

# SCOPING STUDY TO DEVELOP A RESEARCH PROJECT(S) TO INVESTIGATE THE PRESENCE OR ABSENCE OF LYME DISEASE IN AUSTRALIA

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North Coast Lyme Disease & Associated Tick Borne Diseases Support  
Network  
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## All comments are in purple

FINAL REPORT

30<sup>th</sup> September, 2013

John S Mackenzie

Why are there no identification details for Mr Mackenzie?

What are his: credentials, position title, qualifications?

Is there any declaration of conflict of interest?

By what processes and under what criteria was Mr Mackenzie selected and commissioned to undertake this Scoping Study?

Alarming, our investigations have revealed that John S Mackenzie was a co-researcher/author with Richard C Russell and Stephen Doggett (of the infamous Russell et al 1994 study that claims *borrelia* does not exist in Australia) in a 2010 study on Edge Hill virus (Macdonald et al 2010). It is understood that Mr Mackenzie is also an International Editor on the USA CDCs Infectious Diseases Magazine. Lyme disease patients (and indeed any university ethics committees also would) consider this to be a conflict of interest that should have been disclosed with the scoping study as a disclaimer.

We believe it is totally unethical for a colleague of Messrs Russell and Doggett to undertake a study such as this, which must be, by its very purpose, a completely objective and transparent review. It is very obvious from the information provided in this study that it lacks objectivity and is, in places, blatantly biased. This is highlighted in the feedback provided herewith throughout the document.

We have concerns that due to the small number of “specialists” in the tick borne illness field, there is a suggestion of possible “cronyism”, using authors of the Russell

and Doggett study of 1994, and perhaps their ability to remain open and objective was compromised.

**As representatives of LymeLinks, we perceive this as a serious conflict of interest requiring investigation by the appropriate authorities, at a level that will ensure that any conflict of interest, bias, discrimination and/or corruption in the handling of the Lyme disease issue in Australia is exposed.**

## **General Comments**

- Australian research, clinical diagnosis, testing and treatment for Lyme disease is in its infancy and much more research is required before Australians are correctly diagnosed and treated.
- The Scoping Study author is “cherry picking” a small selection all available research testing methodologies, clinical diagnosis and the research on chronic borreliosis to justify its denial of Lyme disease in Australia. There is an urgent need for objectivity regarding this issue. Please consider and reference the full spectrum of what is available rather than what the USA CDC wants you to know.
- The Scoping Study author has not made reference to the serious flaws uncovered in the antitrust investigation initiated by USA Senator Richard Blumenthal into the USA Infectious Disease Society of America (IDSA) process for writing its 2006 Lyme Disease guidelines. “Blumenthal had found “undisclosed financial interests” and that the IDSA guidelines panel “improperly ignored or minimized consideration of alternative medical opinion and evidence regarding chronic Lyme disease, potentially raising serious questions about whether the recommendations reflected all relevant science” 2013. Another key Blumenthal finding was one of “lack of consideration” to varying points of view. Australians deserve better than this.
- **The Australian Government is at risk of repeating these same mistakes if they continue to “cherry pick” research, treatment guidelines etc.**
- A negative blood test does not rule out Lyme disease. The diagnosis of Lyme disease is a clinical diagnosis based on medical history, various blood tests and clinical symptomology, including the individual’s responses to pharmacological treatment, and not based solely on the Australian ELISA and Western blot tests. Many commonly accepted illnesses, such as multiple sclerosis (MS), Parkinson’s disease and lupus, are based on this type of medical history and symptomology and do not receive the level of controversy that the diagnosis of Lyme disease does. (MS Australia.org.au (2013), Parkinsons.org.au (2013), betterhealth.vic.gov.au (2013).

- The Australian medical profession appears to be split into three camps – those that are sceptical of the presence of Lyme in Australia; those that believe there is something out there and are waiting for further research or proof; and those that see the symptoms, see that their patients are sick, and seek a diagnosis and treatment for Lyme-like illness.
- It appears that many Australian health practitioners do not have a clear understanding of the number and prevalence of zoonotic diseases and their varied symptoms. Therefore, they do not consider a zoonotic disease nor ask for specific tests or send bloods to the more specialised labs overseas. Zoonotic diseases are under-studied in Australia, and require far more research to determine the impact on human health.
- There is a growing trend in the Australian medical field to discredit overseas testing particularly from the private labs, and in particular the results from the tick borne disease reference lab IGeneX. The motivation for this situation requires further investigation. Is it based on financial issues (i.e. profits going overseas) or ego/competition (i.e. that overseas labs are finding it, whilst Australian labs are not)?
- Some doctors, including infectious disease specialists, are ignoring these positive test results, or calling them false positives and sending their desperate and sick patients home, often telling them that their illness is psychosomatic. Some affected patients are confused, embarrassed and angry that their illness is not being adequately diagnosed and that their doctor refuses to treat appropriately, if at all.
- There are allegations that private labs are profiting from Australians and are sending them positive results to justify their high testing costs. Basically, Australians who receive positive test results from overseas labs are being told by some Australian medical practitioners that these tests are deceptive or just false positives. One patient was recently told by an infectious disease specialist that; "Some labs present as a fee for service. You pay your money and you're told what you want to hear - much like many of the so-called Lyme practitioners", and subsequently refused to treat this person unless he tested positive to Lyme through Australia's flawed testing. Many Australians simply cannot afford overseas testing, as many are too unwell to work.
- Australian governments promote this attitude and appear not to recognise the growing body of evidence to the contrary. In a letter dated 22 November 2012, from Jillian Skinner MP, Minister for Health and Medical Research in NSW, responding to the author's (Lynn Rees) request for Lyme recognition in Australia, she states;

*"Some people in Australia have been diagnosed with Lyme disease based on test results obtained from overseas laboratories which do not use validated tests and also do not follow internationally recognised testing guidelines. These laboratories are more likely to report Lyme disease test results which are falsely positive, particularly if the person has never travelled to an area where Lyme disease bacteria are known to be present in ticks."*

- The Australian government provides no proof of the allegations made by Ms Skinner above.
- IGeneX in the USA undergoes an accreditation test by Medicare and Medicaid services in the USA to ensure the accuracy of their tests similar to Australian NATA accreditation. For the past nine years IGeneX has had a proficiency testing value of 98% or greater on all their tests.
- There is also a concerning trend that Australian health professionals and researchers who are finding *Borrelia* in Australian patients are being vilified by the medical establishment in an attempt to discredit them. This level of professional hysteria and bullying has not been seen on such a scale since the early days of HIV/AIDS recognition.
- Due to the concern that people are becoming resistant to antibiotics, doctors are discouraged from unnecessarily prescribing them. The treatment of chronic Lyme disease requires multiple, high dose, long term antibiotic therapy. This fact, together with the very high cost of treating chronic Lyme disease, could easily explain why Australian medical authorities allegedly are reluctant to acknowledge the growing body of evidence published on the presence of an Australian species of *Borrelia* (referenced later in this submission feedback) and the increasing number of people who test positive to this disease in overseas specialist labs. In Lyme endemic countries, a four to six week course of antibiotics usually prevents Borreliosis progressing to the chronic stage, if the patient is treated immediately upon receiving a tick bite.
- There are numerous bodies of research and clinical presentations confirming the presence of *Borrelia* spirochetes in Australia, and numerous historical isolations of *Borrelia*, and yet Australian governments appear to continue to cite only the work of Russell et al. (1994), work that only tested for and failed to isolate the one genospecies *Borrelia burgdorferi* sensu stricto.
- The effective treatment of Lyme disease is financially crippling to individuals and would also place enormous pressure on the Australian health system. Perhaps these reasons are detrimentally influencing the Australian government's position that Lyme disease cannot be contracted from ticks in Australia. Due to the high level of misdiagnosis in Australia, with many people suffering from Lyme disease being told they have other degenerative illnesses, possibly the high cost of treating Lyme disease would be cheaper than the long term health care burden of those misdiagnosed.
- In a recent survey of Australians diagnosed with Lyme disease, it took an average of 6 1/2 years before a diagnosis of Lyme disease was made (Lyme Disease Association of Australia (LDAA) 2012). Perhaps a cost benefit analysis needs to be

undertaken, comparing the cost of these years of misdiagnoses with the annual treatment cost for Lyme disease. Such an analysis could show that the costs are comparable. A study was conducted in the USA on the Economic Impact of Lyme Disease (Zhang et al, 2006). It was found that the average cost per Lyme disease case decreased over the 3 year study period, and suggests that it was due to the following reasons: personal protection from tick bites; more frequent visits to physicians when exposed to tick bite; physicians in Lyme disease endemic regions are more likely to test for Lyme disease and provide prompt treatment. Obviously, the prevention of progression of infection to late-stage or chronic Lyme disease will ultimately be more cost effective (in terms of both financial and human costs) than allowing people to develop ongoing, debilitating health conditions that not only affect their ability to work and contribute financially to society, but creates a huge strain on the health care system.

