

# Clinical determinants of Lyme borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort

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**Background:** *Borrelia burgdorferi* is the causative agent of Lyme borreliosis. This spirochete, along with *Babesia*, *Bartonella*, *Anaplasma*, *Ehrlichia*, and the *Rickettsia* spp. are recognized tick-borne pathogens. In this study, the clinical manifestation of these zoonoses in Australia is described.

**Methods:** The clinical presentation of 500 patients over the course of 5 years was examined. Evidence of multisystem disease and cranial nerve neuropathy was sought. Supportive laboratory evidence of infection was examined.

**Results:** Patients from every state of Australia presented with a wide range of symptoms of disease covering multiple systems and a large range of time intervals from onset. Among these patients, 296 (59%) were considered to have a clinical diagnosis of Lyme borreliosis and 273 (54% of the 500) tested positive for the disease, the latter not being a subset of the former. In total, 450 (90%) had either clinical evidence for or laboratory proof of borrelial infection, and the great majority of cases featured neurological symptoms involving the cranial nerves, thus mimicking features of the disease found in Europe and Asia, as distinct from North America (where extracutaneous disease is principally an oligoarticular arthritis). Only 83 patients (17%; number [n]=492) reported never leaving Australia. Of the 500 patients, 317 (63%) had clinical or laboratory-supported evidence of coinfection with *Babesia* or *Bartonella* spp. Infection with *A. phagocytophilum* was detected in three individuals, and *Ehrlichia chaffeensis* was detected in one individual who had never traveled outside Australia. In the cohort, 30 (11%; n=279) had positive rickettsial serology.

**Conclusion:** The study suggests that there is a considerable presence of borreliosis in Australia, and a highly significant burden of coinfections accompanying borreliosis transmission. The concept sometimes advanced of a “Lyme-like illness” on the continent needs to be re-examined as the clinical interplay between all these infections. Evidence is presented for the first report of endemic anaplasmosis and ehrlichiosis on the continent.

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

## Introduction

The existence of human tick-borne disease (TBD) has been previously described in Australia by the author to comprise multiple zoonoses induced by the pathogens *Borrelia*, *Babesia*, *Bartonella*, *Anaplasma*, and *Ehrlichia* spp.<sup>1</sup> Other arboviral infections sometimes found worldwide are *Coxiella burnetii*, *Francisella tularensis*, *Yersinia pestis*, and the *Rickettsia* sp., with the latter comprising significant infection on this continent and having three unique subspecies.<sup>1</sup> Most grow intracellularly, but *Borrelia* sp. is also extracellular and *Y. pestis* is mainly extracellular.<sup>2</sup> In this case series

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of 500 consecutive patients presenting between October 2010 and February 2014, either referred or self-initiated regarding the possibility of “Lyme disease”, an analysis was done to define the clinical characteristics at the time of presentation of these zoonoses compared to those seen in North America, Europe, and Asia, as well as to quantify incidence figures. Patients attended from every state in Australia. In Australia, borrelial infection typically manifests with a neurological presentation, which follows the course seen in Europe and Asia, as discussed earlier.<sup>1</sup> Australian endemic human babesiosis with *B. microti* has been reported clinically, serologically, and at the molecular level.<sup>1,3,4</sup> There has been a recent nomenclature shift, with this piroplasm now classified as a *Theileria* sp. In this manuscript, the former nomenclature will be used. Babesiosis with *B. duncani* has been previously reported as an Australian endemic infection by this author.<sup>1</sup> Herein, the first Australian human endemic Anaplasmataceae infections with *A. phagocytophilum* and *E. chaffeensis* are reported: three cases for the former and one for the latter pathogen. This is intended to show a complex interplay of symptoms and signs between these TBD infections, clinically necessitating a thorough assessment to be able to treat the diseases appropriately. Diagnosing borreliosis has previously been discussed, including the fact that North America singularly has a large proportion of mono or pauci arthritis characterized by extensive swelling, which can be migratory.<sup>1</sup> This arthritic presentation is not seen in Australian borreliosis in this series. Also, it is important to reiterate that the involvement of a large number of cranial nerves will point the differential diagnoses at only borreliosis, bartonellosis, sarcoid, and Guillaine Barre disease.<sup>1</sup> Amyloid should now be added to this list as a further causative factor, but there also appears to be a strong association with neuroborreliosis and sarcoidosis.<sup>5–13</sup> The patient’s clinical history and examination techniques used in diagnosing TBD at a clinical level are discussed and correlated to the level of laboratory-supported diagnosis that can be expected. Particular emphasis is given to diagnosing neuroborreliosis from a clinical perspective. Some early demographic and epidemiological data for this continent are presented.

## Materials and methods

### Demographic and epidemiologic assessment

The data from 500 patient records were analyzed using 55 parameters that were subdivided into demographic, epidemiological, and clinical findings (see Tables 1 and 2). A patient questionnaire was obtained prior to consultation to assess the likelihood of multisystemic disease and multiple

**Table 1** Demographic and epidemiologic parameters extracted from the dataset

Demographic, epidemiologic, and clinical parameters	Number	Percentage	Total number
Average age at presentation	41		500
Average age at onset of illness	35		500
Sex, female	310	62	500
Never left the country	83	17	492
Average illness length in years at time of presentation	7.4		343
Tick bites recorded	240	71	340

cranial nerve abnormality. This was a truncated version of a symptom list from the Burrascano Lyme disease guidelines.<sup>14</sup> Inquiry was made into tick attachment and any associated reddening or swelling of the skin. A travel history outside Australia was sought from the perspective of ever going abroad or being born overseas.

