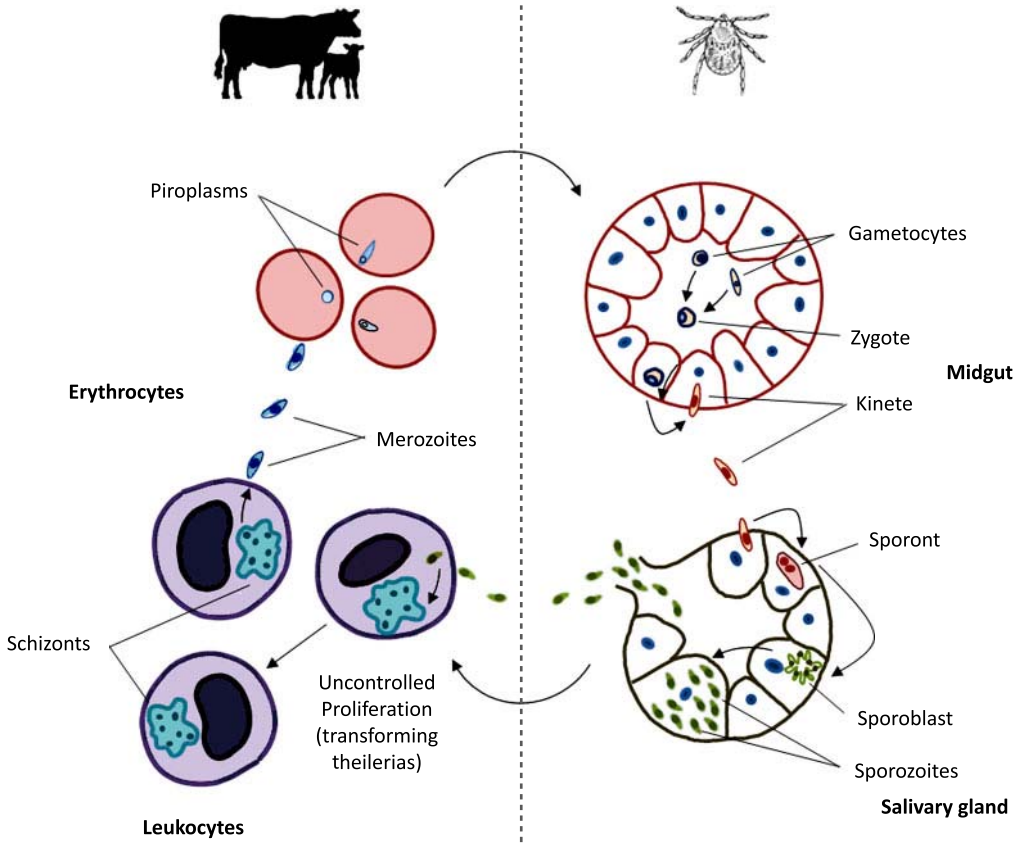

Figure 1. The theilerial intraerythrocytic (piroplasm) phase in the mammalian host is ingested by the tick as it feeds, with gametogenesis occurring in the tick midgut. *Theileria* gametocytes combine to form zygotes in a brief diploid stage within the tick gut lumen. Zygotes which have entered the gut epithelium undergo meiotic division to form motile kinetes which then migrate to the tick salivary gland acini where they differentiate into sporozoites. Sporozoites are the infective stage for the mammalian host and inoculation is achieved as the tick feeds. Sporozoites quickly invade the mammalian host's lymphocytes and develop into multinucleated schizonts. In some species of *Theileria*, known as the transforming theilerias, schizonts induce uncontrolled proliferation of the infected lymphocytes resulting in a lethal cancer-like state. In all *Theileria* species, whether transforming or non-transforming, schizonts go on to produce merozoites that invade erythrocytes to form the piroplasm phase, thus completing the lifecycle.

In Australia, bovine theileriosis is sometimes referred to as bovine anaemia caused by *Theileria orientalis* group (BATOG) to distinguish it from the more severe, exotic bovine theilerial diseases East Coast fever and tropical theileriosis (caused by *Theileria parva* and *Theileria annulata* respectively). Both *T. parva* and *T. annulata* are known as transforming theilerias in that they have their major proliferative stage within bovine leukocytes, inducing a lethal cancer-like state. While *T. orientalis* causes less severe disease than the transforming theilerias, this organism is nonetheless capable of causing up to 5% mortality in affected cattle herds. The major pathogenic effects of *Theileria orientalis* are elicited through the destruction of infected erythrocytes and subsequent anaemia. Therefore, the red blood cell phase (piroplasm), rather than the leukocyte phase (schizont) drives pathogenesis in this species. An enlarged spleen is frequently observed upon post-mortem, along with a large ochre-coloured liver and generalised jaundice[1] brought about by excessive bilirubin from broken down erythrocytes. Animals frequently present with symptoms related to underlying anaemia including lethargy, ataxia and an increased heart and respiratory rate.

The epidemiology of theileriosis in Australia

Prior to 2006, infections with *T. orientalis* in Australian cattle were considered benign. The organism had been observed for over 100 years in blood smears but was considered an incidental finding with very few reports of clinical disease. Early serological surveys suggested the parasite was widespread in NSW and QLD but researchers were unable to induce clinical disease experimentally[1,2]. *T. orientalis* is currently classified into genotypes based on the sequence of the major piroplasm surface protein (MPSP). Up until 2006, MPSP genotypes identified in Australia were Buffeli and Chitose.

Between 2006 and 2008 theileriosis cases were reported in NSW cattle herds with a history of pregnancy or introduction to new herds. Animals presented with abortion, lethargy, jaundice and anaemia. Attention turned to *Theileria* as a cause due to the unusually high numbers of parasites observed in blood smears and after alternative causes of anaemia were ruled out. Follow up molecular testing revealed the presence of a new genotype, *T. orientalis* Ikeda, which was linked to disease in Japan[3]. We

undertook surveillance of a large number of cattle in Australia revealing that this genotype was associated with disease either as the sole agent or in mixed infections with Buffeli and Chitose genotypes[4]. Reports of BATOG increased substantially, and in the intervening years the disease spread throughout coastal NSW and Victoria, south east Qld and into isolated parts of SA, WA and far north Qld (Figure 2). New disease cases have consistently been associated with *T. orientalis* Ikeda.

Immunity

Since 2015, incursions into new areas of the country ceased, although movement of naïve animals into areas where the disease is enzootic remains a major risk factor for disease. Subclinical infections with *T. orientalis* Ikeda are common. In areas where the disease is enzootic it is not unusual to find 100% prevalence in the absence of disease implying a level of immunity, although the immune mechanisms are poorly understood. Animals affected by clinical theileriosis usually seroconvert to the MPSP while subclinically infected animals often lack a detectable humoral response[5]. Calves acquire little protection from the dam via antibodies in colostrum and are highly susceptible to infection[6, 7]. While calves can sometimes be infected transplacentally, this does not appear to be a major route of transmission. Infection dynamics in calves are consistent with tick transmission and animals routinely become highly parasitaemic between 4–9 weeks of age[7]. Given the intracellular nature of the parasite, immunity is likely to be cell-mediated although the potential mechanisms behind this are yet to be explored.