- Horowitz (2013) reports that for people who were accurately diagnosed and treated early for Lyme disease, the annual cost was US\$1,500. And for people NOT diagnosed early, the annual costs of treating their disease was US\$16,000. We understand that Australian Lyme sufferers are finding similar costs. These costs included medical costs, non-medical costs and productivity loss.
- Currently, whether acquired overseas or locally, Australians are not being adequately diagnosed using existing Australian blood testing procedures, and there are no adequate nor comprehensive Australian guidelines advising doctors on how to treat them. Please refer to Mr Mackenzie's own work on addressing infectious diseases, where he has identified this issue. Interestingly, he has not referenced his own work (refer: [www.wpro.who.int/wpsar/volumes/02/1/2011.2.1.006\\_ED\\_MacKenzie.EN.pdf](http://www.wpro.who.int/wpsar/volumes/02/1/2011.2.1.006_ED_MacKenzie.EN.pdf)).
- Due to this perceived lack of action by medical authorities, Australians suffering from a Lyme-like illness rely on overseas testing and guidelines.
- However, patients who are diagnosed by Australian doctors based on clinical symptoms and/or positive blood tests (whether done locally or overseas) are not accepted by mainstream medicine as having Lyme disease, even when they respond positively to Lyme treatment.
- There are other doctors who appear to be open to the possibility, however want more proof. It is the patients who are then left confused and vulnerable and can continue to decline in health.
- Anecdotally, it appears that the few Australian doctors who diagnose and treat Lyme disease patients have been targeted for audits by the health authorities who question their practices in treating Lyme disease patients. Some of these doctors are also subject to vilification from colleagues and the medical establishment, and have been called "charlatans" because they are diagnosing and treating patients for Lyme disease. Unfortunately, this has happened recently to one doctor and is entirely discriminatory. This situation has sent waves of fear and anguish through members of the Lyme community, who already suffer feelings of helplessness and hopelessness on top of their physical ailments, due to the Government's position of denial.

- The current situation requires urgent change. This change would be prompted through new research investigating which species of *Borrelia* are carried by Australian ticks and can be transferred through their bites, and or which animals are hosts to borrelia; the development of sensitive and specific diagnostic tests appropriate for the Australian situation; and further education of our health professionals on diagnosis and treatment of Lyme Borreliosis and other tick borne infections. Public education on tick bite prevention is also an essential component.
- For far too long the voice of the Australian Lyme disease sceptics has been heard. In the meantime, more and more Australians are being diagnosed with a Lyme-like illness (and its many co infections) acquired in Australia. They are receiving appropriate treatment and many are recovering their health.
- It is to the Australian medical community's shame that Australian Lyme sufferers are often misdiagnosed with degenerative autoimmune disorders, dismissed as suffering from psychosomatic disorders, or as is the case for many, both. Misdiagnosis of Lyme disease for an autoimmune disorder often results in patients receiving corticosteroid medication designed to suppress the immune system. This allows the undiagnosed infection to spread rapidly with devastating consequences for the patient.
- It is time that the prevailing assertion amongst the medical industry that Lyme disease cannot be acquired in Australia, is reviewed in light of increasing numbers of Australians being diagnosed. Whether acquired locally or overseas, Australians who have possible Lyme Borreliosis need to be listened to, diagnosed correctly and given appropriate medical assistance, to recover from what is for many, a long-term, debilitating chronic illness that can, and often does, lead to disability or death if untreated.
- In reference to the "Acknowledgements" at the end of the Study, it is noteworthy, and rather disturbing to Lyme patients, that no Lyme-literate medical practitioners appear to have been consulted in the development of this Scoping Study. These professionals are "at the coal-face" of the Lyme disease situation in Australia, and as representatives of Lyme patients, we believe it is essential that in-depth consultation occurs between health authorities and Lyme-literate doctors to determine areas of need for both patients and doctors alike.
- The private lab, Australian Biologics (AB) has been ignored by the author - AB is awaiting NATA accreditation and has 100% on their Quality Control results from Glasgow University. The average across the board was 76%.
- The two-tier testing system for Lyme disease in Australia has many many problems including patents owned by those in IDSA who profess this method of diagnosis.

- The CDC has withdrawn from that method and purports now that it was for surveillance criteria and not diagnosis. They now further purport Lyme to be a clinical diagnosis.
- The two-tier testing system has been widely criticised worldwide. A very good summary of the problems with this method can be found on pages 59-65 in Dr Horowitz's newly published book, "Why can't I get better? Solving the Mystery of Lyme and Chronic Disease". The Scoping Study author should be well advised of these issues and have a copy of Dr Richard Horowitz's book.
- Lyme disease in Australia appears to be a neurological disease, as it is in China and Europe. The scoping author purports it to be an arthritic disease and this means he seems to be presenting his work based on USA test studies and research - which do not always apply here.

**Other comments are made within the text below, also in purple.**

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## TERMS OF REFERENCE

To produce a scoping paper that identifies the research needs for an investigation into whether a causative tick-borne microorganism (*Borrelia*) for Lyme disease exists in Australia. This would include a consultation with relevant stakeholders including:

- The Chief Medical Officer's Clinical Advisory Committee on Lyme disease to determine their views on the possible direction for any future research program;
- Lyme disease and *Borrelia* experts, including those overseas, to seek advice on research directions and current practices; and
- Identify researchers that are currently conducting or considering research projects that examine tick-borne disease in Australia and how their research may complement or inform any research program.

The major outcome from the scoping paper will be the development of an outline for a research project to seek whether a causative agent(s) of Lyme disease exists in Australia. This will also address:

- Optimal methods for identification and bacterial characterisation from appropriate haematophagous arthropod vectors; and
- Provide guidance on a diagnostic pathway.

## INTRODUCTION

It is just over 30 years since the discovery of *Borrelia burgdorferi* as the aetiological agent of Lyme disease in North America (Burgdorfer et al 1982; Steere et al 1983; Benach et al 1983). Since that time, Lyme borreliosis has been recognised as an emerging disease with increasing numbers of cases across much of the temperate zones of the Northern Hemisphere, stretching from the Mexican border to southern Canadian provinces in North America, the whole of Europe and northern Asia (Hubalek 2009; Franke et al 2013). Occasional cases have also been recognised in Central and South America (Gordillo-Perez et al 2007; Carranza-Tamayo et al 2012), and in northern Africa (Hubalek 2009; Franke et al 2013). **And Southern Africa.** It is now recognised to be the most frequent cause of tick-borne disease with an estimated 65,000+ cases in Europe and a further 20,000+ cases in the United States,

August 19, 2013, CDC Press Release states “that the number of Americans diagnosed with Lyme disease each year is around 300,000.” The author has quoted figures that are 15 times less than the actual up-to-date report.

but this may be a significant underestimate with many cases unreported, and compounded by the small number of countries in Europe to make Lyme disease notifiable, and the actual total may be closer to 255,000 cases annually (Rudenko et al 2011; Radolf et al 2012). The clinical presentation varies depending on the stage of the illness: early disease includes erythema migrans and an influenza-like illness; early disseminated disease includes multiple erythema migrans, meningitis, cranial nerve palsies and carditis; and late disease present primarily as arthritis. In most patients, signs and symptoms resolve after appropriate treatment with antimicrobials in 2-4 weeks (Murray and Shapiro 2010), but in some patients a prolonged or ‘late’ disease may occur lasting over several months, and there have also been claims that ‘chronic Lyme disease’ may occur in some individuals with a wide range of unspecific symptoms (Cameron et al 2004; Franke et al 2013). In Australia, the presence of Lyme disease remains uncertain, equivocal, and evidence for the presence of *B. burgdorferi* or any other related aetiological agent remains confused or unsubstantiated.

This is because state and federal governments will not fund further research nor accept earlier research that found *Borrelia*, such as Pope and Carley, Mackerras, Hudson, Wills and Barry and Mayne, Stewart etc, as well as not accepting overseas test results and looking at a person’s clinical symptoms in entirety. What a pity for Lyme patients that there isn’t a multi-billion dollar horse-race gambling industry at stake from the spread of Lyme disease – otherwise we might have already received a huge research grant, as for Hendra Virus!

This uncertainty and confusion has spilt over into the public arena, fuelled in part by emotive and unsubstantiated reporting by the media, and has resulted in substantial public concern.

Lyme disease patients find this statement disrespectful, belittling and patronising, with many suffering far greater loss, pain and hardship than those few cases mentioned in the media. The statement “spilt over into the public arena” is equally offensive, since it is basically only within the public arena that patients in Australia are receiving support, for example, by forming support groups and networks to assist one another. They most certainly cannot depend on any government

systems for support. In fact, many have suffered discrimination and abuse in the government health system, as have the few doctors courageous and caring enough to diagnose and treat Lyme disease patients.

It is becoming increasingly important to resolve this issue in order to provide public assurance,

*“assurance” of what?! That there is nothing to be concerned about?? This seems to be implied here.*

particularly for those whose life-styles or homes are associated with risks for exposure to tick bites, and to provide some degree of certainty to those suffering from symptoms which have been diagnosed as being due to Lyme disease and its many co-infections.

A positive response to Lyme disease treatment provides “certainty”. It is just that simple. It is only the opinions of practitioners and others who are sceptical, uneducated or ill-informed that are “uncertain”. It is of far greater concern that there are thousands of patients in Australia who actually have Lyme disease but are undiagnosed and suffering or, worse still, misdiagnosed and receiving the wrong treatment. Unfortunately, many of these people die due to misdiagnosis and the wrong treatment that will exacerbate their underlying true illness.