### Clinical assessment

Symptoms and signs of cranial nerve deficit were sought using a cranial nerve assessment tool (Table 3). This tool was refined over time. Signs were sought by physical examination. The peripheral nervous system was briefly reviewed, but it was generally not helpful in pinpointing TBD, except that a pathological upwards plantar response (Babinski sign) and hypothenar wasting were not uncommon, which are possible indicators of babesiosis.<sup>15</sup> Particular note was made of large joint pain (hip, knee, shoulder, elbow, wrist, and ankle) with or without swelling compared to small joint pain in the hands, feet, spine, and jaw. The costochondral joints were checked for tenderness. Three psychological parameters in particular were sought: panic attacks lasting more than 30 minutes is associated with borreliosis; in addition, sudden bursts of anger and worsening obsessive compulsive behavior are both associated with bartonellosis. Certain investigation parameters were analyzed in three groups with testing performed at Australian Biologics Sydney (Table 4), IGeneX at Palo Alto, CA, USA (Table 5), and ancillary tests conducted at laboratories across Australia (Table 6; this table also shows single-photon emission computed tomography [SPECT] scans).

### Borrelia

The patients’ symptoms, signs, and examination were checked for neurological problems (as indicated in Table 3), as well as for multisystemic problems over the timeline of the disease (as previously discussed) to form a clinical opinion of the likelihood of the disease based on the involvement of more than three cranial nerves and at least one other system.<sup>1</sup> The

**Table 2** Clinical parameters extracted from the dataset

Clinical parameters extracted from the dataset	Incidence	Percentage	Number
EM rash adequately described	108	78	139
Cranial nerve dysfunction	355	71	500
More than three cranial nerves involved	296	59	500
Cardiac problem of any description	52	58	89
Suspected postural orthostatic tachycardia syndrome	48	54	89
Gastrointestinal symptoms of any kind	80	52	153
Genitourinary symptoms	68	44	153
Dermopathy of any type	55	36	153
Morgellons evidenced by direct skin microscopy	29	6	500
Small joint pain	57	37	153
Large joint pain	2	1	153
Mono arthritis (documented illness in the United States)	2	0	500
Clinical borreliosis – more than three cranial nerves involved	307	61	500
Clinical babesiosis – specific babesiosis symptoms	268	92	292
Clinical bartonellosis – specific bartonellosis symptoms	240	89	270

**Abbreviation:** EM, erythema migrans.

history questionnaire was used to assess patients' symptoms in the first instance in all cases, and some points would require clarification during the consultation, which involved the use of the cranial nerve tool and multisystem review. If the review showed three or fewer cranial nerves involved, then it was determined that borreliosis was a possibility; however, laboratory testing needed to be relied upon for a diagnosis. For more than three cranial nerves involved, a clinical diagnosis of borreliosis or bartonellosis was made. At this juncture, it should also be stated that reliance was placed on a negative computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain to exclude overt intracranial disease, and this was ordered if not done previously.

### *Babesia* and *Bartonella*

Attention was also focused on blocks of symptoms in the patient questionnaire that typically occurred with these TBD coinfections (Table 7). The significant presence of more than just a few of these factors in recognized TBD illness is a harbinger for the presence of coinfection based on clinical grounds. Symptoms/signs that may indicate *Babesia* are tinnitus, chills, flushing, dysphagia, severe neurological disease, thirst, night sweats, chest wall or sternal pain, polydipsia, fatigue, severe nausea, malaise, fever, fine hemangiomas, hypothenar atrophy, papular or vesicular rash, petechiae, anemia, abnormal platelet counts, and the development of these symptoms during borreliosis therapy.<sup>15,16</sup> Two European reviews are also helpful in understanding this disease from a clinical perspective.<sup>17,18</sup> Symptoms/signs that may indicate *Bartonella* are brain fog, ice pick headaches, photophobia, tachycardia, bowel issues, tender subdermal masses (particularly of the neck, forearm, and ileotibial

band), increased obsessive compulsive behavior, anxiety, endocarditis, retinitis, peripheral neuropathy, rapid relapse off antibiotics, immediate systemic illness following tick bite (within days), swollen joints, psychiatric issues, costal margin pain and tenderness, rapid mood shifts, and the development of these symptoms during *Babesia* therapy.<sup>15,16</sup>

### *Anaplasma/Ehrlichia*

*A. phagocytophilum* and *E. chaffeensis* are the etiologic causes of anaplasmosis and ehrlichiosis, and they were formerly known as human granulocytic ehrlichiosis and human monocytic ehrlichiosis, respectively, indicating the white cell line implicated in each infection.<sup>1</sup> The particular symptoms that may guide the diagnostician with these infections are, myalgia, tendon pain, extreme soft tissue tenderness, right upper quadrant pain, slumped posture, hepatomegaly, diaphoresis, muscle spasms, elevated liver enzymes (intrahepatic pattern) and, most importantly, a depressed white cell count.<sup>15,16</sup> Acute infection can be lethal, and treatment must start on clinical suspicion with doxycycline before laboratory confirmation. However, when these TBDs are associated with borreliosis, there appears a much slower chronic and lingering infection with reports of extreme fatigue and feeling severely unwell. With not much in the way of distinctive symptoms, a high degree of suspicion is warranted. Testing is done by serology and polymerase chain reaction (PCR).

