Emerging incidence of Lyme borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia

Background: *Borrelia burgdorferi*, the causative agent of Lyme disease (LD), and *Babesia*, *Bartonella*, and *Ehrlichia* species (spp.) are recognized tick-borne pathogens in humans worldwide. Using serology and molecular testing, the incidence of these pathogens was investigated in symptomatic patients from Australia.

Methods: Sera were analyzed by an immunofluorescent antibody assay (IFA) followed by immunoglobulin (Ig)G and IgM Western blot (WB) assays. Both whole blood and sera were analyzed for detection of specific *Borrelia* spp. DNA using multiplex polymerase chain reaction (PCR) testing. Simultaneously, patients were tested for *Babesia microti*, *Babesia duncani*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and *Bartonella henselae* infection by IgG and IgM IFA serology, PCR, and fluorescent in situ hybridization (FISH).

Results: Most patients reported symptom onset in Australia without recent overseas travel. 28 of 51 (55%) tested positive for LD. Of 41 patients tested for tick-borne coinfections, 13 (32%) were positive for *Babesia* spp. and nine (22%) were positive for *Bartonella* spp. Twenty-five patients were tested for *Ehrlichia* spp. and (16%) were positive for *Anaplasma phagocytophilum* while none were positive for *Ehrlichia chaffeensis*. Among the 51 patients tested for LD, 21 (41%) had evidence of more than one tick-borne infection. Positive tests for LD, *Babesia duncani*, *Babesia microti*, and *Bartonella henselae* were demonstrated in an individual who had never left the state of Queensland. Positive testing for these pathogens was found in three others whose movements were restricted to the east coast of Australia.

Conclusion: The study identified a much larger tick-borne disease (TBD) burden within the Australian community than hitherto reported. In particular, the first cases of endemic human *Babesia* and *Bartonella* disease in Australia with coexisting *Borrelia* infection are described, thus defining current hidden and unrecognized components of TBD and demonstrating local acquisition in patients who have never been abroad.

Keywords: *Borrelia*, Lyme disease, *Babesia*, *Bartonella*, ehrlichiosis, Australia, humans

Introduction

Human tick-borne disease (TBD) is an increasing health burden on the Australian community requiring wider diagnostic recognition. TBD comprises multiple specific zoonoses, some of which are well recognized and documented, including tick paralysis and rickettsial disease as reported at a Sydney University Website (Entomology). The present study addresses recent evidence for emerging *Borrelia*, *Babesia*, *Bartonella*, *Anaplasma*, and *Ehrlichia* infections in Australia. The study confirms previously published work on the existence of human *Borrelia* infection in Australia. There are no further publications on human Lyme disease (LD) in the interval using a search on PubMed on October 3, 2011 for the MeSH headings of humans, Borrelia, and Australia. The study
also examines the incidence of tick-borne coinfections, some of which occur in patients who have never left Australia.

The country has 75 tick species\(^1\) with general acceptance of *Ixodes* spp. and *Ixodes holocyclus* (Ih) in particular, as the main contenders in inducing human TBD in the country.\(^2,3,5\) The entire eastern coastline is habitat to Ih and it is known to vector human disease including rickettsial infection and tick paralysis.\(^1,4,6,7\) The Ih tick is colloquially named paralysis tick, grass tick, shell back tick, and several other names.\(^1\) It thrives in humid conditions along the coast, mainly in flats, from the north of the continent just above Cooktown in Queensland to Lake Entrance in Victoria at the very south of the continent. The region has a very high proportion of Australia’s human population.

The Ih tick has a larval, nymph, and adult form all of which require a blood meal.\(^1\) Larvae typically feed upon small animal hosts whilst nymph and adult will include larger animals. Humans are incidental hosts to the latter two forms. The tick may stay attached for up to 5–6 days before detaching if not found. Many tick bites are not observed or reported.\(^1\) Probably many of the nymph type, about the size of a poppy seed, are simply scratched off particularly if attached to the scalp. In Australia a majority of bites will come to nothing more than a non-elevated erythematous macule on the skin of up to 2 cm diameter resolving completely over a few days providing the tick is removed promptly. Some lesions grow at the bite site to form a 2–3 cm papule by the second day showing a black central eschar.\(^8\) Such a presentation is the typical appearance of Queensland Tick Typhus, a rickettsial infection, at the bite sight and untreated this is followed by a “spotted rash” of widespread distribution known as rickettsial spotted fever, a febrile illness whose erythematous macules are typically 4–5 mm in diameter.\(^6,7\)