The undertone of this statement by the author is anything but “assuring” to Lyme patients! Some report hearing that the presence of Lyme disease in a region will have a detrimental effect on real estate values and tourism, and perhaps this is what the author is implying in his statement, “...particularly for those whose life-styles or homes are associated with risks for exposure to tick bites...”. Surely, the government and others with vested interests in maintaining financial viability of regions would be very grateful for “assurance” that Lyme disease does NOT exist! Consideration of economic factors at the expense of peoples’ health and well-being is nothing short of unethical and immoral and must NOT be allowed to occur in this country. Officials involved in this matter at every level must ensure that there is no “cover up” or “down-playing” of findings or evidence that may inadvertently have financial implications. Adequate education regarding prevention and timely treatment of Lyme disease will most certainly be all that is required to alleviate any “fear-factor” experienced by those with financial interests.

To ensure public confidence, it is essential that any investigation to determine the presence or absence of Lyme disease should be open and uncommitted, **(Agreed! But it is not apparent in this Scoping Study!)**

and that all possible scenarios should be canvassed. In approaching this, the current accepted knowledge of Lyme as it occurs in the United States, Europe and Asia must provide the basis of Australian studies, but with the acknowledgement that an Australian agent responsible for ‘Lyme-like’ disease might be significantly different from those described elsewhere in the world, including the possibility that it might be due to an infectious agent other than a *Borrelia* species, and that this might extend to differences in modes of transmission and to possible treatment protocols.

Scoping a research programme to prove or disprove the presence of Lyme borreliosis in Australia requires an understanding of the incidence, cause, transmission, pathogenesis, diagnosis, and epidemiology of Lyme disease. Thus this scoping study begins with a brief review of Lyme disease in

the Northern Hemisphere, especially the United States and Europe, and then defines some of the questions needed to address the rationale of the study. Finally these questions are expanded to form a research programme. Sections in the Background specifically directed at the Australian scene or particularly relevant to determining the presence or absence of Lyme borreliosis in Australia, are shown in bold type.

## **BACKGROUND: BRIEF REVIEW OF LYME BORRELIOSIS.**

### **(a) *Borrelia* species in Lyme disease and their vectors, reservoirs and genomes.**

Lyme disease is a zoonotic tick-borne disease caused by a certain members of a group of related spirochaetes – *Borrelia burgdorferi* sensu lato (s.l.) – that are transmitted by specific *Ixodes* spp. ticks. The *B. burgdorferi* s.l. complex is a diverse group of more than 18 spirochaete species. Four species comprising *B. americana*, *B. andersonii*, *B. californiensis*, and *B. kurtenbachii* are found only in North America; eleven species occur in and are restricted to Eurasia comprising *B. afzelii*, *B. bavariensis*, *B. garinii*, *B. japonica*, *B. lusitaniae*, *B. sinica*, *B. spielmanii*, *B. tanukii*, *B. turdi*, *B. valaisiana*, and *B. yangtse*; and three species occur in both North America and Europe, *B. burgdorferi* sensu stricto (s.s.), *B. bissettii*, and *B. carolinensis* (Rudenko et al 2011). In North America, the primary and by far the most frequent cause of Lyme borreliosis has been *Borrelia burgdorferi* s.s. (Radolf et al 2012; Stanek and Reiter 2011; Rudenko et al 2011), although very occasional cases may be due to *B. andersonii* and *B. americana* (eg. Clark et al 2013), whereas in Europe five species, *B. afzelii*, *B. garinii*, *B. burgdorferi*, *B. spielmanii*, and *B. bavariensis*, have been shown to cause Lyme disease, with occasional cases associated with three other species, *B. bissettii*, *B. lusitaniae*, and *B. valaisiana* (Rudenko et al 2011; Stanek et al 2012). In ticks, *B. afzelii* and *B. garinii* are the most common genospecies circulating in Europe, followed by *B. burgdorferi* s.s. and *B. valaisiana* (Rauter and Hartung 2005). North American strains of *B. burgdorferi* s.s. are significantly more heterogeneous than those from Europe, with genetic diversity demonstrated by PCR-restriction fragment length polymorphism (Liversis et al 1999) and by sequence typing (Bunikis et al 2004), and different genotypes have been associated with disease severity (Travinsky et al 2010). Sequence typing has also shown genetic diversity for *B. afzelii* in Europe (Bunikis et al 2004). Of three main genospecies, *B. garinii* and *B. afzelii* are antigenically distinct from *B. burgdorferi* s.s., which may account for some of the variation in clinical presentation in different geographic regions. The *Borrelia* spp. in the Lyme borreliosis group, together with their vectors and reservoirs, are tabulated and discussed by Franke et al (2013).

There are 300 known species of Bb, 24 of which have so far proven to be pathogenic to humans.

New genospecies in the Lyme *Borrelia* complex are being recognised almost every year (Stanek and Reiter 2011) and more would be undoubtedly found if a concerted effort was made in collecting and processing ticks, especially in new areas. Examples of this have been demonstrated in Canada (Scott et al 2010; Ogden et al 2011), and in Uruguay (Barbieri et al 2013). The latter report is the first isolation of indigenous *B. burgdorferi* s.l. in the Southern Hemisphere, and also demonstrates that novel *Borrelia* genospecies in the *B. burgdorferi* s.l. complex may occur in new geographic areas.

Transmission of Lyme borreliosis is through injection of tick saliva during feeding. The disease is transmitted largely by four species of hard ticks in the *Ixodes ricinus* complex: the major vector in Europe is *I. ricinus* and in Asia is *I. persulcatus*, whereas in the United States the major vector in the

north-eastern and mid-western states is *I. scapularis*, and in western US is *I. pacificus* (Stanek et al 2012; Radolf et al 2012). Other hard ticks do not appear to play any significant role in Lyme borreliosis; they are either inefficient in the acquisition of *Borrelia* spirochaetes from blood meals, or they are unable to maintain the spirochaete. The risk of infection in humans increases with length of time of exposure to the tick, approaching 100% on the third day (Biesiada et al 2012). It usually requires a feeding period of more than 36 hr for transmission of *B. burgdorferi* by *I. scapularis* or *I. pacificus* ticks in North America, but can be significantly shorter, often less than 24hr, for transmission of *B. afzelii* by *I. ricinus* ticks in Europe (Hubalek 2009). Although *Borrelia* spirochaetes can be transmitted to humans by all three stages of tick development, the nymphal stage is responsible for the vast majority of infections, partly because it is often overlooked by those being bitten due to its small size, followed by the female adult tick. An exception to this is found with *I. persulcatus* in which adult female ticks are most frequently responsible for transmission (Stanek et al 2012). The mean prevalence of *Borrelia* infection in ticks is difficult to quantify with any accuracy as it depends on locality, suitable sources of blood meals to ensure tick maintenance, prevalence of suitable reservoir species, climate, herbage and other environmental factors. Recent meta-analysis of surveillance data from Europe indicated that the overall mean prevalence was 13.7%, with a range from 0 to 49% (Rauter and Hartung 2005). Similar results have been observed in other European studies (eg: Reye et al 2010; Wilhelmsson et al 2010; Mysterud et al 2013) and in endemic areas of North America (Morshead et al 2006), although some studies found a higher incidence (32-39%) in questing ticks (Walker et al 1994; Hanincova et al 2006). The number of spirochaetes per *Borrelia*-infected tick ranged from  $2 \times 10^2$  to  $4.9 \times 10^5$  with a median of  $7.8 \times 10^3$  (Wilhelmsson et al 2010). Similar levels of infection per tick were reported from the north-east United States (Wang et al 2003).

Although other tick species are believed to play no significant role in Lyme borreliosis, some species of *Amblyomma* can yield spirochaetes in the *B. burgdorferi* s.l. complex. In the United States, *B. burgdorferi* s.s. has been reported in *A. americanum*, the Lone Star tick (eg Schulze et al 2006; Clark et al 2013), but this tick species has been shown to be unable to transmit Lyme *Borrelia* (Piesman and Sinsky 1988; Ryder et al 1992).

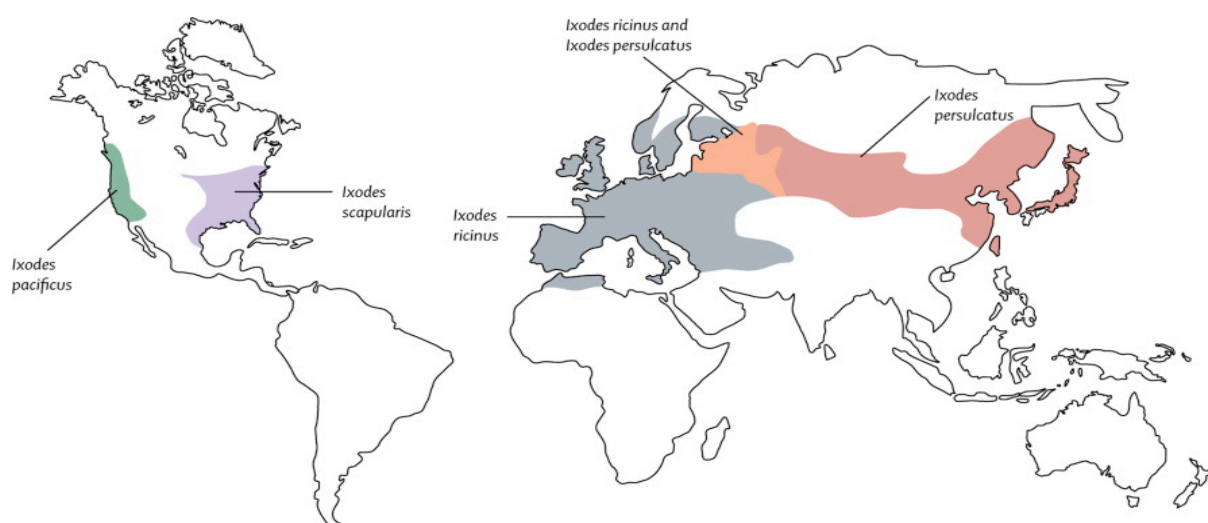


Figure 1. Global distribution of the vectors (*Ixodes ricinus* species complex) of Lyme *Borrelia*. From Stanek et al (2012), Lancet 379: 461-473.