### Other infections

*Mycoplasma pneumoniae* and *Mycoplasma fermentans* will lead to fatigue, fever, insomnia, headache, bowel issues, psychiatric disturbances, and arthritis (both large and

**Table 3** Cranial nerve assessment tool for neuroborreliosis and bartonellosis

Cranial nerve	Reported symptoms	Examination
1	Phantom smells	Anosmia partial/full
1	Any loss of smell	
1	Left or right	Which side
2		Visual acuity
2	Loss of any part of vision	Visual fields
2	Any floaters	Check patient's understanding
2	Any loss of night vision	
3		Full pupil assessment
3		Focusing difficulty with small print
3	Dilated pupil(s)	
3	Double vision – directional	Diplopia – medial or upwards
3	Drooping eyelids	Ptosis
4	Double vision – downwards direction	Diplopia – downwards direction
6	Double vision – lateral with weak affected eye	Diplopia – lateral with weak affected eye
5	Facial pain	Facial pain trigeminal divisions
5		Loss of corneal reflex
5	Taste changes	
5	Dental pain	
5	Jaw pain	
7	Facial paralysis (Bell's palsy)	Facial paralysis (Bell's palsy)
7	Facial muscle weakness or muscle twitching	Facial muscle weakness or muscle twitching
7		Test masseters
7		Mental reflex
8	Loss of hearing during illness	
8	More sensitive to loud sound or pain	
8	Tinnitus	Tinnitus
8	Vertigo	Vertigo, check on true meaning
8	Imbalance	Imbalance
8		Rombergism
8		Nystagmus (rapid is affected side)
9	Throat sore	
9	Difficulty swallowing	
9		Elevates larynx swallowing
9		Gag reflex present
9	Lack of saliva	
10	Neck pain around the throat	
10	Slurring of speech	Slurring of speech
10	Mispronunciation	Mispronunciation
10	Rapid beating of the heart or irregularity	Rapid beating of the heart or irregularity standing up quickly
10	Feeling faint	Feeling faint
10		Clinical evidence or suggestion of POTS
11	Cannot shrug shoulders	
11		SCM and trapezius strength
12	Difficulty with tongue movements	Tongue movement and strength full
12	Biting tongue	

**Abbreviations:** POTS, postural orthostatic tachycardia syndrome; SCM, sternocleidomastoid.

**Table 4** Borreliosis and coinfection investigation parameters extracted from the data for AB

AB testing	Positives	Percentage	Number
<b>Borrelia</b>			
Whole-blood PCR	81	55	146
Serum PCR	18	58	31
Urine PCR	46	84	55
Immunoblot IgM	7	47	15
Immunoblot IgG	4	27	15
Total immunoblot positive	10	67	15
ELISPOT LTT	16	84	19
AB <i>Borrelia</i> total positive testing	127	59	216
AB <i>Borrelia</i> total negative testing	89	41	216
Mycoplasma PCR positive	30	43	69
Mycoplasma fermentans PCR positive	18	26	69
AB <i>Chlamydia</i> Pn PCR positive	9	18	50

**Abbreviations:** AB, Australian Biologics; PCR, polymerase chain reaction; Ig, immunoglobulin; ELISPOT, enzyme-linked immunosorbent spot test; LTT, lymphocyte transformation test; Pn, pneumonia.

small joints) with joint swelling.<sup>16</sup> *M. fermentans* can show a negative response to prior antibiotic use with tetracyclines and macrolides. *Chlamydophila pneumoniae*, formerly designated as *Chlamydia pneumoniae* (CPN), is a known cause of respiratory illness, but it may also cause widespread arthritic pains in patients with TBD, as can *C. trachomatis*.<sup>19</sup> Of particular note

**Table 5** Borreliosis and coinfection parameters from IGeneX data

Investigation parameters at IGeneX	Positives	Percentage	Number
<b>Borrelia</b>			
IFA	19	95	20
PCR serum	18	60	30
PCR blood	9	43	21
Western blot IgM	93	79	117
Western blot IgG	71	69	103
IGeneX <i>Borrelia</i> total testing positive	146	58	252
IGeneX <i>Borrelia</i> total testing negative	106	42	252
<b>Babesia</b>			
<i>Babesia duncani</i> serology	43	35	123
<i>Babesia microti</i> serology	18	15	123
PCR <i>Babesia</i>	1	1	123
FISH <i>Babesia</i>	5	4	123
IGeneX <i>Babesia</i> total testing positive	66	54	123
<b>Bartonella</b>			
<i>Bartonella</i> sero	23	19	122
<i>Bartonella</i> PCR	0	0	122
<i>Bartonella</i> FISH	6	5	122
IGeneX <i>Bartonella</i> total testing positive	29	24	122
Anaplasmosis	8	31	26
<i>Ehrlichia chaffeensis</i>	5	19	26

**Abbreviations:** IFA, immunofluorescent antibody assay; PCR, polymerase chain reaction; Ig, immunoglobulin; FISH, fluorescence in situ hybridization.

**Table 6** Other investigations

Other investigations	Incidence	Percentage	Number
Abnormal CT SPECT brain from 55 requests	40	73	55
<i>Chlamydia</i> Pn serology positive (positive for IgG or IgM)	48	32	152
<i>Mycoplasma</i> sero (positive for IgG or IgM)	44	25	174
EBV sero (positive for IgG or IgM)	140	89	158
<i>Rickettsia</i> sero (positive for IgG or IgM)	30	11	279
Initial CD57 number assessed	418		
Average CD57 count	78		

**Abbreviations:** CT, computed tomography; SPECT, single-photon emission computed tomography; Pn, pneumonia; Ig, immunoglobulin; EBV, Epstein–Barr virus.

in the current study shows 68% monoarthropathy and 32% polyarthropathy. *M. pneumoniae* and *Chlamydia* serum PCR investigations returned positive findings in spite of negative serology for those infections in the patient series. *Rickettsia* infections sometimes appeared, and they routinely had neither been tested nor treated. Anecdotally, Epstein–Barr virus infection is said to be problematic in the presence of borreliosis and can flare up during treatment. Brucellosis, human herpesvirus 6, human immunodeficiency virus (HIV), leptospirosis, parvovirus, Q fever, and syphilis must be considered when determining the status of the patient, particularly in the context of immune suppression, as will be discussed.