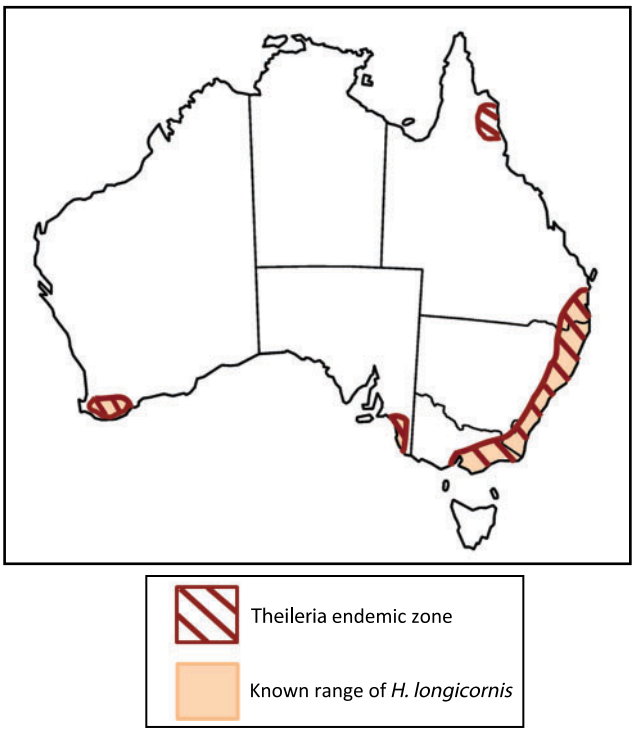

Figure 2. The *T. orientalis* endemic zone and the known range of *H. longicornis* in Australia.

Transmission

A range of tick species have been implicated in transmission of theileriosis overseas, but members of the genus *Haemaphysalis* are considered the main vectors. Studies conducted in Japan demonstrated transmission of *T. orientalis* Ikeda with *Haemaphysalis longicornis*, which was introduced to Australia in the 19th or 20th century. Conversely, transmission work conducted in Australia in the 1980s demonstrated that *H. bancrofti* and *H. humerosa* (latterly believed to be *H. bremneri*) were competent transmitters of *T. orientalis*, while *H. longicornis* was not[2]. The likely explanation for this discrepancy lies in the fact that Japanese studies were conducted with *T. orientalis* Ikeda stock, while studies in Australia were conducted with *T. orientalis* Buffeli. To investigate this further we undertook sampling of ticks from cattle and other domestic and wildlife species within the endemic area, identified the tick species with DNA barcoding, and screened the mouthparts by PCR for *T. orientalis*. A total of 135 ticks were collected representing eight different species; however, only *H. longicornis* ticks tested positive for *T. orientalis*, lending further weight to *H. longicornis* as the likely vector for theileriosis in Australia[8]. Indeed the extent of disease spread in Australia is almost perfectly defined by the known range of *H. longicornis*, which prefers the wetter areas of east coast and is rarely found west of the Great Dividing Range (Figure 2). Small pockets of *H. longicornis* occur in the moist areas of southwest WA and southeast SA where *T. orientalis* Ikeda outbreaks have also been reported. Thus, while never directly demonstrated, the evidence overwhelmingly points to *H. longicornis* as the major vector for bovine theileriosis.

In addition to tick transmission, mechanical transmission can be achieved experimentally with as little as 0.1 mL of blood when parasite levels are high and can induce clinically relevant levels of *Theileria* in the recipient animal[6]. While merozoites can undergo several rounds of proliferation within erythrocytes, mechanical transfer (via non-tick arthropods or iatrogenic means) is unlikely to support parasite persistence in the long term as it bypasses the sexual stage of the lifecycle that is required to maintain genetic diversity within the population.

Control

There are currently no effective chemotherapeutics or vaccines for the control of bovine theileriosis in Australia. Treatment of

(*a*)

T. orientalis Ikeda (Australia)	*T. orientalis* Chitose (Australia)	*T. orientalis* Buffeli (Australia)	*T. parva*	*T. annulata*	
99.2%	82.6%	82.6%	66.1%	66.0%	*T. orientalis* Shintoku (Japan)
	82.8%	83.1%	66.1%	66.1%	*T. orientalis* Ikeda (Australia)
		86.5%	66.3%	66.2%	*T. orientalis* Chitose (Australia)
			67.0%	66.9%	*T. orientalis* Buffeli (Australia)
				79.5%	*T. parva*
					T. annulata

(*b*)

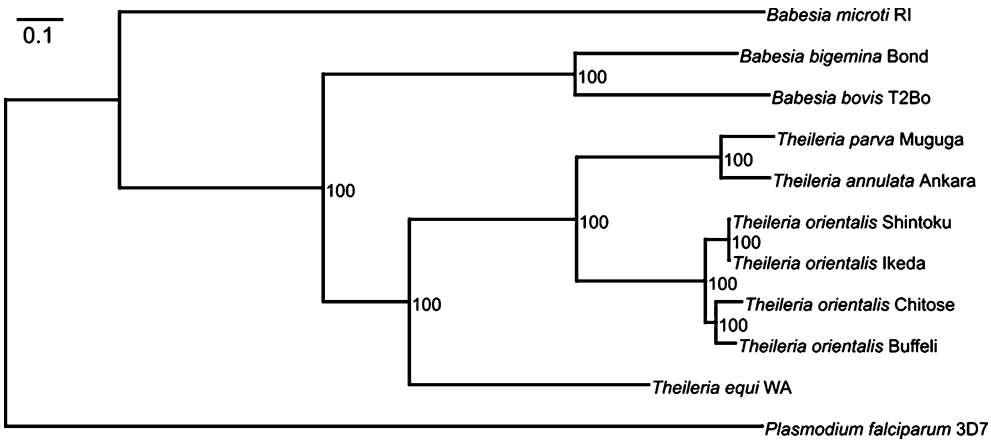

Figure 3. (*a*) Average nucleotide identity of key *Theileria* strains as determined from genome sequences. (*b*) Phylogenomic analysis of *T. orientalis* Ikeda, Chitose and Buffeli relative to reference strains, including *T. orientalis* Ikeda (Shintoku strain) from Japan, based on 654 protein coding genes. Figure adapted from Bogema *et al*.[13].