Erythema Migrans (EM) is a local skin reaction to a tick bite caused by *Borrelia burgdorferi* sensu lato (Bb s.l.) infection.\(^9,10\) At this stage of infection the term Early Lyme Disease is also used and this terminology includes early systemic manifestations such as meningitis or cranial nerve neuropathy. After an initial 1-cm macular erythematous lesion, at day 2–5 or even later, the lesion will commence to grow in diameter and thicken for anywhere up to 14 days if left untreated. In Australia, the lesion will often become itchy and painful and rarely there will be central induration and paling of color which gives the bull’s eye classically referred to in LD literature. This has been the author’s observation over 20 years in his current practice in Australia. A single primary lesion must reach more than 5 cm in size to be classified as an EM and then it is considered to be pathognomonic.\(^11\) When documented in a known tick area it is also considered sufficient for a clinical diagnosis of LD.

LD is a protean multisystem illness that develops 6 months to 8 years after a tick bite that carries the borrelia spirochaete.\(^12\) Nomenclature of the stages of the disease includes the use of the terminology early disseminated LD and late disseminated disease.\(^13\) In North America a principal symptom of the disease is an arthritic illness that may have severe pain and swelling, more of large joints, and can be associated with marked general fatigue and other somatic features.\(^13\) The US Centers for Disease Control and Prevention (CDC) website further states that in the musculoskeletal system, LD produces recurrent attacks of arthritis with objective joint swelling in one or a few joints, sometimes followed by chronic arthritis.\(^13\) LD in North America may also be principally a neurological disease which is generally accepted to be the case throughout Europe and Asia. In the case load presented here it is entirely a neurological disease with symptoms of meningism, cranial neuropathy, and sometimes encephalopathy. Of importance is the large number of cranial nerves involved including sensory. Such a factor precludes diagnoses of multiple sclerosis, motor neuron disease, amyotrophic lateral sclerosis, chronic fatigue, fibromyalgia, and several others in the author’s opinion, leaving only sarcoid, bartonellosis, borreliosis, and the autoimmune neurological diseases (one of which is commonly known to every medical graduate as Guillain–Barré syndrome) as differential diagnoses. The latter residual differential diagnosis is put forward by the author after discussion with neurological colleagues.

Treatment of EM and early localized disease is usually very effective. Treatment of the later disseminated stage of the disease will usually be straightforward when identified early. Other cases can be protracted and treatment much more difficult. In this latter group there can be immune suppression known to be an innate response to the Lyme bacterium. Stricker and Winger first identified immune dysfunction demonstrated by lowered CD57 natural killer cell counts associated with LD.\(^14\)

* Babesia* spp. are piroplasms that invade red blood cells.\(^15\) In the developing world babesiosis is frequently mistaken for malaria which it symptomatically mimics in the acute phase. Most healthy individuals recover completely from infection with this piroplasm over 4 or more weeks. A very few go on to chronic infection. The disease can be fatal in the acute stage with multorgan failure due to intravascular hemolysis and stasis, particularly in splenectomised individuals. There is a large emerging incidence of low-grade babesiosis in association with LD in North America. Treatment for babesiosis in the presence of Lyme borreliosis is very difficult.
Drug therapy is similar to that for malaria. Clinical symptoms and signs as a TBD are intermingled with those of LD, are complex, and beyond the scope of this work.