## Imaging studies

Many patients presented with prior brain CT scans and MRIs. When absent, a CT of the brain was ordered. In some patients who appeared to have a greater degree of neurological deficit but normal prior scanning, SPECT CT of the brain was done.

**Table 7** Coinfection questionnaire assessment tool for *Babesia* and *Bartonella*

Fevers at night*
Saturated bed clothing from sweating*
Chest wall pain*
Sternum central chest pain*
Shortness of breath air hunger*
Headache at the top of the head or crown*
Dark urine*
Cough chronic and dry excessive bruising*
Vivid dreaming*
Small joint pains***
Fevers in morning**
Tender lumps under skin**
Electric shocks in limbs**
Skin stretch marks**
Glands swollen – particularly neck**
Ice pick headaches**
Irritability**
Angry outbursts**
Pain or soreness in the soles of the feet when getting out of bed**

**Notes:** \**Babesia*; \*\**Bartonella*; \*\*\*both.

## Laboratory assessment

Two laboratories capable of extensive investigation of *Borrelia* and other TBD coinfections were used.

### Australian biologics

Australian Biologics Laboratory Testing Services in Sydney, Australia was used initially in the series, and whole blood and serum analyses were performed for the detection of specific *Borrelia* sp. DNA using multiplex PCR for *B. burgdorferi*, *B. afzelii*, and *B. garinii*. Samples were analyzed in duplicate with positive and negative controls using primers AB–B1 (proprietary to Australian Biologics) for the *Borrelia* 16S rRNA gene target. The thermal profile for both analyses involved incubation for 2 minutes at 50°C, polymerase activation for 10 minutes at 95°C, then PCR cycling for 40 cycles of 10 seconds at 95°C, dropping to 60°C sustained for 45 seconds. The positive control used was an American Type Culture Collection (ATCC) *B. burgdorferi* genomic control. The magnitude of the PCR signal generated ( $\Delta R$ ) for each sample was interpreted as positive or negative, as compared to positive and negative controls. Validation of the assay was produced firstly by the use of external sequencing, and secondly through participation in quality assurance programs for the detection of *Borrelia* by PCR with Quality Control for Molecular Diagnostics (Glasgow, Scotland, UK), a joint research facility of Glasgow and Strathclyde Universities (Glasgow, Scotland, UK). Later, urine PCR analysis was added. From February 2012, some patients were also investigated by Mikrogen immunoblot for IgM and IgG, and they were also tested for borrelial T-lymphocyte transformation via the enzyme-linked immunosorbent spot test (ELISPOT). This laboratory also offered *M. pneumoniae*, *M. fermentans*, and chlamydo-philic PCR investigations.

### IGeneX

The other laboratory principally used for borreliosis investigation was IGeneX in Palo Alto, CA, USA. IGeneX also



offered testing for *Babesia*, *Bartonella*, *A. phagocytophilum*, and *E. chaffeensis*. IGeneX is a major laboratory specializing in TBDs. It is a reference laboratory recognized by the American College of Pathologists, and it is also Clinical Laboratory Improvement Amendments (CLIA), Medicare, and Medicaid approved, thus satisfying licensing requirements for testing throughout most of the United States to perform highly complex clinical testing.<sup>20</sup> The US Food and Drug Administration and Centers for Disease Control and Prevention (CDC) oversee the performance of the CLIA.<sup>20</sup> IGeneX has also met licensing requirements for testing in the states that require additional licensing: California; Florida; Maryland; New York; and Pennsylvania. Statements concerning laboratory performance and validation in the area of quality assurance in borreliosis testing are available on the IGeneX Website.<sup>21</sup> Collection of sera and timely shipment was previously discussed.<sup>1</sup> Sera were analyzed by an immunofluorescent antibody assay (IFA), followed by IgG and IgM Western blot (WB) assays. IGeneX WB uses two different strains B-31 and B-297. OspA and OspB bands are retained in the tests.<sup>22</sup> These bands were removed from the CDC reporting criteria because they were used in the Lyme vaccine, LYMERix™. The result is a sensitivity level >90% for the IGeneX WB test compared to 46% sensitivity for commercial tests.<sup>22</sup> Both whole blood and sera were analyzed for the detection of specific *Borrelia* sp. DNA using multiplex PCR testing for *B. burgdorferi*-specific DNA sequences from OspA A plasmid and flagellin genes. The test is not *B. burgdorferi* specific, and it also detects *B. afzelii*, *B. andersoni*, and *B. garinii*. Simultaneously, some patients were tested for *B. microti*, *B. duncani*, *A. phagocytophilum*, *E. chaffeensis*, and *B. henselae* infection by IgG and IgM IFA serology, PCR, and fluorescent in situ hybridization.

#### Other laboratories

Several standard accredited laboratories across Australia were used for CD57 estimation and the several nonborreliosis serologies discussed within.