vector ticks via acaricides and minimising the movement of cattle from non-endemic to endemic areas are the main methods of disease management. Imidocarb, erythromycin or oxytetracycline are sometimes administered to affected animals, but to little effect[9]. In New Zealand, blood transfusion is regularly undertaken on animals that are moderately to severely anaemic, but this practice is costly and time consuming. Buparvaquone, a known anti-protozoal is used to treat BATOG in New Zealand and is also used to treat East Coast fever in Africa. When administered in a timely fashion, buparvaquone is effective against *T. orientalis*, yet this drug is not approved for use in Australia due to its tendency to leave residues in meat and milk[10] and the need to observe lengthy withholding periods. Vaccination would be the preferred option for disease control but there has been little progress towards a vaccine for this disease worldwide. Despite assertions that a live vaccine based on the benign Buffeli genotype would be a potential way forward[11], there is little hard evidence that this genotype provides protection in naturally infected animals. In other *Theileria* species, immunisation with one variant does not result in heterologous immunity against other variants. Furthermore, high seroprevalence of animals to *T. orientalis* Buffeli and/or Chitose in NSW prior to 2006[1] failed to prevent widespread outbreaks of disease caused by *T. orientalis* Ikeda. Development of subunit vaccines is generally regarded as problematic for apicomplexan parasites due to genetic diversity within parasite populations. Nonetheless some early work in Japan demonstrated partial protection against theileriosis using a subunit vaccine formulation of the Ikeda MPSP antigen with Freund's adjuvant or liposomes[12]. Despite these

initially promising results, no further vaccine development has been undertaken with this or with other antigens.

Lessons from genome sequencing

Recent draft genomes of Ikeda, Chitose and Buffeli genotypes of *T. orientalis* may assist in providing insights into the differential pathogenesis of these subtypes[13]. Surprisingly, these genomes revealed potential species level diversity within *T. orientalis* with average nucleotide identities almost as low as observed between *T. annulata* and *T. parva* (Figure 3). Phylogenetic analysis of 654 protein-coding genes also showed that *T. orientalis* Ikeda forms its own lineage relative to *T. orientalis* Buffeli and Chitose, while the Japanese and Australian strains of *T. orientalis* Ikeda are remarkably similar[13]. The origin of *T. orientalis* Ikeda in Australia has never been elucidated although the importation of a small number of Wagyu breed cattle into Australia from Japan in the late 1990s has been proposed as one potential route of introduction. Genome sequencing of further international isolates of *T. orientalis* may lend weight to this theory. The origin of introduction is potentially highly relevant to the issue of vaccine development. If *T. orientalis* Ikeda in Australia arose from only a limited parasite population, then genetic diversity would be expected to be relatively low, making the prospect of developing a long-lasting vaccine for this disease more likely. Further genome-based studies are currently being undertaken to establish the genetic diversity within the Ikeda genotype in Australia.

Conflicts of interest

The author declares no conflicts of interest.

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Biography

Dr Cheryl Jenkins is a Principal Research Scientist at the NSW Department of Primary Industries with an interest in parasitic and bacterial diseases of production animals.

Variables affecting laboratory diagnosis of acute rickettsial infection

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The reference standard for the confirmation of a recent rickettsial infection is by the observation of a four-fold or greater rise in antibody titres when testing paired acute and convalescent (two to four weeks after illness resolution) sera by serological assays (Figure 1). At the acute stage of illness, diagnosis is performed by molecular detection methods most effectively on DNA extracted from tissue biopsies (eschars, skin rash, and organs) or eschar swabs. Less invasive and more convenient samples such as blood and serum may also be used for detection; however, the low number of circulating bacteria raises the possibility of false negative results. Optimal sampling practices and enhanced sensitivity must therefore be considered in order to provide a more accurate laboratory diagnosis.

Human pathogenic bacteria from the genus *Rickettsiae* cause mild to severe diseases worldwide. The rickettsial agents (spotted fever group) found in Australia include *Rickettsia australis, R. honei* subsp. *marmionii,* and *R. felis* typically cause mild to moderately severe illness. Nonspecific symptoms of all rickettsioses at the early stage of illness confound clinical diagnosis. Patients should be given appropriate antibiotic therapy upon suspicion of having a rickettsial disease because it is essential for effective treatment especially in more severe rickettsioses, such as Rocky Mountain spotted fever (RMSF), where a delay in doxycycline treatment correlates with more dire outcomes and death. RMSF is caused by the Gram-negative Alphaproteobacteria, *R. rickettsii*. With fatality rates from 5% to 42% in paediatric cases in the US and Mexico[1,2], early clinical diagnosis and doxycycline treatment are essential for a positive prognosis. However, clinical diagnosis is difficult because symptoms at the initial stage of illness are nonspecific and may include fever, chills, headache, and malaise; and the characteristic maculopapular rash, which spreads centripetally and can involve soles and palms, may not be seen or may only present later at two to four days after symptom onset[2]. Although RMSF is not endemic to Australia, international travel and exposure to arthropods should be considered during clinical diagnosis.

Molecular detection is readily reported and may be used for the confirmation of disease at the acute stage of illness. However, because *Rickettsia* are obligate intracellular bacteria, these organisms localise in endothelial cells and the level circulating in blood is believed to be low at the early stage of infection, less than 100 copies per mL of blood[3,4]. The low bacteremia may equate to less than 1 genome equivalent per 10 µL of blood. Therefore, rickettsial DNA may not be in the test reaction, or may be present below the reproducible limit of detection[5]. Positive results confirm disease, but negative results can only describe that there was no detectable target DNA in the reaction. Other factors affecting molecular detection of *Rickettsia* in blood includes the timing of sample draw, patient antibiotic treatment, sample age, sample stabiliser, and assay sensitivity.

Timing of sample draw and antibiotic treatment

For the detection of rickettsial DNA in blood by molecular methods, the sample must be taken before or within 48 hours of appropriate antibiotic treatment to minimise false-negative results[2]. Note: antibiotics must not be withheld and patients should be empirically treated upon suspicion of rickettsial infection. Due to the fast progression and potential severity of these diseases, early treatment is essential for the best possible outcome[6]. False negatives due to low rickettsial bacteremia are difficult to verify so the level of detection efficiency at the acute stage of illness is not clear at this time.

Sample age and blood collection tubes

The standard retention time of blood for PCR testing is within seven days of sample draw. Ethylenediaminetetraacetic acid (EDTA) blood collection tubes are used in haematology testing and are reported most often for the molecular detection of *Rickettsia*[2] as well as other infectious diseases. Acid citrate synthase anticoagulants have also been described as acceptable for molecular testing, while heparin has been described as having an