*Bartonella henselae* is a Gram-negative intracellular bacterium.\(^1\)\(^6\)\(^1\)\(^6\) Alone it has been known to be the cause of cat scratch fever particularly in pediatric medicine where it is defined by its hallmark of pink striae that are quite unmistakable. The infection causes an acute febrile illness in solitary infection. However it can also be tick-borne, and whilst attention is given to EM and LD, it can be overlooked, just as *Babesia* is in the acute phase. Bartonellosis may go on to cause chronic symptoms identical to or confounding those of neuroborreliosis. Also it is extremely important in all documented cases to consider thorough cardiac assessment for both endocardial and myocardial damage. CDC gives a minimum length of illness was 8 months, while the maximum was 12 years. Investigation was recommended for all cases of neuroborreliosis. Bartonellosis may go on to cause chronic symptoms identical to or confounding those of neuroborreliosis. Also it is extremely important in all documented cases to consider thorough cardiac assessment for both endocardial and myocardial damage. CDC gives a good overview.\(^1\)\(^7\)

Human granulocytic ehrlichiosis (HGE) alternatively known as human granulocytic anaplasmosis is an infection of the neutrophil line of white blood cells.\(^1\)\(^6\)\(^,\)\(^1\)\(^8\) The agent is a rickettsia. Onset of symptoms can be as bland as a febrile illness after exposure by typically 4 weeks. HGE can present much more seriously and can be fatal. The only significant routine laboratory finding is a low white blood cell count. The infection will only be confirmed by suspicion and further serological testing. Treatment should commence whilst waiting for serology.

Human monocytic ehrlichiosis (HME) like HGE, is an infection caused by a rickettsial spp. but of the monocyte rather than of the neutrophil cell line.\(^1\)\(^8\)\(^,\)\(^1\)\(^9\) Though reported in North America, HME was not found in this study.

**Materials and methods**

The author is a general practitioner with specific interest and training in dermatology and LD. Most of the patients in the series were self-referred having found the author’s interest in LD. Some were referred from the University of Newcastle, and some from other doctors. The demographic spread of these patients involved the entire eastern seaboard of Australia for either residency or holidaying. Many had travelled overseas and walked or hiked in nature through Asia, Europe, or Northern America. While only some had a clear memory of tick bites, and even fewer any recollection of a rash the size of EM, most were unable to pinpoint where they could have been exposed, among several possible countries and outdoor exposure including Australia. Patients presented with a broad range of clinical symptoms consistent with multisystem disorder. The minimum length of illness was 8 months, while the maximum was 12 years. Investigation was recommended for all cases where LD was considered possible from a clinical perspective.

All cases of potential tick-borne disease referred from November 2009 to August 2011 for serological and PCR testing of any description for the above diseases at IGeneX in Palo Alto, CA, were included in this survey. Three patients presented with these tests already done and are included. Two patients attended from New Zealand. At the same time others were tested by Australian laboratories. Testing for *Babesia, Bartonella, and Ehrlichia* spp. could be offered only by IGeneX, however. A total of 51 patients had testing at IGeneX over the 18 months of the survey. In the same period a smaller number had other tests in Australia for *Bb* enzyme-linked immunosorbent assay ELISA, all of which were negative, and a number underwent polymerase chain reaction (PCR) analysis for *Babesia* DNA, some of whom tested positive. A further subset of those positives had confirmation by sequencing. This non-IGeneX group is the subject of future research. Many had no testing of this type because of patient cost.

IGeneX is a major laboratory specializing in TBDs. It is a reference laboratory recognized by the American College of Pathologists, and is Clinical Laboratory Improvement Amendments, Medicare, and Medicaid approved thus satisfying licensing requirements for most of the US states to perform high complexity clinical testing. IGeneX has also met licensing requirements in the states requiring additional licensing: California, Florida, Maryland, New York, and Pennsylvania. Statements concerning laboratory performance and validation in the area of quality assurance in LD testing are available on the IGeneX website.\(^2\)\(^0\)

Blood was collected in EDTA tubes and serum separator tubes, stood for 30 minutes, and then centrifuged before dispatch. A hemolyzed specimen was discarded. Sera were analyzed by an immunofluorescent antibody assay (IFA) followed by immunoglobulin (Ig)G and IgM Western blot (WB) assays. Both whole blood and sera were analyzed for detection of specific *Borrelia* spp. DNA using multiplex PCR testing. IGeneX multiplex B. *burgdorferi* PCR detects specific DNA sequences from Osp A plasmid and flagellin genomic genes. The test is not B. *burgdorferi* specific and also detects *B. afzelii, B. andersoni,* and *B. garinii.* Simultaneously patients were tested for *Babesia microti, Babesia duncanii,* *Anaplasma phagocytophilum,* *Ehrlichia chaffeensis,* and *Bartonella henselae* infection by IgG and IgM IFA serology, PCR, and fluorescent in situ hybridization (FISH).