Mites have also occasionally been implicated in the transmission of *B. burgdorferi* s.l., but their role in transmitting the spirochaetes to humans remains to be determined (Lopatina et al 1999; Kampen et al 2004; Netusil et al 2005; Literak et al 2008). Mites (*Dermanyssus gallinae*) have been suspected of transmitting *B. anserina* to avian hosts in eastern Australia (J.Curnow, personal communication).

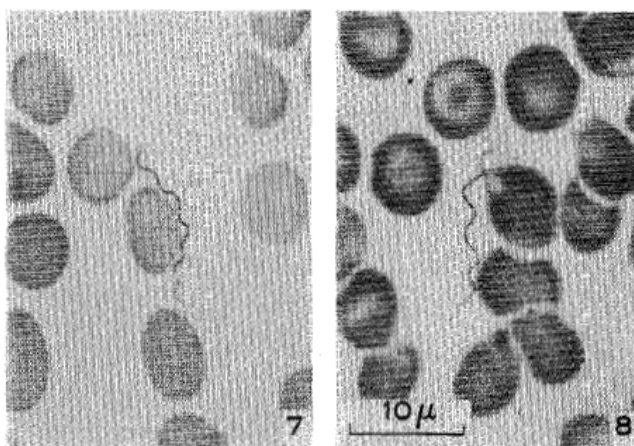
**No member of the *I. ricinus* complex occurs in Australia, but the most plausible indigenous vector is *I. holocyclus* which is known to parasitise native vertebrate hosts, domestic animals and humans, and is the most common tick biting humans. Known as the paralysis tick, it is the most important medically, causing local irritation and allergic reactions, but paralysis in humans is now uncommon, and occurs most often in domestic animals. It is found in a 20-30 kilometre strip along the eastern coast of Australia, as well as in pockets up to 100km inland. To this date, there has only been one report of *Borrelia* species being found in *I. holocyclus* ticks, but the cultures were not confirmed and were unsustainable (Wills and Barry 1991). *Borrelia* Research in Australia**

Mulhearn (1946) noted two parasites found in the blood of cattle (*Spirocheta theileri* and *Bartonella bovis*) recorded for the first time. A bartonella parasite is a known co-infection of humans with Lyme disease.

In 1953 Mackerras, (Mackerras, Mackerras, Sanders 1953) identified a spirochete bacteria in the blood of a bandicoot (*Isodon obesulus*) named *Theileria taehyglossi*.

In 1956 researchers Carley and Pope (Carley and Pope 1962) isolated a species of *Borrelia* from native rats, in north-western Queensland. This *Borrelia* species was named *Borrelia queenslandica*.

A few years later Mackerras (1959 and 1953) after reviewing published accounts of blood parasites of Australian animals and conducting further research identified *Borrelia* in the blood of bandicoots and kangaroos. Evidence of the spirochaete from these animals was provided in plates 7 & 8, in the published paper and reproduced below.



Mackerras's review revealed numerous previous accounts of *Borrelia* found in animals in Australia dating from as early as 1797 as follows: 1797 - bandicoot; 1800 – eastern grey kangaroo; 1822 - red kangaroo; 1897 – rats; 1946 - rodents, kangaroos and cattle, and 1952 – cattle.

Mackerras's study also identified a number of other tick-borne pathogens (known co-infections of Lyme disease), including *Babesia* and *Anaplasma*. These pathogens are



believed to have spread steadily southward after being introduced from Java to the Northern Territory in 1872 by 12 Brahmin cattle infected with cattle ticks. *Babesia* was only formally acknowledged again by the medical community when it was isolated in a man from the South Coast of NSW, who died in 2012, (Senanayake et al 2012).

During the late 1980s and early 1990s two separate research projects were undertaken to search for *Borrelia* in Australia, with contradictory findings. The first research project (reported by Wills & Barry, 1991) found a number of *Borrelia* species and subsequently developed a screening test for them. This was the work of microbiologists Professor Richard Barry and his then student Michelle Wills from University of Newcastle, work which although accepted in the thesis which earned her a PhD (Wills 1995), was never formally published in the scientific literature.

The second research project was led by entomologists, Richard Russell and Stephen Doggett at the Institute for Clinical Pathology and Medical Research, Westmead, with Sydney University. No *Borrelia* species were identified in their team's study which used PCR (polymerase chain reaction - a highly specific test) to test 1038 ticks of the 12,000 collected from the East Coast of Australia. A total of 10,970 ticks were also dissected for spirochaete isolation (Russell et al 1994).

However this study had two major shortcomings. Only 570 *Ixodes holocyclus* ticks were tested using PCR, despite this species being the tick of most concern to human health in Australia. In addition, they only tested for *Borrelia burgdorferi*, the American strain, and not other strains of *Borrelia*. Russell and Doggett isolated unidentified "spirochaete -like objects" in fed ticks they dismissed them as artefacts and concluded: "There is no definitive evidence for the existence in Australia of *B. burgdorferi* ... or any other tick-borne spirochaete...". (Russell et al. 1994).

Most of these ticks tested were unfed. In the work of Radolf (2012) he found that the biological transformation of borrelia in ticks immediately upon attachment to mammals and the subsequent OspC production in the salivary apparatus of the hypostome and gut wall, was not yet known.

Further to the Russell and Doggett study, Piesman and Stone (1991) published research which set out to implicate *I. holocyclus* as a vector for Lyme disease transmission. They fed Australian larvae ticks the North American Bb isolate B31, and then studied the ensuing nymph forms. None of these nymphs were found to carry spirochetes. The authors of this research stated that, "These experiments should be repeated with Australian strains of spirochetes". It is understood that these experiments were not repeated or have not been published.

Unfortunately, these two studies which failed to isolate an Australian species of *Borrelia*, both using outdated methods by today's standards, have continued to hold the position that Lyme disease is not acquired in Australia.

It is understood that in the Russell and Doggett study (1994), no spirochetes were found in the midgut content of 570 *I. holocyclus* of mostly unfed ticks using dark field microscopy. Of these 570 ticks, 279 were nymphs and 289 were adults. There were only 92 fed ticks in the entire series.

The authors did not state how many fed *I. holocyclus* ticks were subjected to PCR examination. Radolf (2012) found that spirochetes within unfed nymphs exist in low numbers and in a poorly understood metabolic state that enables them to endure prolonged periods of nutrient deprivation. We draw this reference to the Scoping author's attention.

However, before this research was published in 1994, another researcher picked up their initial results and claimed, “Doggett had made over 70 isolates of spirochaete-like organisms from more than 30 separate coastal areas stretching from southern Queensland to northern Victoria” (Alpers 1992). These were later dismissed by Russell et al as “...artefacts, probably bacterial flagellae,” (Russell et al. 1994).

I wonder if overseas experts examined these isolates, and if they are still available for examination overseas.

Given the small number of ticks sampled, and the manner and strain for which they were tested, this is hardly a convincing scientific conclusion.

In her PhD study, Michelle Wills tested 168 cultures from ticks: 74 had spiral shaped organisms that were comparable in size and shape to *Borrelia burgdorferi*. Wills isolated spirochaete from Australian ticks, amplifying the outer surface protein A gene in three of the isolates. Two matched with 100% identity to *Borrelia burgdorferi* sensu stricto, the other one was not identical to the American species, but was considered a spirochete closely related to *Borrelia burgdorferi* sensu lato. A fourth isolate more closely resembled *Borrelia garinii* based on its reactivity to monoclonal antibodies.

The Newcastle researchers also did a sero prevalence study. Over 2000 people were tested and they found that, while 62% returned antibodies to a flagellin, a very large number (28%) had antibodies against outer surface protein A. This was measured by the Western blot test using proteins from all three species of *B burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*.

The Newcastle work highlighted that Australian testing should be looking for all three strains of *Borrelia*, these being *B burgdorferi*, *B. garinii* and *B. afzelii*. (McFadzean 2012).

Wills also contributed to a paper that confirmed the presence of an Australian strain of *Borrelia* closely related to *B. garinii* and *B. afzelii*, and described clinical presentations of a Lyme-like illness (Hudson et al. 1994).