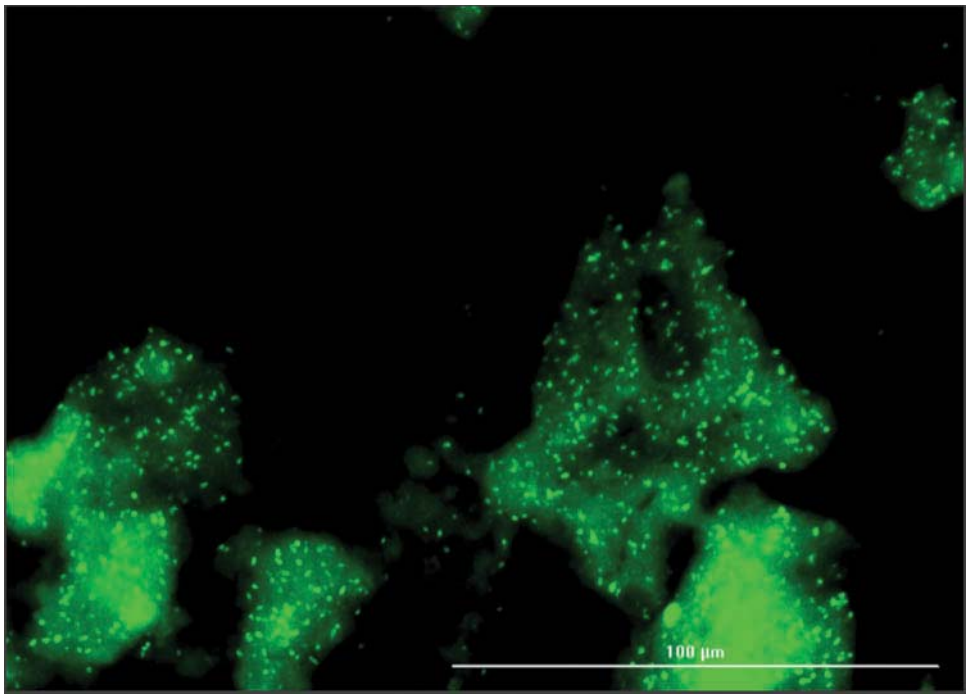

Figure 1. The reference standard for rickettsial disease confirmation, indirect immunofluorescence antibody (IFA) showing human antibody response to *R. rickettsii* antigen.

inhibitory effect on polymerase activity. The literature describing PCR anticoagulant compatibility originated with protocols using phenol-chloroform extractions and early master mix formulations[7]. Since these early reports of PCR/blood stabiliser compatibility, new reaction mix and extraction chemistries and technologies have been developed and must be examined with the reagents and methodologies within individual labs. We verified that the performance of the reagents and extraction products used in our testing are compatible with all three stabilisers stored at 4°C and our current methods[8]. We examined the level of detection of rickettsial target in contrived samples using blood treated with EDTA, acid citrate dextrose solution A (ACD-A), and sodium heparin over seven days and have verified the use of these blood stabilisation types as compatible with our testing methods. While ACD-A and heparin additives provided testable extraction products comparable to or better than the current collection tube standard, the EDTA samples showed a decline in target detection within the seven day period (Figure 2).

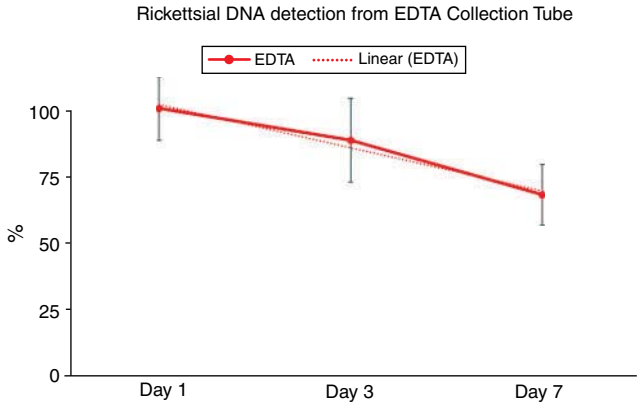

Figure 2. The average percentage of *R. rickettsii* DNA detected from contrived samples stored at 4°C on days 1, 3, and 7 from blood collected in EDTA tube.

Assay sensitivity

Current molecular detection assays for rickettsial diseases include real-time PCR and isothermal amplification protocols with specificities varying from 78% to 99% and limits of detection from one to 10 copies per reaction[5]. These methodologies are at the limit of detection for these targets and technologies. This calculates to 200 to 2000 genome equivalents per millilitre of blood, which is still above the detection range needed (less than 100 copies per mL of blood) at the early stage of illness. Due to the variation in protocols it is unclear if the differences in sensitivities are due to amplification targets, reagents, instrumentation, extraction methodologies, or assessment strategies. RNA detection has increased the detectable range, as the target numbers may be higher than the DNA copies as long as labile RNA transcripts have not degraded.

Conclusion

There is currently an undefined level of accuracy for molecular detection methods in blood due to current DNA assay sensitivity and overall variation in best practices for sampling, stabilisation, and preparation. It is important to be mindful of the following when testing blood.

(1) Draw sample during the symptomatic stage of illness, before or within 48 hours of doxycycline treatment.
(2) Samples must be processed as soon as possible or within days to avoid template degradation, especially if EDTA is the blood stabiliser.
(3) Assessment of alternative targets might increase assay sensitivity. RNA detection is a promising target and its utility and limitations are yet to be defined.

The optimisation of all preanalytical and analytical processes may improve rickettsial molecular detection in blood at the acute stage of illness. Further validation is needed to determine a standard for sample collection and handling to improve integrity of specimens suspected of rickettsial infection.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgements

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Biography

Dr Cecilia Kato is the *Rickettsia* Diagnostics Team Lead and Director of the Reference Diagnostic Laboratory at the Centers for Disease Control and Prevention, Division of Vector Borne Diseases, Rickettsial Zoonoses Branch in Atlanta Georgia, in the United States. Cecilia has been at the CDC since 2008 and has served as the lead of the Diagnostics Lab since 2012. The *Rickettsia* Diagnostics Team supports United States and international Public Health Labs with the laboratory diagnosis of rickettsiosis, ehrlichiosis, anaplasmosis, scrub typhus, and Q fever. Her research emphasis is on assay development, which includes a provisional patent for enhanced sensitivity for *Rickettsia* species detection in patient samples, a FDA cleared *Rickettsia* real-time PCR kit, point of care diagnostics, and enhanced surveillance. She also works with international partners to build diagnostic capacity and supports epidemiological studies and outbreak response.

Rethinking *Coxiella* infections in Australia





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***Coxiella burnetii* is the causative agent of coxiellosis in animals and Q fever in humans. Despite being a vaccine preventable disease, Q fever remains a frequently reported zoonotic infection in Australia. Recently, a *Coxiella* species was identified in brown dog ticks (*Rhipicephalus sanguineus*) in urban and rural regions of Australia. Further molecular characterisation revealed that it is genetically identical to '*Candidatus* Coxiella massiliensis' (KM079627) described in *R. sanguineus* ticks removed from humans with eschars in France and serologic cross-reactivity among '*Ca.* Coxiella massiliensis' and *C. burnetii* may occur. This report highlights the need for molecular testing of seropositive companion animals and humans to determine which species of *Coxiella* they are infected with, in order to further assess *Coxiella* species associated with *Coxiella* infections in Australia.**

Coxiella burnetii is a small, obligate intracellular, Gram-negative coccobacillus found worldwide (except in New Zealand) and has a sylvatic lifecycle involving wildlife and domestic mammals, birds, and arthropods[1,2]. *Coxiella burnetii* was first described in the 1930s as the causative agent of Q (query) fever in abattoir workers in Brisbane, Queensland, Australia[3]. *Coxiella burnetii* is also the known cause of coxiellosis in animals and is persistently shed by infected animals in secretions and parturient by-products. Transmission occurs predominantly through direct or indirect contact with infected tissues from domestic ruminants and companion animals, rather than as a consequence of tick bite[4]. Clinical presentations of Q fever range from acute to chronic, and can lead to post-Q fever fatigue syndrome, although asymptomatic Q fever represents >54–60% of infections[3]. High annual reports of human Q fever in Australia persist despite a readily available vaccine[5]; over 4800 cases were reported between 2007 and 2017, with 716 notifications of Q fever in the past 18 months[6].