**Results**

**Borrelia**

Fifty-one patients were tested for Lyme *Borrelia* and 28 showed positive test results (Table 1). IFA (IgG/M/A)
serologic reactivity to Bb antigens was demonstrated in 13 patients; one with a titer of 1:160, three with titers of 1:80 and nine patients with titers of 1:40. WB banding patterns are provided on reports and are interpreted both by IgeneX standards and CDC surveillance standards, as discussed below. Patterns for IgM WB revealed three patients seroreactive to the 23–25 kDa protein, seven to the 31 kDa protein, four to the 34 kDa protein, four to the 39 kDa protein, 28 to the 41 kDa protein, and three to the 83–93 kDa protein. Patterns for IgG WBs revealed three patients seroreactive to the 23–25 kDa protein, two at the 30 kDa protein, seven to the 31 kDa protein, four to the 34 kDa protein, five to the 39 kDa protein, 16 to the 41 kDa protein, and zero to the 83–93 kDa protein (Table 1). PCR assays detected plasmid DNA in serum samples from eight patients and in whole blood samples from two patients (Table 1). Twenty-eight patients had positive WB by IgeneX standards (Table 1). Twenty patients had WBs that were positive to the strict CDC WB surveillance criteria (discussed below); six lacked sufficient IgG reactivity to be CDC-positive and a further two lacked IgM reactivity. Reactivity to the flagellin band 41 kDa was universal on all WB results but this is a common denominator across all spirochetes. A high incidence of reactivity to the 31 and 58 kDa proteins merits further evaluation. In a report from China in 2010, Jiang et al studied 127 Lyme borreliosis-positive patients (B. garinii strain PD91) and 504 negative controls and proposed that band 58 is important diagnostically in that country for their major strain of Borrelia infection. In this Australian study, 14 out of 28 positives showed band 58 on either IgG or IgM. Band 58 may signify B. garinii in Australia.

IFA, WB, and PCR test results vary from patient to patient with no clear pattern emerging as to which technique is more likely to show evidence of LD. Isolated WB positivity (no IFA or PCR positivity) was found in 12 of the 28 cases. Only one case of isolated PCR positivity (IFA and WB negative) was found. Of most importance is the fact that 13 patients with diagnostically positive WB and negative IFA would have been undiagnosed if IFA had been used as an

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lyme IFA titer</th>
<th>IgM WB</th>
<th>IgG WB</th>
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<td>1</td>
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<td>9 at 1:40</td>
<td>15 pos 6</td>
<td>4 at 1:80 5 CDC</td>
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**Notes:** Pos, positive Western blot bands by IgeneX standards; CDC, positive Western blot bands by CDC surveillance criteria: combined count showing kDa 58 protein is 14.

**Abbreviations:** IFA, immunofluorescent antibody assay; Ig, immunoglobulin; PCR, polymerase chain reaction; WB, Western blot.
obligatory screening test prior to WB testing. See tabulation of results in Table 1.

Babesia

Forty-one patients were tested for babesiosis (both B. duncani and B. microti) and 13 tested positive (Table 2). Two of these patients had positive Babesia Fluorescent in situ Hybridization (FISH) results. Two patients demonstrated positive IgM reactivity for B. duncani, one with a 1:160 titer and one with a 1:80 titer. Six patients demonstrated IgG reactivity, three with titers of 1:80 and three with titers of 1:40. B. Duncani DNA was detected by PCR in one patient. IgM seroreactivity was demonstrated in two patients, and a titer of 1:20 and IgG seroreactivity was demonstrated in four patients with a titer of 1:40. IFA was used to detect antibody/antigen reactivity. In no instance was serology done at acute onset.

Bartonella

Forty-one patients were tested for bartonellosis, nine of whom tested positive (Table 2). IFA was used to detect antibody/antigen reactivity. Two demonstrated positive IgM reactivity at a titer of 1:40 and two at a titer of 1:20. Four patients demonstrated IgG reactivity: one at titer of 1:80 and three at a titer of 1:40. Bartonella PCR and FISH assays were performed but were negative in all cases. In no instance was serology done at acute onset. For these and also the babesiosis findings above, many months of symptoms had passed before all specimen collections. Positive results from any of the tests triggered clinical suspicion of disease and consideration of treatment for those identifiable clinical features that had triggered the investigation.