In a later paper, another team led by Hudson described laboratory evidence of *Borrelia* from a man who had become sick with a Lyme-like illness 16 days after being bitten by a tick in the Pittwater area of Sydney (Hudson et al. 1998). They argued that clinical details indicated that local acquisition of Lyme disease was possible, despite the fact that the patient had travelled to Europe 17 months prior to being symptomatic. They also identified an issue with conventional blood and tissue tests which were negative, despite an illness duration of at least two years. However, because of this man’s previous travel history, local acquisition of Borreliosis was dismissed.

The Australian medical community is still questioning and dismissing the possibility of local infection (even when positive *Borrelia* test results are obtained) if a patient has travelled overseas, regardless of either the time since travel or the recent clinical symptoms to a tick bite in Australia and the lack of any such tick bite or symptoms while overseas.

**In summary, there are numerous bodies of research and clinical presentations confirming the presence of *Borrelia* spirochetes in Australia, and numerous historical isolations of *Borrelia*, and yet Australian governments appear to continue to cite only the work of Russell et al. (1994), work that only tested for and failed to isolate the one genospecies *Borrelia burgdorferi* sensu stricto.**

In her recently published book, *Lyme Disease in Australia* (2012), Dr Nicola McFadzean comments, “...perhaps the findings of Russell and Doggett should be a side note rather than the source of a national denial of a major, and growing, health crisis” (McFadzean 2012).



Experimental vector competence studies have demonstrated that this species of tick is unable to be infected by a North American isolate of *B. burgdorferi* (Piesman and Stone 1991), but further studies with other *Borrelia* species is warranted. The high spirochaete loads reported in infected ticks in both Europe and North America would have suggested that a similar finding might be expected in Australian ticks, so it is all the more surprising that they haven't been regularly detected in *I. holocyclus* or any other Australian tick species. In Western Australia where cases of Lyme-like disease have also been reported, the most common ticks biting humans are *A. albolimbatus*, *A. triguttatum* and *I. australiensis* (Mark Harvey, personal communication), but none of these have been associated with borreliosis. Most of the 75+ species of Australian ticks are hard ticks, and other widespread examples are the brown dog tick (*Rhipicephalus sanguineus*) and the bush tick (*Haemaphysalis longicornis*), although there has been a report that Lyme spirochaetes have been detected in this latter species in China (Meng et al 2008). There are fewer soft ticks in Australia, and the most important belong to the *Ornithodoros* genus, examples being the *O. gurneyi*, the Kangaroo soft tick; *O. capensis*, a tick of birds, and *O. moubata* complex. Some members of this latter complex feed on animals, with one species infecting pigs and occasionally biting humans. The avian *O. capensis* has been shown to harbour the flavivirus, Saumarez Reef (see below). This genus of soft ticks is more important for the transmission of the relapsing fever *Borrelia* species, but *B. burgdorferi* s.l. have occasionally been found in *Ornithodoros* species (eg. Lane et al 2010; Adham et al 2010).

#### (b) The natural reservoirs of Lyme *Borrelia* species.

The reservoirs of Lyme *Borrelia* spp. are small mammals and some birds (reviewed in Piesman and Gern 2004; Rizzoli et al 2011; Franke et al 2013). Deer are not competent reservoirs but are essential in many areas for the maintenance of tick populations because they are one of a few wildlife hosts able to feed sufficient numbers of adult ticks (Stanek et al 2012). Other large domestic animals such as cattle and sheep are also not competent reservoirs. Lyme *Borrelia* do not cause disease in reservoir hosts, and other than humans, the only mammals known at this time to show disease symptoms are dogs and possibly horses. Indeed the use of ticks taken from dogs provides a good indication of the presence of Lyme disease in a given location, and dogs are an excellent sentinel species for estimating Lyme disease risk (Hamer et al 2009; Smith et al 2012). Although a broad spectrum of clinical signs have been attributed to infection with *Borrelia* in horses, actual cases of equine Lyme borreliosis are rare if they exist at all (Butler et al 2005).

Birds can act both as biological carriers of *Borrelia* and transporters of infected ticks, and aid in the dispersal and spread of *B. garinii* to new foci (Comstedt et al 2011). Certain Passerine songbirds are capable of being reservoir hosts of *Borrelia* species in the United States and Europe, especially members of the Family *Turdidae*, which includes the thrushes, Blackbirds, and the American robin, many of which are migratory so thus dispersing infected ticks and expanding the geographic range of the spirochaete (eg: Olsen et al 1995; Comstedt et al 2006; Kipp et al 2006; Poupon et al 2006; Dubska et al 2009; Brinkerhoff et al 2010; Scott et al 2010; Brinkerhoff et al 2011; Scott et al 2012). The most common *Borrelia* species found in *Ixodes ricinus* ticks taken from Passerine birds in Europe were *B. garinii*, *B. lusitaniae*, and *B. valaisiana*, whereas those in United States were *B. burgdorferi* in

*I. scapularis* ticks, and occasionally in *I. pacificus*. A second bird-associated tick, *I. auritulus*, was also found to be infected with *Borrelia* species including *B. burgdorferi* s.l. and some novel *Borrelia* species in ticks collected from a number of songbird species from various sites across Canada (Morshed et al 2005; Scott et al 2010; Scott et al 2012). *I. auritulus* ticks have been found on birds in many parts of the world (eg: Gonzalez-Acuna et al; 2005; Eisen et al 2006; Kolonin 2009), but while they may not bite humans, they appear to play a significant role in Canada in the dispersal and maintenance of *Borrelia* species. With respect to dispersal by migratory passerines, it has been found that birds are able to carry the Lyme disease as a latent infection for several months which can be reactivated by migratory stress and passed on to ticks making the birds long-distance carriers of the spirochaete (Gylfe et al 2000).

The seabird tick, *I. uriae*, also maintains *B. garinii* in silent enzootic cycles in seabirds at their nesting sites demonstrating that these non-passerine seabirds also play a role in long-distance dispersal of *Borrelia* species over a wide area in both the Northern and Southern Hemispheres (Olsen et al 1993), and suggested a transhemispheric exchange of Lyme disease spirochaetes (Olsen et al 1995). However, *I. uriae* ticks (and *I. holocyclus*) might possibly have an Australian origin, and with a parsimony analysis indicating that southern hemisphere *I. uriae* ticks are paraphyletic in respect to northern *I. uriae* ticks, and the northern ticks are monophyletic, it would suggest that this tick is no longer carried across the equator, but further work is needed to clarify this (Gylfe et al 2001). More recently, phylogenetic studies of *Borrelia* species circulating in seabirds in the Northern Hemisphere have been undertaken by Duneau et al (2008) demonstrating the presence of two main clades, one associated with *B. garinii* and the other with *B. lusitaniae*, but there was no clear association between different *Borrelia* species and a given host seabird species. An additional sequence also clustered most closely with *B. burgdorferi* s.s. Other studies have described various tick-borne viruses in *I. uriae* ticks collected on Macquarie Island, but no attempt was made to examine the ticks for *Borrelia* infections (St George et al 1985; Major et al 2009). *I. uriae* do not bite humans, but it is probable that seabirds carrying the ticks could interact with shore birds at many sites which could introduce Lyme *Borrelia* to native avifauna, or the ticks could be introduced by gulls to shorebirds, but as avian ticks are not known to bite humans in Australia, it is likely that only occasional, sporadic human cases could result.

*Ixodes holocyclus* ticks parasitise Australian birds, therefore it is possible that any of the abovementioned pathogens in migratory birds may be transferred to *I. holocyclus*.

**It is therefore plausible that certain *B. burgdorferi* s.l. strains could be brought to the Southern Hemisphere and enter local Australian ecosystems through intermingling between seabirds and land-based avian species, but most bird ticks do not bite humans, and if they did, would rapidly drop off before the opportunity to transmit the spirochaete. If cases of human infection were to result, they would be very occasional and localised.**

### **(c) Some comments of the *Borrelia* genome.**

*Borrelia* species have the most complex genomes of all known bacteria (Radolf et al 2012), comprising a relatively small chromosome and 20 or more linear and circular plasmids. The complete genome sequences of three major species of *Borrelia* have been described; strains of *B. burgdorferi*

s.s. (Fraser et al 1997; Schutzer et al 2011), *B. garinii* (Glockner et al 2004; Casjens et al 2011), and *B. afzelii* (Glockner et al 2006; Casjens et al 2011), together with a number of their plasmids (Fraser et al 1997; Casjens et al 2000; Glockner et al 2006). In addition, whole genome sequences have also recently been reported of *B. bissettii*, *B. valaisiana*, and *B. spielmanii* (Schutzer et al 2012). Most of the housekeeping genes are on the chromosome which is fairly constant in organization and content across the genus (Casjens et al 2010). In contrast, the plasmids exhibit much greater variability in gene content and are not equally represented between all strains and are not all essential for the maintenance of the enzootic cycle. The core of all *Borrelia* species consists of the chromosome and two plasmids (1p54 and cp26). The key genes thought to contribute to maintenance of the *B. burgdorferi* enzootic cycle, including the position of each with respect to the genetic element, and the function of their encoded protein, have been reviewed by Radolf et al (2012). As an obligate parasite, *B. burgdorferi* lacks the conventionally recognizable machinery for synthesizing nucleotides, amino acids, fatty acids, and enzyme cofactors, apparently scavenging these necessities from the host (Fraser et al 1997).