## Results

### Demographic and epidemiologic factors

The average patient's age at presentation was 41 years, with a median of 42 years and a standard deviation (SD) of 18 years. Refer to Table 1 and see Figure 1 for a rather widely splayed cumulative distribution curve. There were 310 females (62%) in the study. Only 83 of 492 respondents (16.5%) reported never leaving the country. Illness length in years was an average of 7.4, with a maximum of 47 years, a minimum of 0.17 years, a median of 3.0 years, and a SD of 9.5 years from a sample size of 341. The average age of the commencement of illness was therefore 35 years, with a median of 35 years and a SD of 19 years. Thirteen patients' illness could be traced from birth or in the first year of life; six were siblings, two were from one family, and four were from another, indicating the possibility of maternal transmission. In addition, borreliosis was laboratory proven in both mothers. A recognized history of tick bites was found in 240 of 340 individuals (71%), though the time span from bite to illness could not be reliably estimated in the majority of cases.

### Clinical findings

#### *Borrelia*

Certain clinical parameters are shown in Table 2. Of the 240 individuals reporting a tick bite, 108 (22% of the total cohort size) reported an associated rash large enough to have been considered as erythema migrans. Clinically, the diagnostic criterion for this rash is a minimum of 5 cm.

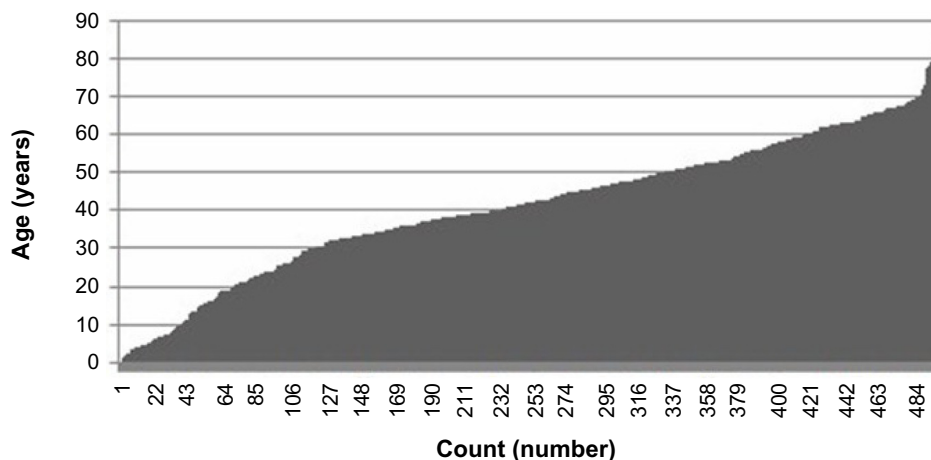


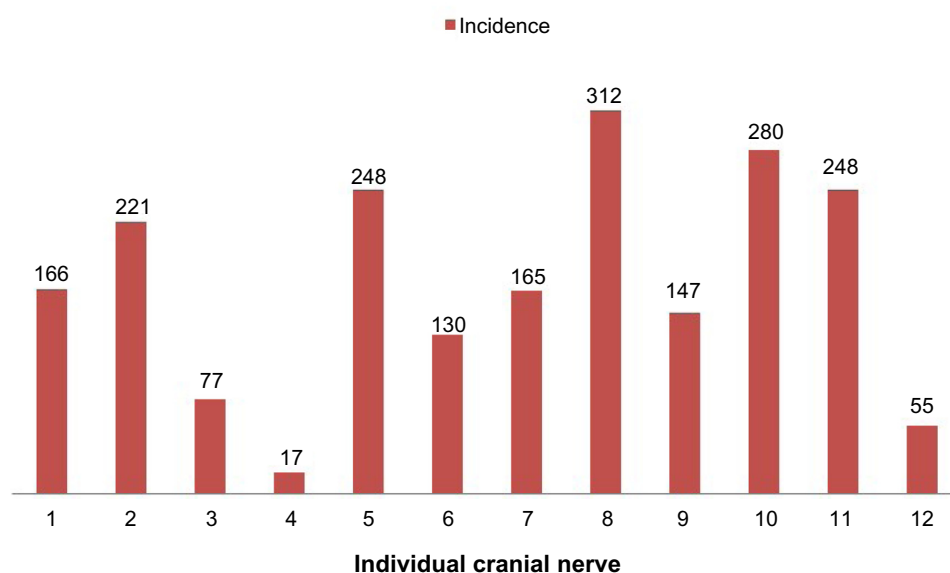
Figure 1 Cumulative age histogram of patients in years.

Cranial nerve involvement was extensive and is charted as a histogram for each individual nerve (Figure 2). Cranial nerves 5, 8, 10, and 11 were involved in at least 50% of respondents. It was previously proposed that when diagnosing neuroborreliosis clinically, only *Borrelia*, *Bartonella*, sarcoid, amyloid, and Guillain–Barre disease could affect more than two cranial nerves at a time, and that three nerves appear to be a crucial number in this series.<sup>1</sup> Involvement of more than three cranial nerves was found in 296 patients (59%), while involvement of more than two cranial nerves was found in 310 patients (62%) (see Figures 3, 4A, and 4B). The incidence of clinically-supported borreliosis jumps dramatically at the involvement of four cranial nerves (refer to Table 8 and Figure 4). Cardiac history commonly revealed tachycardia or arrhythmia. All patients presented with electrocardiograms that were already performed to assess for heart block (a known complication of borreliosis), and to determine the baseline QTc for prescribing purposes.<sup>23</sup> No incidence of third-degree heart block was found. Seriously delayed QTc was found in only a very small number of patients. There were some patients that were already diagnosed with postural orthostatic tachycardia syndrome (POTS), and adding suspected cases from the patient histories brought that number to 48. Many went on to have a positive tilt table test. POTS is a recognized complication of borreliosis.<sup>24,25</sup> Neural involvement with lymphoplasmocellular infiltrates can be found in the autonomic ganglia.<sup>26</sup> More centrally, cardiac rhythm has been shown to be controllable from the reticular formation in the midbrain, as well as from the posterior hypothalamus.<sup>27</sup>

There are no literature reports associating bartonellosis to POTS on a PubMed search. The author, however, suspects this association on the basis of Herxheimer-type reactions (which will be discussed) during initial *Bartonella*-targeted treatment, which is administered prior to borreliosis treatment.