Human granulocytic ehrlichiosis (HGE) and human monocytic ehrlichiosis (HME)

Twenty-five patients were tested for HGE and HME. Of these, four were serologically positive. All these patients had travelled outside Australia, but one reported travel only to Fiji for 2 weeks and had documented multiple tick bites on 1 day in Australia. HME is an emerging TBD in North America but will not be discussed further in this document as the presence of Ehrlichia chaffensis was not detected in laboratory tests of any of the 25 patients.

HGE positive IFA serological results were as follows: IgM, two patients at a titer 1:20, IgG, one patient at 1:80 and one patient at 1:40. In no instance was serology done at acute onset. Many months of symptoms had passed before all specimen collections. Of note is the fact that all these patients had negative Australian Rickettsial studies.

Finally the 21 patients who demonstrated evidence of the studied coinfections formed a discrete subset of the 28 Bb-positive laboratory test patients.

Patients who had never travelled outside Australia

A group of four patients reported having never travelled outside Australia. Their geographic movements within Australia portray a lifelong picture. See Table 3 for results summary.

Patient A is a child (number 27 in table 1) who travelled from Byron Bay NSW to Eastlakes Victoria by caravan but resided on the mid-north coast of NSW. This patient had Lyme IgM WB positive at bands 23–25, 39 and 41 KDa, while B. henselae serology for IgM was positive at a titer of 1:40.

Patient B is an adult (number 24 in Table 1) who has always lived in Queensland but has travelled to Northern NSW, Sydney, Melbourne, and Hobart. Yeppoon Queensland is the furthest north he has been. Lyme IFA was 1:40, Lyme WB IgM was positive at bands 31 and 41 kDa, Borrelia PCR was positive for plasmid. B. duncani, IgG serology was positive at 1:40, B. microti IgG serology was positive at 1:40 and B. henselae IgM serology was positive at 1:40.

Patient C is an adult (number 19 in Table 1) who has lived in Queensland, except Karratha Western Australia from age 2–7 and used to holiday repeatedly at Armstrong Beach Queensland which is the site of a cluster of LD already

<table>
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<th>Table 2 Babesia and Bartonella positive laboratory test results</th>
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<tr>
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<tr>
<td>B. duncani</td>
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<td>B. microti</td>
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<td>Babesia FISH</td>
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<td>Bartonella FISH</td>
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Notes: 41 patients tested for both Babesia and Bartonella; discrete patient positives were Babesia 13 and Bartonello 9.

Abbreviations: FISH, fluorescent in situ hybridization; IFA, immunofluorescent antibody assay; Ig, immunoglobulin.
identified by the author. Lyme IFA was 1:80, WB IgM was positive at 34 and 41 kDa, and Bartonella IgG serology was positive at 1:40.

Patient D is an adult (number 6 in Table 1) who was born in Victoria and then moved to NSW, from where he had travelled to Queensland as far north as Airlie Beach. He travelled into South Australia for 1 day once. Lyme WB was positive at 31 and 41 kDa and B. duncani was IgG positive 1:40 titer.

Discussion

There has been considerable debate about the existence of LD in Australia. Published reports of both EM and locally acquired and proven human Borrelia burgdorferi infection in Australia are limited.2 At the time of the McCrossin study2 in 1986 only serological diagnostic tests were available. For the Hudson study3 in 1998 Borrelia were cultured and identified with PCR in one patient with the results suggesting the bacteria were similar to European B. garinii in derivation rather than Asiatic spp. That person had previously been overseas. In the current setting we need first to examine the reporting of WB. By IGeneX standards the IgM or IgG WB is positive if two or more of the bands 23–25, 31, 34, 39, 41, 83–93, are present. For CDC surveillance criteria for IgM, two or more of the bands 23–25, 39, and 41 must be positive, while the CDC IgG surveillance criteria for WB is positive if five of the following bands are present: 18, 23–25, 28, 30, 39, 41, 45, 58, 66, 83–93. Importantly it must be noted that the CDC has just amended its statement of criteria to include clinical impression. According to the website revised in January 2011: “Surveillance case definitions establish uniform criteria for disease reporting and should not be used as the sole criteria for establishing clinical diagnoses, determining the standard of care necessary for a particular patient, setting guidelines for quality assurance, or providing standards for reimbursement.” These criteria were developed for reporting purposes (case definition). They were never meant to encompass the entire possible spectrum of LD and it is a scientific error to exclude a diagnosis of LD clinically when there are less than five bands on IgG WB as has been occurring at Australian laboratories.