#### **(d) Lyme *Borrelia* and human disease.**

When an infected tick takes a blood meal, the ingested bacteria multiply and undergo a number of phenotypic changes, including the expression of the outer surface protein C (OspC) which allows them to invade the host tick's salivary glands – a process which takes several days and explains why transmission only occurs after a significant delay (Stanek et al 2012). The initial clinical manifestations of Lyme disease in both North America and Europe share some common features such as an erythema migrans (EM) rash and an influenza-like illness, with fatigue, headache, myalgia, arthralgia, and malaise, although some early infections may be completely asymptomatic. In about 90% of cases, the development of the characteristic skin lesion, EM, occurs at the site of the tick bite. The incubation period between tick bite and appearance of EM is typically 7 to 14 days, but may be as short as a day and as long as 30 days (Marques 2010). Atypical EM can occur in some patients, and be uncharacteristic (Marques 2010; Schutzer et al 2013). Erythematous lesions occurring within a few hours of a tick bite represent hypersensitivity reactions rather than EM. Subsequent manifestations may develop and correlate approximately with the infecting species: *B. burgdorferi* s.s. infection frequently leads to arthritis, whereas *B. garinii* leads more often to neurological manifestations, and *B. afzelii* to skin disorders, although these clinical associations are not absolute (van Dam et al 1993). A summary of clinical case definitions and a discussion of the various manifestations of Lyme borreliosis has been provided by Stanek et al (2011), and the primary and supporting diagnostic testing and supporting clinical findings for each Lyme manifestation were tabulated in summary form in Stanek et al (2012). As Lyme disease can take various forms, differential diagnosis is essential, and the most common of these are described by Stanek et al (2012).

To exemplify the presentation and disease course, one particular series of cases is described here that occurred over a 12 month period in an area of central Germany where Lyme disease is very common (Huppertz et al 1999). A total of 313 cases were diagnosed as Lyme disease, based on strict case definition criteria which required the presence of either EM lesions or lymphocytoma, or a positive serological test with the presence of another specific manifestation of Lyme borreliosis,

giving an incidence of 111 cases/100,000 population. The highest rates of infection were in children (<18) and elderly adults (60-65 years old). EM was the most common clinical manifestation, occurring in 288 (92%) patients. It was the only manifestation in 279 (89%) patients of whom 26 had multiple EM lesions. Other specific manifestations of Lyme borreliosis with or without EM occurred in 34 (11%) patients. Fever was noted in only 26 patients. Of the 34 patients with manifestations other than EM alone, 15 had arthritis, 9 had neuroborreliosis, 6 had lymphocytoma, 4 had acrodermatitis chronica atrophicans (ACA), and one patient had carditis. In the 15 patients with Lyme arthritis, the large joints were most frequently involved, most commonly the knee, and most patients had had symptoms in more than one joint, usually the shoulders or elbows. Of the 9 patients with neuroborreliosis, 5 had symptoms of meningitis, one of whom also had facial nerve palsy; one patient had uveitis and oedema of the optic disk; and the remaining three had symptoms of radiculoneuritis, such as paresthesia or palsy of the lower extremities. Children were more likely to have manifestations other than EM alone, whereas adults were more likely to have EM as their only manifestation. A total of 185 (60%) patients reported a recent tick bite; those in children were more likely to be on the head and neck compared to adults where the bite was most frequently on the legs. All patients with symptoms other than EM alone experienced improvement or resolution after antibiotic therapy, except one patient with ACA who refused therapy.

The results in the above case study were similar to those in other studies in Europe, but differ slightly from enzootic areas in North-Eastern United States. The incidence of EM is similar in some studies (Shapiro and Gerber 2000) but is lower in others (Bacon et al 2008; Ertel et al 2012) in the United States. The most common late manifestation in the United States is arthritis; but neurological manifestations are less common, and ACA and lymphocytomas are extremely rare. The latter may be a reflection of the aetiological agent as they are usually caused by the European *Borrelia* species, *B. afzelii* or *B. garinii* (Huppertz et al 1999; Shapiro and Gerber 2000).

As with **all** infectious diseases, infection with *B. burgdorferi* s.l. leads initially to an IgM antibody response, followed 2-4 weeks later by an IgG antibody response. The IgM response tends to be relatively short-lived in most patients, but the IgG response remains for decades following infection (Glatz et al 2008; Kalish et al 2001).

A Lyme-like disease in Brazil, the Baggio-Yoshinari syndrome, has been associated with *B. burgdorferi* s.l. infection serologically and by PCR. The Brazilian cases present with EM and with other Lyme-like manifestations, such as arthritis, but with an increasing frequency of relapsing episodes in untreated patients. The main vector is *Amblyomma cajennense*, although ticks in the genus *Rhipicephalus* may also be involved (Gouveia et al 2010; Carranza-Tamayo et al 2012; Mantovani et al 2012). These Brazilian cases demonstrate that other disease manifestations may be important in Lyme borreliosis in novel geographic habitats, including problems in isolating and identifying infectious agents and in the role of tick species other than members of the *I. ricinus* complex.

Late Lyme disease occurs in a few patients with specific symptoms that can take several months to fully resolve. Thus Lyme neuroborreliosis may take weeks or months to resolve in a small number of patients with residual paresthesias or facial paresis, and in cases when Lyme neuroborreliosis diagnosis was made late in the course of disease, recovery from severe neural disease may be incomplete. Patients with acrodermatitis chronica atrophicans who sustained severe tissue damage prior to treatment may have atrophic lesions, peripheral neuropathy, and joint deformities. In a

small number of Lyme arthritis patients, the arthritis may not respond to further antibiotic treatment, and it is likely that the arthritis in these patients is driven by immune-pathological mechanisms (Stanek et al 2011). Similar slow resolution of symptoms and signs can occur in patients with many other systemic infections (Hickie et al 2006), even if some symptoms persist. It should also be recognised that Lyme is not a fatal disease, although a few case reports have suggested that Lyme carditis might have contributed to a patient's death (Halparin et al 2013).

Chronic Lyme disease is a widely used but poorly defined term. It is frequently used as a diagnosis for patients with persistent pain, fatigue, or neurocognitive complaints without clinical evidence of previous acute Lyme borreliosis and in some instances even without serological identification of borrelial infection. This is a very bold statement by the author. Please provide references or statistics to verify and substantiate this claim. The author's wholesale quoting from articles is misleading Australians into thinking if they DON'T have these manifestations, then they DON'T have Lyme disease. This may be perceived as corrupt. There is nothing "frequent" about Lyme disease diagnosis in Australia. It is quite the opposite, in fact. Surveys of Lyme disease patients will confirm this (refer LDAA). The symptoms referred to are far more frequently clinically diagnosed as anything from: MS, chronic fatigue, somatic disorder, depression, fibromyalgia, Aspergers disorder, early dementia, and so on. Most patients in our support group report being misdiagnosed for years, then finally responded to Lyme disease treatment after seeking out a doctor who understood the complexities of Lyme disease diagnosis and treatment. The statement "without clinical evidence" is extremely disrespectful to Lyme literate doctors and their patients who spend literally hours in consultation to determine the correct diagnosis and rule out other causes. This shows ignorance on the part of the author, who has clearly not conversed with any Lyme literate doctors in Australia, as demonstrated in the list of Acknowledgements at the end of the report, nor provided references for his statement. It needs to be clearly understood by all involved with this Scoping Study and policies that may be developed as a result, that Lyme disease and co-infections IS A CLINICAL DIAGNOSIS with serological testing being used to support diagnosis, rule out other causes, etc – as is the protocol for many other well recognised illnesses, including but not limited to: MS, Parkinsons' disease and lupus. This reflects international protocols for Lyme disease and co-infection treatment.

However, some consider Lyme borreliosis to be a disease that may lead to an irreversible chronic stage, potentially leading to fibromyalgia or chronic fatigue syndrome, or worse. It is important for all concerned that every attempt is made to verify or not the diagnosis of chronic Lyme disease, and also that the 'Jury is out' over this contentious issue. The "Jury is out" only by the Australian government. The author is "cherry picking" the science here. The wide volume of research that is held with the International Lyme and Associated Diseases Society (ILADS) and the studies on the long term persistence of Borrelia in monkeys etc must also be considered. There IS clear evidence of chronic Lyme disease.

There needs to be confirmatory testing in accredited laboratories to provide scientific evidence-based support to these cases; it is well-accepted that specific IgG remains at detectable levels for many years (Glatz et al 2008; Kalish et al 2001). Serological test results are indeed useful to support a Lyme diagnosis, but are often unreliable, and therefore cannot be considered "confirmatory testing". Ang et al (2011) note "ELISAs and immunoblots for detecting anti-Borrelia antibodies have widely divergent sensitivity and specificity, and immunoblots for detecting anti-Borrelia antibodies have only limited agreement". This has certainly been the

case to date in Australia. Many patients in our support group report that a doctor suspected Lyme disease based on clinical evidence, however, upon the presentation of a negative serological test result, the diagnosis was dismissed and the patient was thrown back onto the medical “round-a-bout”. The implications of relying on serological test results for Lyme as a diagnostic criterion has already proven to be devastating for Lyme patients in Australia. PLEASE do not allow this to happen any more. Please educate our doctors to consider every aspect of the presenting patient - do not train them to be “robots” who will only observe test results.