Australian serological surveys have reported the number of infected dogs with *C. burnetii* has increased over 26 years to nearly 22%[7], with free-roaming dogs within Indigenous communities having the highest seroprevalence compared with breeding, pet, or shelter dogs, in a most recent study[8]. It has been proposed that dogs become infected with *C. burnetii* through consumption of infected raw meat, hunting, and scavenging wildlife, or due to heavy tick infestations[8], most commonly with *Rhipicephalus sanguineus* ticks[9]. While our knowledge about the epidemiology of *C. burnetii* in companion animals continues to increase, it is unclear whether the high *C. burnetii*-seropositivity observed in these animals contributes to increasing reports of Q fever cases in humans.

In addition to *C. burnetii,* several other *Coxiella* species and subtypes of the genus have been identified in a range of different hosts, including *C. cheraxi,* the cause of mass mortalities in Australian redclaw crayfish, (*Cherax quadricarinatus*)[10]; *Coxiella* spp. endosymbionts of ticks[11]; and more recently, '*Candidatus* Coxiella massiliensis', associated with ticks removed from humans with eschars[12]. Molecular evidence suggests that *C. burnetii* originated from an inherited symbiont in soft ticks and acquired virulence factors enabling it to infect vertebrate cells[11]. To date, over 40 tick species have been associated with *C. burnetii* and *Coxiella* spp. *Amblyomma, Dermacentor, Ixodes,* and *Rhipicephalus* species are the most frequently implicated vectors[11,13].

Tick-associated *Coxiella* spp. have a role in maintaining tick health and influence the vertical transmission of other tick-borne pathogens[14]. Due to their symbiotic role in ticks, *Coxiella* spp. endosymbionts of ticks are considered non-pathogenic to vertebrates, however, the dogma of what is considered an endosymbiont versus a pathogen has been challenged recently though the observation of serological reactions to a number of tick-associated endosymbionts in people following a tick bite[14,15]. Furthermore, a retrospective study identified *Coxiella* sp. ('*Ca.* Coxiella massiliensis') in several tick species, including *R. sanguineus* ticks removed from patients presenting with scalp eschars, cervical lymphadenopathy, fever, increased C-reactive protein and thrombocytopenia[11,12]. Following the recent molecular characterisation of a *Coxiella* sp. in *R. sanguineus* ticks in Australia[16], this present study screened 41 *R. sanguineus* ticks with a *Coxiella*-specific *GroEL* PCR assay to determine the genetic relatedness to '*Ca.* Coxiella massiliensis'.

A *Coxiella*-specific PCR assay, targeting a 659 bp region of the *GroEL* gene was performed using the primers Cox-660f (GGCGCICARATGGTTAARGA) and Cox-1320r (AACATCGCTTTACGACGA) according to Angelakis *et al*.[12], with the following modifications: each 25 µL PCR reaction contained 1× Perfect Taq buffer (5 Prime, Germany), 1 mg/mL BSA (Fisher Biotech, Australia), 2.5 mM $MgCl_2$, 1 mM dNTPs, 400 nM of each primer, 1.25 U Perfect Taq polymerase (5 Prime, Germany) and 2 µL of undiluted DNA. All samples were performed under the following thermal conditions: initial