In addition to POTS and heart block, borreliosis can cause carditis. A PubMed search for borreliosis + carditis conducted on September 6, 2014 yielded 326 articles. Severe cardiac arrhythmia and arrest has been reported.<sup>28–33</sup> Bartonellosis may cause carditis and also cardiac valvular disease. A PubMed search on the same date for cardiac + bartonellosis + human returned 123 articles.

In the study gastrointestinal symptoms were recorded for nausea, belching, flatus, diarrhea, constipation, and abdominal pain. *Borrelia* may cause bladder dysfunction. A key urinary symptom includes returning immediately after urinating but finding nothing to pass. Skin problems were reported in 11% of the cohort; they were usually widespread and often presented with itching. In 6% of patients, Morgellons could be demonstrated by skin microscopy. Morgellons has recently been closely associated with borreliosis infection.<sup>34–37</sup> There was no previous report of the incidence of Morgellons in a large borreliosis population cohort. Moreover, 11% of patients presented with small joint pain. Most often, one had to see which treatment worked best to abate this symptom to be sure of its diagnostic etiology. The two patients with large joint pains had returned from living in the US where they had contracted and undergone treatment for their infection. Using cranial nerve criteria of the involvement of more than three cranial nerves,



**Figure 2** Histogram of the incident of involvement for each cranial nerve.

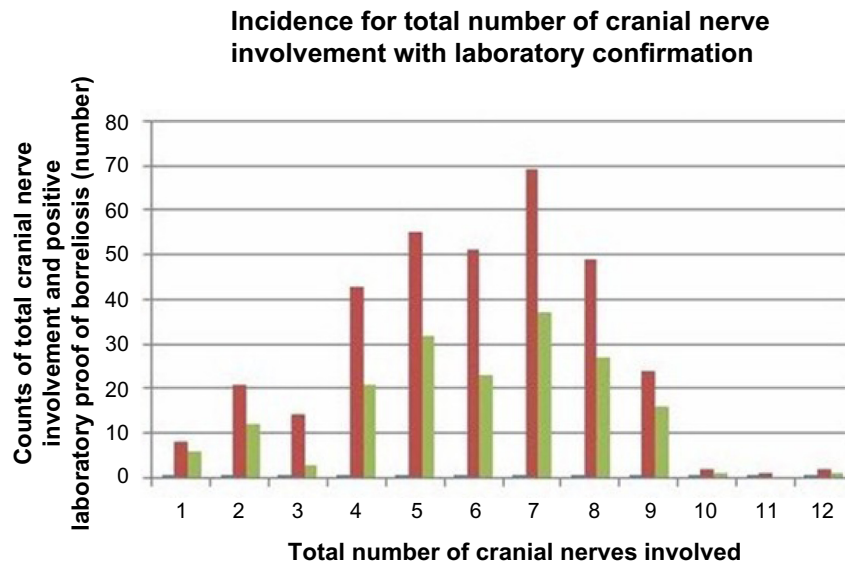


Figure 3 Histogram showing the incidence of the total number of cranial nerves involved in the dataset (red) and respective laboratory proof of borreliosis (green).

there was a clinical diagnosis of borreliosis in 296 individuals and laboratory proof in 158 of those cases.

**Babesiosis**

Examining the possible constellation of symptoms and signs with respect to babesiosis there were 268 cases clinically suspected and 46 laboratory proven. The babesiosis figures can be further subdivided into infection by *B. microti* (number [n]=13) and *B. duncani* (n=33), respectively, from 123 patients; four individuals had both simultaneously. These coinfections are more difficult to prove.

**Bartonella**

For bartonellosis, when examining the possible constellation of specific symptoms and signs, it was found that there were 240 clinically-suspected cases and 23 laboratory-proven cases. With *Bartonella*, we suspect that there is more than the genus *B. henselae* involved, which is the only one tested in this series. In Australia, *B. koehlerae* has been reported to cause human disease.<sup>38</sup> *B. bacilliformis*,

*B. quintana*, and *B. vinsonii* subspecies *berkhoffii* are known to cause human disease as well.<sup>39-42</sup> Similarly, it is suspected that in Australia, there are other *Babesia* sp. causing human infection, and *B. divergens* is known to cause human disease.<sup>40</sup>

Combined symptomatology of the two coinfections, as well as borreliosis alone, was found in 307 (61%) individuals. Finally, by aligning the data for clinical and laboratory factors for all three infections, only 50 (10%) individuals in the entire cohort had no diagnosis. This assumes that *Babesia* and *Bartonella*, where they had occurred, were transmitted with borreliosis as a TBD. For *Bartonella*, this is not necessarily true, and among the case load there were two incidences of pure *Bartonella* infection, requiring only *Bartonella*-targeted treatment to achieve full recovery.

**Other infections**

Clinical factors for other infections were not reliably recorded in the individual files.