In addition, there will need to be a long and extensive discussion to establish the correct balance between the demands of evidence-based medicine and other healing concepts (Stanek and Strle 2009), as was undertaken recently by the Robert Koch Institute, Germany’s national Public Health Institute.

**Lyme borreliosis has been reported in Australia (eg Mayne 2011; Hudson et al 1998; D Dickeson, personal communication; B Hudson, personal communication), but the vast majority of cases were patients who had travelled to Lyme endemic areas overseas. Confirmatory testing is needed for patients with no travel history, and where additional testing of putative positive specimens has been done in NATA-accredited Australian laboratories, the results could not be confirmed to international standards for Lyme diagnoses (D Dickeson, personal communication).** The author does not specify to what “international standards” he refers. The USA CDC has recently clarified that its Lyme disease case definition criteria is for surveillance purposes only – it is NOT intended as diagnostic criteria (CDC 2013). The use of CDC case definition criteria for interpretation of Lyme disease test results is a common mistake by physicians in Australia who are not Lyme literate, mainly because this is the stance adopted by NSW Health.

Further, in relation to the author’s paragraph above, the use of the word “putative” is suggestive of disbelief. There is a strong undertone of scepticism in the personal communication quoted from D Dickeson, Westmead Hospital, and this is the same undertone that has been detected at various points in this Scoping Study. Upon further investigation, it appears that “D Dickeson” quoted here is in fact David Dickeson, one of the researchers of the infamous “Russell & Doggett” study that has stalled the progress of Lyme disease research, diagnosis and treatment in Australia for the past 20 years. (*Russell RC, Doggett SL, Munro R, Ellis J, Avery D, Hunt C, Dickeson D. (1994) Lyme disease: search for a causative agent in ticks in south-eastern Australia. Epidemiology and Infection 112:375-384*). Naturally, Mr Dickeson will feel the need to defend his research that did not find *Borrelia* in Australia. This has ethical and immoral undertones. From a patient’s perspective, to be told that your positive test results are not convincing enough is appalling – especially if it just to “save face” for the researchers. In fact, it is completely unethical. This Scoping Study lacks objectivity in places, and as already mentioned, there has been no liaison with Lyme literate, treating doctors in Australia, nor patients. This country must not uphold biases on this issue any longer.

**A possible case of Lyme disease was recently reported with a neuropsychiatric presentation but no detail was provided on standard Lyme borreliosis diagnostic test procedures (Maud and Berk, 2013).** Australia appears to be discriminative with this view that only Australian NATA accredited labs should be used to confirm *Borrelia*. Is this rule also applied to every other disease known to man?

The Australian medical community is still questioning and dismissing the possibility of local infection (even when positive *Borrelia* test results are obtained) if a patient has travelled overseas, regardless of either the time since travel or the recent clinical symptoms to a tick bite in Australia and the lack of any such tick bite or symptoms while overseas. This is very

poor clinical reasoning at best, and again shows a bias towards an ingrained belief that Lyme disease does not exist in Australia.

(e) **Other *Borrelia* species associated with disease.**

Other species of *Borrelia* have been associated with louse-borne and tick-borne relapsing fever (Larsson et al 2009; Cutler 2010). Louse-borne relapsing fever is caused by *B. recurrentis* and transmitted by the human body louse (*Pediculus humanus*), and is found in limited areas of Asia, Africa and South America. It is usually associated with crowding and poor hygiene, and with periods of famine, social disruption, and war, as well as among refugees from these events (Brouqui 2011; Badiaga and Brouqui 2012). However, homeless populations in developed countries may also be at risk. There is a sudden onset of fever lasting 3-6 days, and this is usually followed by a single, milder episode. The fever often ends in “crisis”, consisting of chills, followed by intense sweating, falling body temperature and low blood pressure. Louse-borne relapsing fever is mainly a disease of the developing world, but without antibiotic treatment can result in a 10-70% fatality rate.

Tick-borne relapsing fever is caused by various *Borrelia* species depending on geographic area, and is found in Africa, parts of Asia and southern Europe, and in North and South America. It is characterised by multiple episodes of fever. The first episode of fever usually occurs after an incubation period of about 7 days, and lasts from 4 to 7 days. Subsequent episodes occur with up to 2 weeks between episodes. Frequent complaints include nausea, malaise, headaches and body aches, and sometimes with skin rashes and hepatomegaly, sometimes with jaundice. Neurological complications may occur as well as lymphocytic meningitis. Without antibiotic treatment, the disease may result in a 4-10% fatality rate. Soft ticks of the family Argasidae, usually *Ornithodoros* species, are the vectors of the tick-borne relapsing fever group, which includes *B. duttonii* in Africa and *B. hermsii* and *B. turicatae* in the United States. Unlike Ixodid ticks which usually attach to their host for days, *Ornithodoros* ticks feed quickly, completing their blood meal in less than an hour, and as their saliva contain painkillers, patients may be unaware that they have been bitten.

A second clade of *Borrelia* spirochaetes closely related to the relapsing fever spirochaetes phylogenetically are associated with hard tick vectors, including *B. theileri*, *B. lonestari*, and *B. miyamotoi*. *B. theileri* is a pathogen of cattle, the aetiological agent of bovine borreliosis, and transmitted by *Rhipicephalus microplus*, the cattle tick. It is found widely in Australia but is not believed to infect humans. *B. lonestari* is transmitted by *Amblyomma americanum* and has been associated with a Lyme-like disease in south-eastern and south-central United States which resembles Lyme disease clinically, but the patients have no evidence of infection with *B. burgdorferi* and do not develop the sequelae associated with Lyme disease (Armstrong et al 2001; James et al 2001). This syndrome is called southern tick-associated rash illness (STARI) (James et al 2001; Masters et al 1998). However, recent detailed studies of 10 patients with STARI have suggested that some cases of Lyme borreliosis in Florida and Georgia might be due to *Borrelia burgdorferi* s.l., and indeed DNA sequencing indicated *B. burgdorferi*, *B. andersonii*, and *B. americana* as infecting agents (Clark et al 2013). *B. miyamotoi* can be transmitted by a variety of *Ixodes* species including *I. persulcatus* in Japan, *I. ricinus* in Europe, *I. scapularis* and *I. pacificus* in North America. In the 8 years since *B. miyamotoi* was discovered in Japan, it has been found to have a wide geographic range in Eurasia and North America, but its role in human disease has only recently been demonstrated in Russia (Platonov et al 2011); of the 46 patients, all presented with an influenza-like illness with fever



as high as 39.5°C, and relapsing fever occurred in 5 (11%) patients, and erythema migrans in 4 (9%). A second study in Russia found that over 50% of cases of Lyme disease without erythema migrans were caused by *B. miyamotoi*, whereas *B. burgdorferi* s.l. predominated as a causative agent of the erythemic form of borreliosis (Kara et al 2010). Another recent case attributed to *B. miyamotoi* has been reported in a case of progressive mental deterioration in an older, immunocompromised patient when the spirochaete was detected by microscopy and PCR in cerebrospinal fluid (CSF) (Gugliotta et al 2013). To date, *B. miyamotoi* has not been able to be cultured *in vitro*. It has recently been shown to occur in ticks collected from passerine birds, particularly Northern Cardinals (*Cardinalis cardinalis*) in the United States, which might indicate that birds have a role in the geographic dispersal of this species (Hamer et al 2012).

Other new *Borrelia* species are being reported which are different to the *B. burgdorferi* s.l. or to the relapsing fever *Borrelia* species, although not yet associated with disease (eg Takano et al 2011; Mediannikov et al 2013), in a number of different tick species including *Amblyomma* spp. and *Hyalomma* spp..

The Louse-borne and tick-borne relapsing fevers have not been reported in Australia or New Zealand. *B. miyamotoi* appears to be widely dispersed now in the Northern Hemisphere, but there is no evidence of it being in the Southern Hemisphere. It would seem that an examination of soft ticks, particularly *Ornithodoros* species, in Australia for *Borrelia* and other pathogens is well overdue. **Absolutely. Absence of evidence is NOT evidence of absence!**

(f) *Borrelia* species in Australia.