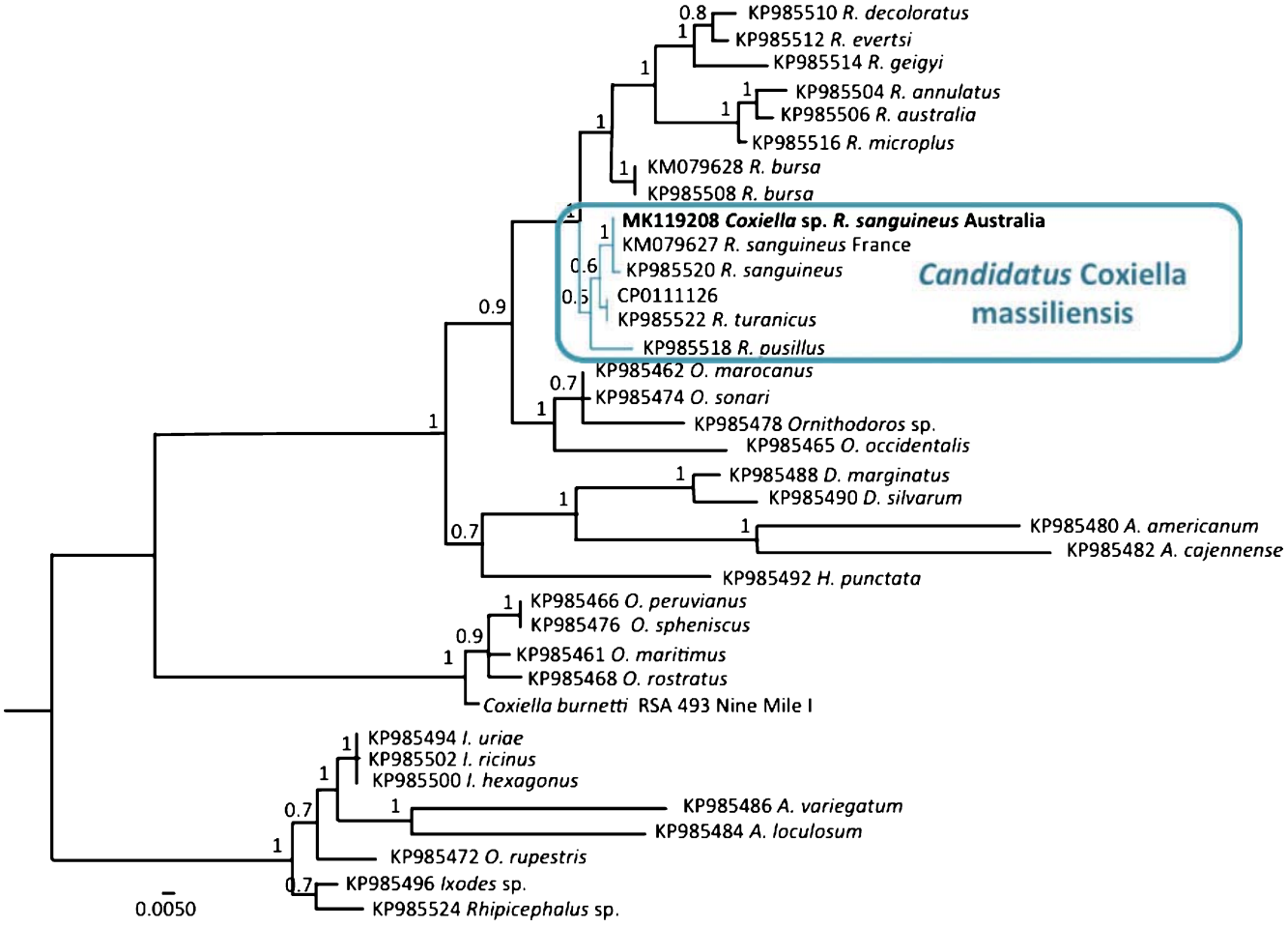

Figure 1. Phylogenetic tree based on 547 bp *GroEL* gene sequences including *Coxiella* associated with ticks, *C. burnetii* reference strain and an outgroup, *Rickettsiella gyrll* (cropped). The proposed '*Candidatus* Coxiella Massiliensis'[12] is highlighted by the teal box. The Bayesian tree was constructed using MrBayes 3.2.6[16] with posterior probabilities and the following parameters were used: substitution model GTR, gamma category 5, chain length 1,100,000, sampling every 200 trees and burn-in length 100,000. Bold type indicates the consensus sequence from this study. Abbreviations: *A.*, *Amblyomma*; *D.*, *Dermacentor*; *I.*, *Ixodes*; *O.*, *Ornithodoros*; *R.*, *Rhipicephalus*.

denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 30s, annealing at 52°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. A phylogenetic tree was constructed with a 547 bp trimmed alignment of all known *Coxiella GroEL* sequences, including those obtained in this study, with MrBayes 3.2.6[17].

DNA was successfully amplified in 80% (33/41) of the *R. sanguineus* ticks and Sanger sequencing was conducted on 10 positive samples according to Oskam *et al*.[16]. All 10 sequences were identical to each other (MK119208), and 100% similar to '*Ca.* Coxiella massiliensis' isolated from *R. sanguineus* in France (KM079627). Phylogenetic analysis revealed the '*Ca.* Coxiella massiliensis' identified in this study had high support (posterior probability 1.0) to '*Ca.* Coxiella massiliensis' found within other *R. sanguineus* ticks (Figure 1)[12]. The prevalence of '*Ca.* Coxiella massiliensis' in this study was higher than the '*Ca*. Coxiella massiliensis' prevalence of 35% (7/20) reported by Angelakis *et al*. in *R. sanguineus*[12].

It is still unknown whether '*Ca.* Coxiella massiliensis' can be transmitted to humans via tick bite or aerosol inhalation in Australia, however it prompts further investigation to determine if cross-reactions can occur among other *Coxiella* sp. in Q fever serological tests. This study highlights the need for molecular testing of companion animals and humans that are seropositive for *C. burnetii* to determine which species of *Coxiella* they are infected with and to comprehensively assess all species of *Coxiella* in Australia for health risks.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Dr Charlotte Oskam is a senior lecturer and team leader in the Vector and Waterborne Pathogens Research Group at Murdoch University. Her research interests extend from ancient DNA, microbiomes, ticks, to zoonoses.

Jadyn Owens is a Murdoch University graduate in Molecular Biology and completed an independent study contract supervised by Dr Oskam in the Vector and Waterborne Pathogens Research Group at Murdoch University.

Annachiara Codello was a research assistant during this project in the Vector and Waterborne Pathogens Research Group at Murdoch University.

Alexander Gofton is a PhD student in the Vector and Waterborne Pathogens Research Group at Murdoch University. His research interests are in tick microbiomes and tick-borne pathogens of animals and humans.

Telleasha Greay is a PhD student in the Vector and Waterborne Pathogens Research Group at Murdoch University. Her research interests are in tick microbiomes and tick-borne pathogens of companion animals.

EduCon 2018

Karena Waller
ASM Ed SIG Chair

This year's ASM EduCon was held on Wednesday 4 and Thursday 5 July 2018 in Brisbane at the Mantra South Bank Hotel. It was a fabulous meeting, attended by 28 registrants from around the nation in the fields of microbiology education, and education more broadly. Over the 2-day program, registrants enjoyed a diverse program of engaging presentations on teaching and learning, and issues in higher education while enjoying the plentiful and tasty catering supplied by the venue.

The meeting commenced with Associate Professor Tracey Bretag, from the University of South Australia, delivering an engaging and enlightening, yet very sobering, presentation on *Contract cheating in Australian higher education: Results from a nation-wide survey of students and staff.* Tracey presented her research findings (funded by the Australian Government Department of Education and Training) regarding the nature, extent and motivations for student engagement in contract cheating within the context of the Australian higher education environment.

Dr Terrence Mulhern, from The University of Melbourne, summarised his work on student misconceptions in a presentation titled *Learn from your mistakes. How to use misconceptions to trigger student learning*. In his presentation, Terry explained what misconceptions are, where they come from, and some handy strategies of identify and overcoming them in the context of large STEM classes.

Thursday's program commenced with Dr Raina Mason, from Southern Cross University (Gold Coast) delivering a thought provoking presentation, titled '*This assessment makes my brain hurt!*' – *accounting for cognitive load in assessment*. Raina's presentation succinctly summarised cognitive load theory, and how learning can be negatively impacted when the cognitive load of a student is exceeded. Raina also detailed some examples from her own teaching and research outcomes, aimed at identifying and avoiding cognitive overload in student assessment tasks.

Dr Karena Waller, recipient of the 2017 ASM David White Excellence in Teaching Award, delivered a presentation titled *Reaching out in Microbiology*, describing her passion for developing and delivering laboratory-based outreach activities for local and international high school student programs visiting the microbiology labs at The University of Melbourne.

Associate Professor Kelly Matthews, from the University of Queensland, presented her research findings regarding engaging students as partners in their learning. Kelly's presentation, titled *Challenging and expanding our beliefs about the role of students in scholarly learning and teaching practices,* described some practical examples of engaging students as partners in their learning, and the positive outcomes for students within STEM disciplines.

The program concluded with Ms Lyris Snowden, from the University of the Sunshine Coast, delivering a presentation titled *The pros, cons and diversity of Work Integrated Learning (WIL): the experience of putting WIL into practice.* Lyris provided great insight into the potential trials, tribulations and incredible benefits of incorporating WIL opportunities into teaching and learning programs in higher education.

Our meeting was very proudly sponsored by McGrawHill Education, Monash University and The University of Melbourne. We are extremely grateful for their very generous support. Given the huge success of EduCon this year, in addition to the many wonderful conversations and networking opportunities it provided, I am already looking forward to seeing you all at the next ASM EduCon, to be held in Adelaide in July 2019.