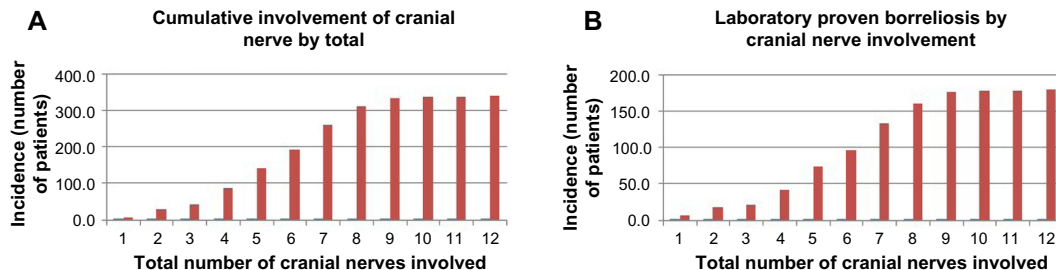


Figure 4 Cumulative involvement of the cranial nerves by total and laboratory-proven borreliosis by cranial nerve involvement. Notes: (A) Cumulative involvement of the cranial nerves by total; (B) laboratory-proven borreliosis by cranial nerve involvement.



**Table 8** Incidence and cumulative data for the cranial nerves with laboratory proof

Cranial nerve	1	2	3	4	5	6	7	8	9	10	11	12
Incidence of involvement	8	21	14	43	55	51	69	49	24	2	1	2
Cumulative count	8	29	43	86	141	192	261	310	334	336	337	339
Laboratory confirmed	6	12	3	21	32	23	37	27	16	1	0	1
Cumulative count	6	18	21	42	74	97	134	161	177	178	178	179

## Laboratory findings

The specific methods of laboratory testing for borreliosis, bartonellosis, babesiosis, *Anaplasma*, and *E. chaffeensis* have been previously discussed.<sup>1</sup> Results showing the incidence of the respective findings are set out in Table 4 for Australian Biologics; of note, 127 of 216 individuals were borreliosis positive (59%). In Table 5, which presents data for IGeneX, it was found that 146 of 252 individuals were borreliosis positive (58%). Also, IGeneX testing showed that 66 of 123 individuals were positive for *Babesia* (54%), 29 of 122 were positive for *Bartonella* (24%), eight of 26 were positive for anaplasmosis (31%), and five of 26 were positive for *E. chaffeensis* infection (19%). A review of the sequencing information of the data from Australian Biologics showed that the following species were found: *B. burgdorferi* CA382; N40; ZS7; *B. garinii*; *B. afzelii*; and *B. valaisiana*. The initial CD57 count was assessed in 418 individuals; the average was 78. Three were as low as zero. Normal is considered higher than 110.

## Imaging

Invariably, CT and MRI were normal, except that occasionally an MRI brain scan would report flecks or spots of white in white matter, which were dismissed as unimportant, incidental, or of no clinical significance. SPECT CT of the brain is a valuable method for documenting the degree of neuroborreliosis.<sup>43</sup> Fifty-five patients underwent the procedure. Some sites in Australia reliably reported the defects of this disease as neuroborreliosis. Other sites reported defects, but they dismissed these as nonspecific, in spite of the diagnosis on the request form. A review of the patient data indicated that 40 scans (73%) showed regional defects in perfusion, as reported by radiologists, with some reports confirming neuroborreliosis.

## *Anaplasma Ehrlichia* infection case reports

Many patients were tested by serology and PCR for *A. phagocytophilum* and *E. chaffeensis* in Australia with completely negative results. Recently, the author requested this testing from IGeneX, and positive results were obtained again. Eight patients had *Anaplasma* infection and five had *E. chaffeensis* infection. Importantly though, four very

sick individuals were found: one with *E. chaffeensis* and three with *Anaplasma*; these patients had never left Australia. This becomes the first case report of endemic human infection in Australia with these two bacteria. Two had low white cell counts of 3.4 and 2.9 by 10<sup>9</sup>/L, respectively. All four were markedly intolerant of doxycycline and had to start 50 mg doses, which slowly increased incrementally. All had laboratory-proven coexistent borreliosis, indirectly providing evidence for tick transmission.

## Rickettsial infections

Of the 500 patients in this study, 30 of 279 (11%) individuals had positive rickettsial serology (either IgM or IgG), demonstrating past or present infection. Allowing for some degree of cross-reactivity, this is still an appreciable number.

## Other parameters

Other factors known to suppress the immune system in borrelial infection are the administration of cortisone and chemotherapy.<sup>44,45</sup> Many coexisting infections are found in borreliosis, the incidence of which is reported in Table 6. *C. pneumoniae* testing by serology was positive in 48 out of 152 individuals and by PCR in nine out of 50 individuals on serologically-negative patients. Serology detected that 44 of 174 individuals were positive for *M. pneumoniae*, while PCR detected that 30 out of 65 were positive among serologically-negative patients. *M. fermentans* by PCR detected 18 positives out of the 69 tested. Other infections that were screened for included Q fever, brucellosis, leptospirosis, and HIV; viral encephalopathies may need to be considered.<sup>46</sup>

## Discussion

Distinguishing between borreliosis and bartonellosis in a clinical setting is extremely difficult, and reliance on specific *Bartonella* symptoms is important. It appears that both diseases often coexist and are tick-borne in Australia. A comparison can be made with a recent publication by Berghoff<sup>47</sup> on the differential diagnoses of these infections. There is a wide age distribution and, importantly, an extensive delay in the recognition and diagnosis of neuroborreliosis. This could be improved with detection of cranial nerve

symptoms and deficits. The cumulative histogram in Figure 4 shows, firstly, an exponential increase in the clinical diagnosis of neuroborreliosis with increasing cranial nerve involvement and, secondly, the number of positive laboratory findings that rise correspondingly with the involvement of four cranial nerves. The histograms show a sigma curve, which is virtually identical for both a clinical diagnosis versus a laboratory-supported diagnosis. When examining the figures for a clinical diagnosis, the X-axis simply represents an ordinal increase in count and the gradient ( $\tan \theta$ ) is simply the difference between each Y-axis count: 14 for 3; 43 for 4; 55 for 5; 51 for 6; and 69 for 7, before falling again as the sigma curve flattens. Linear regression analysis of the data yielded a coefficient of 0.999485. It is proposed that the involvement of three cranial nerves is thus a critical number, which could pivot the diagnosis of clinical borreliosis or bartonellosis in either direction; however, the involvement of four or more cranial nerves correlates well with a clinical diagnosis of either borreliosis or bartonellosis.