Lyme serological testing methods (ELISA, IFA, and WBs) although specific, lack sensitivity. Differences in WB performance and interpretation account for some of the variation in specificity and sensitivity reported among laboratories. PCR has become a gold standard of proof of infection, not just for LD, but all infections. A positive culture remains the absolute proof of infection, but a negative culture does not rule it out.

In this series, 51 patients were tested for the presence of Bb DNA with 8 patients demonstrating positive results for Borrelia multiplex PCR assays for plasmid DNA. One of these, patient B above, demonstrated positive PCR test results from a serum sample, yet had never left Australia. Patient B’s Lyme IFA was titered at 1:40, while the IgM WB showed positive bands at 31 and 41 kDa. In the author’s opinion this is the first published evidence of Bb infection verified by PCR where the infection was acquired within Australia. Of note is the fact that the infection progressed over a period of 3 years and the patient manifested the full classic triad of associated tick-borne diseases as discussed further below.

No cases of human babesiosis have been documented, either from B. duncani or B. microti in Australia. This study provides serological and DNA evidence that babesiosis exists in Australia, as demonstrated by the positive findings for IgG and IgM reactivity, FISH and PCR in 14 individuals tested out of 41 in Table 2. Among those who had never left Australia, 2 patients, B and D, manifested weak serological evidence of babesiosis with negative PCR and FISH indicating local acquisition of both B. microti and B. duncani coexisting with LD, and possible transmission by ticks.

B. henselae infection has been considered rare in Australia. This study provides evidence that it should be considered as a TBD co-infection at any age as indicated by nine of the 41 patients (seven adults and two children) manifesting positive serology, though to date PCR and FISH
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have been negative (see Table 1). Furthermore patients A and C, who have never left Australia, provide evidence of local acquisition of *B. henselae* coexisting with LD and possible transmission by ticks.

Although there is currently no evidence of locally acquired ehrlichiosis it is evident from the study that in travellers and immigrants it could be brought into the country from overseas, and that the two tick-borne forms of the disease, HGE and HME, need to be considered in diagnostic evaluation.

All the above diseases are reported as emerging infectious diseases worldwide, particularly in North America. This study shows Australia is no exception to the pattern. The broader topic warrants wider research than is presented here.

**Conclusion**

These results show a burden of TBD in Australia, which requires not only further research and evaluation but also a management response from the medical profession and Public Health entities of the eastern states. The long running discussion about the existence of LD in Australia needs to be put in a new perspective. This study demonstrates that it is endemic in Australia as is also babesiosis. Regardless of whether LD is acquired locally in Australia or acquired from abroad, the fact remains that Australian citizens are living in this country afflicted with this disease and are in need of treatment.

LD is protean in its manifestation and difficult to treat if protracted. The medical profession of Australia needs now to be alert to the possibility of detecting this disease early and in anyone with protracted illness who has neurologic symptoms particularly of the cranial nerves. The possibility of LD coinfection also needs to be examined in all cases given the findings here of 21 patients with a coinfection out of the 51 tested. Some patients were not tested for coinfection (cost consideration), and the incidence of these coinfecting pathogens could be higher. Australia currently lacks facilities to investigate all aspects of LD, a matter which needs to be redressed, and testing funded appropriately for Australians as a health priority. State Public Health Authorities in all states of the Commonwealth need to develop an adequate program for detection, monitoring and treatment of the TBDs discussed here as a matter of urgency. Public health education programs need to be put in place in an effort to reduce the current disease burden regardless of whether its acquisition is local or foreign.

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**Disclosure**

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**References**