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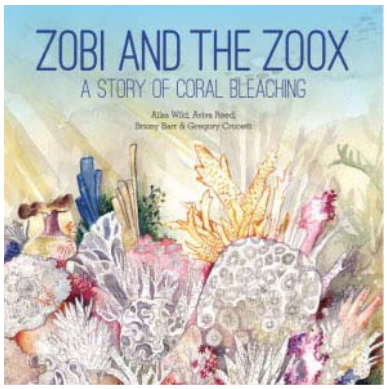
Book reviews

Zobi and the Zoox; A Story of Coral Bleaching

Ailsa Wild, Aviva Reed, Briony Barr and Gregory Crocetti
CSIRO Publishing, 2018



It sounds like a great name for a rock and roll band, but it's a highly educational and quite entertaining story book about the microbial ecology of coral-microbial symbioses and coral bleaching. Zobi (a rhizobium) and the zoox (dinoflagellate zooxanthellae) live symbiotically with a coral polyp named Darian, who is suffering from heat stress. Their problems are explained and illustrated creatively and beautifully in colourful, large artistic drawings of chemical reactions, cellular processes, cells and tissue layers. Zobi and Cy (a cyanobacterium in the coral mucus) watch in horror as Darian expels thousands of zoox from his gastrodermis into the surrounding ocean. The microbial consortium in the mucus starts to die off as slimy green algae invade. Can the rhizobia assist the zoox in time to save Darian and his colony, which is home to them all?

I'll leave you in suspense, but rest assured the story has a satisfying ending and a good message about threats to coral reefs from climate change. A strong theme throughout the story is biological symbiosis and the value of working together for mutually beneficial goals. Another effective theme is that of spatial scale, emphasising interacting processes within and across molecular, cellular, organismal, reef, and even planetary levels. Much of the exceptional and delightful artwork shows enlargements of microbes or chemical reactions (in blow-up circles akin to a microscope's field of view) set against the larger context of a coral polyp or a reef community of corals, fish, and sea turtles.

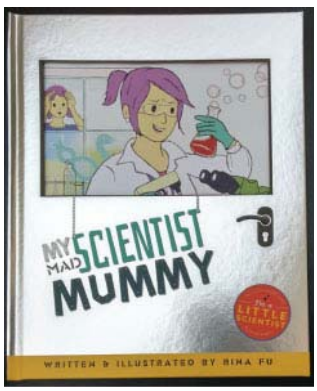
The 28-page story is followed by a 17-page tutorial on 'The Science Behind the Story', which explains the biology of the organisms and symbioses, the chemistry of processes like photosynthesis and nitrogen fixation, the microbial ecology of mucous layers, coral reefs and coral bleaching at a quite sophisticated level for young readers, yet in simple language and with additional beautiful artwork and micrographs. Other highlights are the pronunciation guide at the start and the illustrated glossary at the back.

The book serves a variety of audiences and purposes. The story itself would be engaging and educational for older primary school children, while the tutorial would be instructive, enjoyable, and sufficiently challenging for secondary school students. Although, to high school students it might feel too much like a children's book overall. For professional microbiologists, the book will be fun and helpful to those wanting to promote an understanding of microbial ecology and environmental issues to a young audience, and the book serves as a model of effective science communication to the public. The artwork itself would be pleasing and a great conversation starter on a microbial ecologist's office wall!

Associate Professor Jeff Shimeta is a marine scientist at School of Science, RMIT University, Melbourne, Australia.

My Mad Scientist Mummy

Rinu Fu
Little Steps Books, 2018



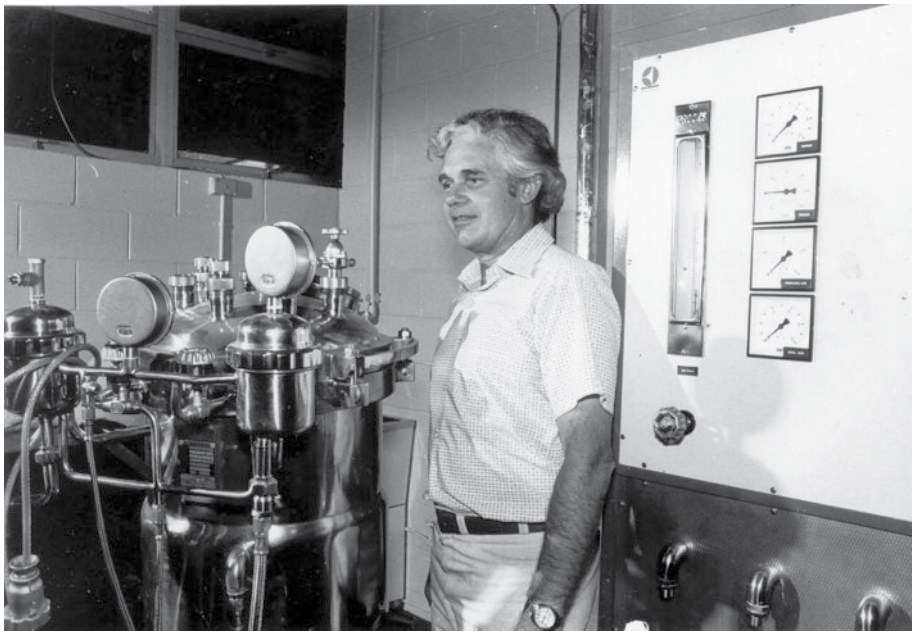
My Mad Scientist Mummy is a cool book for kids. It is a great way to tell young children at home and school about the great world of work as a scientist who, by the way, is also a MUM. Dr Madeline Mummy is happily ensconced in her laboratory with her flasks and microscope as a research scientist. She has lots of smiling scientist friends she works with, even some laboratory staff with big rabbit ears and long tails. None of them look mad, but Dr Mummy's little daughter must have heard whispers about 'mad scientists' because she does wonder if her Mummy might be mad. Well, after exciting adventures to the lab, where she is decked out in cool safety gear she watches open mouthed with wide eyes as Mummy's exciting experiments eventually come to life. All is well – Mummy is not a mad scientist – but – turn the page – she can get mad!

Having had three children myself who struggled to picture my non-mum scientist life, this children's book, with great illustrations, is a novel idea that should be embraced by young scientist mums everywhere. A good bed-time story for the kids when you come home from work!

Prue Bramwell is a microbiologist and academic at RMIT University.