The ELISA/IFA/WB controversy has been discussed before.<sup>1</sup> The current CDC position statement on Lyme disease case surveillance can be found on the CDC Website.<sup>48</sup> It has been acknowledged by the CDC that there is a large burden of disease that can only be diagnosed clinically. Specifically, the CDC states the following “For purposes of surveillance”:<sup>48</sup>

Nervous system. Any of the following, alone or in combination: lymphocytic meningitis; cranial neuritis, particularly facial palsy (may be bilateral); radiculoneuropathy; or, rarely, encephalomyelitis.

In this report, there is very close concordance between the sensitivities of IGeneX and Australian Biologics running at 58% and 59% of the total tested, respectively. In Europe borreliosis is associated sometimes with acrodermatitis chronica atrophicans which is caused by *B. afzelii*. In this series, only two patients had acrodermatitis chronica atrophicans and one had never traveled outside Australia. Diagnosing the correct infections to prioritize appropriate treatment is essential. It is important to clear coinfections before treating infections caused by *Borrelia*.<sup>15,16</sup> Treating *Borrelia* infections first can, in fact, make the patient deteriorate further.<sup>15,16</sup> This is supported by observation of the emergence of the specific coinfection symptoms of bartonellosis or babesiosis during borreliosis treatment. Not only must the two coinfections be cleared, but any of the other current infections should also be treated, and sometimes this can be viral. A quick check on the CD4 count will tell if the patient is fighting a viral load. Almost surprisingly, the incidence of HIV positivity in

the cohort was zero where tested. As can be seen by the data presented, pure borreliosis is not common in Australia, and consideration must be given to a host of infections when assessing TBD. The data were reliable for this finding from patient 300 onwards. In this subset, 17 (8.5%) patients had pure borreliosis confirmed by testing, and no clinical suspicion or laboratory proof of coinfection was observed. Fifty patients (10%) could not be diagnosed as having one of the three key infections in the series, either clinically or by investigation. It is highly concerning that 11% of patients had positive rickettsial serology with a protracted disease complex and had never been tested for this infection.

White matter flecks seen on MRI in proven neuroborreliosis can be a result of that infection.<sup>49–52</sup> Further, Kanekar and Devgun<sup>53</sup> state that in neuroborreliosis,

The most common abnormality seen on MR is multiple bilateral periventricular and/or subcortical foci of T2 prolongation. These findings usually mimic MS (multiple sclerosis); however, multiple enhancing cranial nerves (third, fifth, and seventh cranial nerve), nerve root, or meningeal enhancement may favor Lyme over MS.<sup>43,53–56</sup>

The incidence of SPECT abnormality in neuroborreliosis was 75% in one study.<sup>43</sup> The current study's data of 40 out of 55 scans (73%) appear to conform to those findings if the reported areas of reduced perfusion are considered as an abnormal scan. CD57 is a measure of immune suppression in the presence of borreliosis;<sup>57</sup> normal is considered a value  $\geq 110$  cells/mm<sup>3</sup> using the Beckman Coulter antibody (catalog A74779; Beckman Coulter, Inc., Brea, CA, USA), with  $<60$  considered very significant borreliosis. The potential diversity of tick-borne infections was recently enumerated at 237 pathogens in a recent study of *Ixodes perscultatus* in the People's Republic of China;<sup>58</sup> this has modeling applications for Australia.

## Jarisch–Herxheimer reaction

This reaction is well described in the literature, but particularly well by Horowitz.<sup>16</sup> In summary, the breakdown products of the cellular components of *Borrelia*, *Babesia*, and *Bartonella* (in particular, the cell wall products of *Borrelia*) produce a temporary IL storm of IL-1, IL-6, and TNF- $\alpha$ . The effect is similar to having viral influenza, which induces the same storm, plus a patient will experience some of the symptom set associated with their own disease complex. The reaction can be very severe in some individuals and is dependent upon bacterial load versus treatment dosage. In treatment, a balance is sought between what a patient can

comfortably endure using as many remedial measures as possible to relieve distress, and sufficient therapy to be effective against the disease being targeted.

## The vectors

Recently Mayne et al have unequivocally demonstrated evidence for *Ixodes holocyclus*, the paralysis tick, as a vector for human borreliosis.<sup>59</sup> The vector is found along the entire eastern seaboard of the continent and across into central Victoria. Other vectors need to be sought in this region and across Australia.

## Conclusion

Neuroborreliosis can affect large numbers of cranial nerves. Herewith, data were presented demonstrating that a thorough search for symptoms and signs of cranial nerve abnormality can clinically pinpoint neuroborreliosis and/or bartonellosis when more than three nerves are involved. The total results highlight an underestimated set of TBD in Australia that requires further research and evaluation. Borreliosis is protean in its manifestation and difficult to treat if protracted. Those in the medical profession in Australia need to be alert to the possibility of detecting this disease and its associated coinfections early to initiate effective short-lasting treatment, thus preventing a cascade into severe chronic borreliosis, which can be devastating in all aspects of life.

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## Disclosure

From 2014 the author has served as a medical adviser at Australian Biologics as a free service. The author reports no other conflicts of interest in this work.

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