Vale A/Professor Horst Werner Doelle (1/9/1932–6/9/2018)

Horst graduated with a degree in Botany and Microbiology in 1954 from the University of Jena in Germany, followed by a 'Dr. Rer. Nat.' which is a Doctor rerum naturalium, literally: Doctor of the things of nature, doctor of natural sciences, which is a postgraduate academic degree awarded by universities in European countries equivalent to a PhD. Dr Doelle started at the University of Queensland (UQ) in 1964 as a Senior Lecturer in Microbiology at the Department of Microbiology as one of the founding members of this Department. He was subsequently awarded a PhD from UQ in 1966 and a DSc in 1975 for recognition of his publication outputs. From 1974 until his retirement in 1992 he held the position of Associate Professor at the Department of Microbiology at UQ. Professor Victor Skerman enabled Horst to develop his program of research. He was invited to deliver more than 70 lectures and courses worldwide, with more than 10 of these post retirement. He was a an active member of ~20 societies and organisations including the Australian and American Societies for Microbiology, British Society of General Microbiology, Australian and British Biochemistry Societies, World Federation of Culture Collections, International Cell Research Organisation, New York Academy of Sciences, International Organisation for Biotechnology and Biochemical Engineering, American Biographical Insistute, Australian Institute of Energy, and Uniquest's 'Consultant Club'. At UQ he also served as a Faculty of Science Executive and Acting Dean, an honorary member of the Department of Chemical Engineering, and the Director of MIRCEN-Biotechnology. He published avidly and also patented eight processes he discovered which led to the development of five companies in the 1980s. Ultimately Dr Doelle loved teaching and developed microbial physiology/biochemistry programs delivered to biochemists, microbiologists and chemical engineers at UQ. The lectures encompassed thermodynamics of biological systems in relation to yeast and mammalian systems as well as detailed lectures on catabolic and biosynthetic events together with their regulatory mechanisms and applications for the improvement of mankind. In 1979 he created the microbial technology and biotechnology program at UQ at the postgraduate level and in 1985 at the undergraduate level. In his day Horst was happy that the course was approved by UNESCO (the United Nations Educational, Scientific and Cultural Organization) and also listed as one of UNESCO's Microbial Resource Centre's for Biotechnology (MIRCENs are academic/research institutes co-operations to harness international scientific cooperation). He had a passion to elevate science in developing countries and he visited many countries across Asia and Africa during his career. His vision was vast and now UQ's Biotechnology program is ranked 7th in the world out of 4000 Universities/Institutions and number one in Australia. He officially retired from UQ in 1992 and continued working as a consultant and publisher with a Biotechnology e-book edited by him as recently as 2008.



Vale Dr David Leslie

VIDRL Staff mourn the loss of Dr David Leslie, Medical Microbiologist, who passed away on 4 July after a long illness. David will be remembered for his keen intellect, his commitment, and his passion for his microbiology discipline, with specific expertise in Mycobacteria and Syphilis where he was a leading authority within Australia. He was greatly respected and valued as a source of advice by colleagues around Melbourne, and further afield. David's leadership was always welcoming and encouraging to the many scientists and clinicians that were fortunate enough to work with him.

David's relationship with VIDRL has been a long one. In 1986 he trained as a registrar in the Microbiology Department, Fairfield Hospital, which has gone on to become part of VIDRL. He was then appointed as Medical Microbiologist and Head of the Department of Clinical Pathology in 1993, and Deputy Director of VIDRL in 1995. He was instrumental in the computerisation of the Clinical Pathology Department; and greatly facilitated the unification of Clinical Pathology and Virology in 1993 under Stephen Locarnini's leadership to form VIDRL. David had a strong belief in the concept and importance of a public health reference laboratory during a challenging climate of economic rationalism.

David was active in committee work, serving lengthy periods on Victorian advisory committees for Tuberculosis, Syphilis, and Sexually Transmitted Diseases and Blood-Borne Viruses. He was an expert reviewer for many peer-reviewed journals including the Journal of Clinical Microbiology, and Clinical Infectious Diseases. He was a long-standing examiner for the Royal College of Pathologists of Australasia (RCPA); and reviewed serology quality assurance programs for RCPA in Syphilis, Legionella and Hydatids.

David was also closely involved in registrar training. There are a great many clinical microbiologists and infectious diseases physicians, around Melbourne, and Australia who have spent time as registrars at VIDRL, and have been supervised by David. That generation of expertise is part of David's legacy.

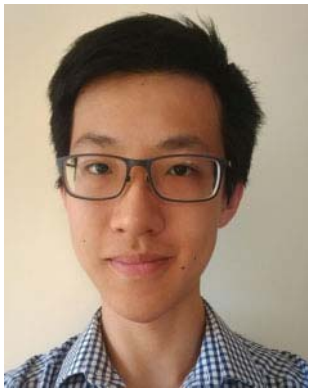
After some years in New South Wales, David returned to VIDRL in 2002 as Medical Microbiologist and Head of the Division of Microbiology and Laboratory Services. He resumed his leadership of the Victorian Mycobacterium Reference Laboratory at VIDRL, and his representation on the Victorian Tuberculosis Advisory Committee and more recently the Victorian Syphilis Advisory Committee. Syphilis having long been a rare disease, had undergone resurgence by this time, and David's expert advice has been in high demand. David foresaw the need for improved Syphilis diagnostics, and was instrumental in the introduction of Syphilis nucleic acid testing at VIDRL, and championing syphilis research. Between 2010 and 2014 as part of VIDRL's leadership David contributed to design of new facilities at the Doherty Institute, and the successful move of his group. David has since remained on staff through a long battle with illness up until his recent passing.

In his personal life David was an avid bird watcher and lover of nature; in later years spending much time on his property on French Island. He also loved cars and enjoyed live music. David was generally a private man but happy to express an opinion on topics he was passionate about, like microbiology, politics and the environment.

He will be greatly missed as a colleague and a friend.

Corrigendum

In *Microbiology Australia* (Volume 39, Issue 3, page 175) Jack Wang rather than Talitha Santini, who was the supervisor of this work, should be recognised as the ASM summer student who performed this research. Photo of Jack below.



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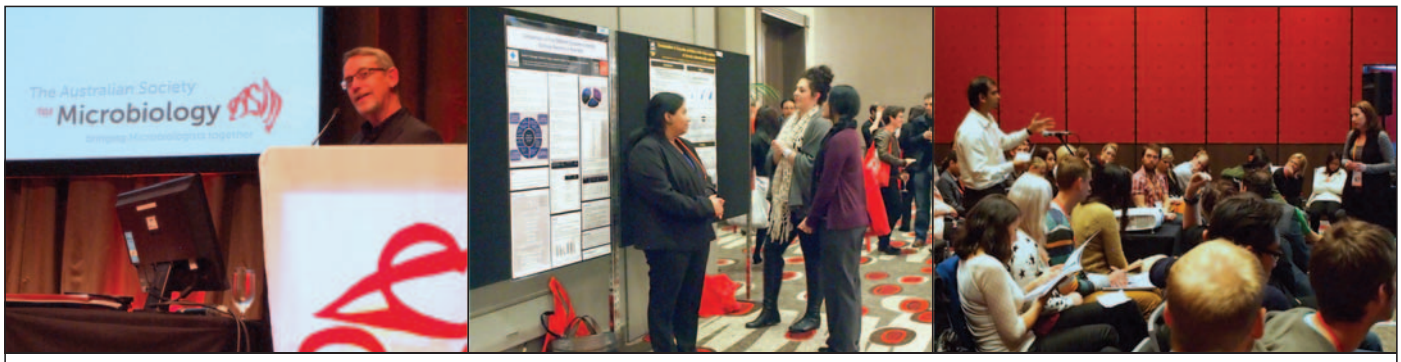

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