Considerable amount of work was carried out from the late 1930s through to the early 1970s on *B. anserina*, the agent responsible for fowl spirochaetosis, transmitted by an *Argas* spp. tick in eastern and south-eastern Australia. The disease was largely controlled by controlling the tick vector, but it remains in some species in which it is transmitted by mites (J Curnow, personal communication). It had recently been thought to be a disease only of domestic species and wild birds were believed to be resistant (Mackenzie 1994; Ladds 2009), but this may be untrue, and the spirochaete may be found in various species of doves (J Curnow, personal communication). There have been two early reports of the detection of *Borrelia* species in rodents, native mammals and cattle (Mackerras 1959; Carley and Pope 1962; Pope and Carley 1956). Carley and Pope were able to culture a *Borrelia* species, *B. queenslandica*, from *Rattus villosissimus* collected near Richmond in north-western Queensland. However, they were not able to maintain it in culture. Spirochaetes morphologically similar and antigenically related to *Borrelia burgdorferi* were cultured from the gut contents of *I. holocyclus* and *Haemaphysalis* spp. ticks by Wills and Barry (1991), but the cultures weren't sustainable and these results have not been able to be repeated from ticks collected more recently. However, there is little recent information, with the exception of a limited study by Russell (1995) in native rats, bandicoots and a marsupial mouse trapped on the south coast of NSW, but no evidence of any spirochaete was found. A major study of 12,000 ticks collected along the coastal strip of NSW was undertaken by Russell and Colleagues to investigate the presence of *Borrelia* species. About 11,000 ticks comprising more than 12 species, especially *Haemaphysalis bancrofti*, *H. longicornis*, *I. holocyclus*, and various other *Ixodes* species, were dissected and the gut contents examined by dark field microscopy and, in some cases, culture but no spirochaete of any kind was detected, although spirochaete-like objects were visualised from



by dark-field microscopy. A further 1000 ticks were tested by PCR for the presence of *Borrelia* species, but once again, all were negative (Russell et al 1994; Russell 1995). Please refer to “Lyme Disease – A Counter Argument of the Australian Government’s Denial” by Karen Smith, document attached. There are many flaws in this study that are highlighted very succinctly by Ms Smith.

More recently Mayne (2012) reported the detection of *B. burgdorferi* from four patients by PCR of EM biopsy specimens; surprisingly the PCR results indicated considerable diversity with sequence data suggesting three distinct strains of spirochaete. Wills and Barry also found 3 strains in their research as discussed earlier.

Despite the recent indication of possible *B. burgdorferi* strains in Australia, further work is needed to verify these claims, and confirmatory evidence should be obtained in a second NATA-accredited laboratory. Once again, it seems odd that the negative findings of the Russell et al (1994) have been so readily accepted without challenge for 20 years, however, when a positive result is obtained, the recommendation is that the “claims” be “verified”. In the 20 years since this study, there have been another six strains of *borrelia* identified – Australia has not kept pace with research in this area. There is no other area of medical practice that so strongly holds onto a single piece of research without challenge nor validation by further studies – surely the author would never accept medical services from any specialist who bases his/her practices on research conducted 20 years ago!!! Can you imagine a neurosurgeon that operated today using 20 year old methods?! Australia’s approach to Lyme disease must not continue in this shameful, unprofessional and unethical manner. Please ensure this Scoping Study results in unbiased and ethical outcomes.

#### **(g) Laboratory diagnosis.**

Laboratory support is an essential component of clinical diagnosis of Lyme borreliosis because of the non-specific nature of many clinical manifestations. This should read, “Laboratory support is a useful component of clinical diagnosis...” In the face of unreliable testing in Australia, it has been the experience of many patients in our support group that trialling of different medications in the hands of a well-trained, Lyme-literate doctor, and gauging the response to treatment, is an extremely effective diagnostic tool. Positive laboratory testing can certainly reduce the “trial and error” effect, as the doctor can go straight to the correct treatment, which is obviously better for the patient. However, it is NOT “an essential component” as outlined above. As already mentioned, this is exactly the same situation for many other well recognised illness that also have non-specific clinical manifestations, that are routinely clinically diagnosed eg MS, Parkinsons disease, lupus, etc.

A wide range of methods have been developed for the direct detection of *B. burgdorferi* s.l in clinical tissue specimens. These include microscopic examination, detection of specific proteins or nucleic acids, and cultivation. Culture of spirochaetes from patient specimens remains the gold standard for specificity, but it is a slow process with long incubation times, and because of the low numbers of viable spirochaetes in most biopsies and the fastidious nature of the organism, the results can be very variable, ranging from 1% in Lyme arthritis to 70% in EM skin lesions, and importantly, negative results may not exclude active infections (Stanek et al 2010; Brouqui et al 2004). However culture is seldom done or available because it is unnecessary for patients with EM and too insensitive for patients with extracutaneous manifestations (Stanek et al 2012). Direct nucleic acid tests utilise PCR-

based molecular techniques that can rapidly and specifically confirm clinical diagnosis of Lyme disease, and identify the genospecies in clinical specimens and cultures (eg: Cerar et al 2008a; Cerar et al 2008b; Liveris et al 2002; Liveris et al 2012; Nocton et al 1996; O'Rourke et al 2013). The sensitivity varies depending on methodology and specimen source (Marques 2010), and will probably depend on genospecies responsible for the infection, particularly in non-endemic areas. In a study comparing the sensitivities of two PCR assays and culture for detection of *Borrelia* spp. in skin biopsies from patients with typical EM, nested PCR was found to be the most sensitive method for detecting *Borrelia* in skin lesions, followed by culture and a PCR targeting the flagellin gene. Standardisation is required as there are significant differences in methodologies and gene targets, and more clinical validations are needed, but the direct detection of *B. burgdorferi* s.l. by PCR is much more desirable than serology if the method can be developed to be reliable, easy-to-perform, economical, and sensitive (Stanek and Strle 2009). It is also important to recognise that a negative PCR result does not necessarily indicate the absence of *Borrelia* (Aguero-Rosenfeld et al 2005). Nevertheless, next generation PCR methodologies promise to make diagnosis of Lyme borreliosis more accurate and reliable in the future.

Indirect tests through serological assays for antibodies to *B. burgdorferi* s.l. are the mainstay of laboratory diagnosis, and the most common diagnostic methodologies employed; not only are the prerequisite laboratory facilities widely available, but specimens are easy to obtain. However, the complexity of the antigenic composition of *B. burgdorferi* s.l., and the temporal appearance of antibodies to different antigens, have made the sensitivity and specificity of serological tests questionable, although the use of newer recombinant antigens rather than whole cell lysates have substantially improved their reliability. Nevertheless, the limitations of serological tests must be recognised (Murray and Shapiro 2010; Evans et al 2010); the antibody response may be weak or absent, especially in EM and early in infection (Steere et al 2008) which due to a delayed IgM response or seroconversion may be ablated by early antibiotic treatment (Stanek and Strle 2003; Glatz et al 2006), and they do not distinguish between active and inactive infections (Kalish et al 2001). Most serological diagnostic protocols in the United States and Europe use a two tier system with the first stage most commonly an enzyme-linked immunosorbent assay or sometimes an indirect immunofluorescent-antibody assay, although the former is preferable as it is quantifiable and is significantly more sensitive. This is followed by a Western blot (CDC 1995; Aguero-Rosenfeld et al 2005; Brouqui et al 2004; Wilske et al 2007; Stanek et al 2011). If the serological test is positive or equivocal, then separate IgM and IgG immunoblots are done on the same serum sample. If symptoms have persisted for 4 weeks, then the IgG Western blot should be positive – untreated patients who remain seronegative despite persisting symptoms are unlikely to have Lyme disease and other potential diagnoses should be considered (Stanek et al 2012). A positive specific antibody response persists for many years (Gratz et al 2008; Kalish et al 2001). Western blots are interpreted using standardised criteria, requiring at least two of three bands for a positive IgM Western blot, and five of ten bands for a positive IgG Western blot (Marques 2010), but the criteria for the United States (CDC 1995) are not applicable for European patients (Robertson et al 2000) as their immune response is restricted to a narrower spectrum of *Borrelia* proteins compared with that shown by American patients (Dressler et al 1994). **The CDC clearly states that its case definition criteria is for surveillance purposes only, and NOT for diagnostic purposes. As stated repeatedly, Lyme disease is a CLINICAL diagnosis.**

There are problems in interpreting Western blots, particularly in Europe because of the number of different genospecies of *B. burgdorferi* s.l. with variant antigens being expressed with slightly different antigen sizes between different genospecies, and even between strains of a single species, which make standardisation of blotting procedures difficult (Robertson et al 2000; Evans et al 2011; Mavin et al 2011).

It is important that the 2-tier protocol is undertaken; if the first tier ELISA is omitted or interpretation of the Western blot is carried out using criteria that are not evidence-based, this will potentially decrease the specificity of the testing and lead to misdiagnosis. Interpreting the IgM Western blot can lead to false positive results if insufficient care is taken as non-specific weak bands can often occur.

The use of recombinant antigens, principally VlsE lipoprotein of *B. burgdorferi*, and the C6 peptide, which reproduces the invariable region 6 of VlsE, has been a major advance in Lyme disease serology. The C6 peptide ELISA has excellent sensitivity for acute-, convalescent-, and late-phase specimens as well as excellent specificity (Liang et al 1999; Bacon et al 2003; Marangoni et al 2008; Steere et al 2008; Wormser et al 2008). It has been suggested that the new recombinant antigen tests, particularly with C6, may make the 2-tier protocol redundant, but most evidence would indicate that this decision is too early and the 2-tier testing should continue for the foreseeable future, especially in Europe with several pathogenic genospecies with variability of immunodominant antigens (Stanek et al 2011). Other new methodologies show promise to provide new diagnostics in the future (Kraiczky et al 2008; O'Rourke et al 2013; Colman et al 2011). Immunoblots should use recombinant antigens p100, p58, p41i, VlsE, OspC, and DbpA, including those expressed primarily in vivo (VlsE and DbpA) instead of whole cell lysates.

The accuracy and reproducibility of commercially produced Lyme disease kits has been generally poor (eg: Bakken et al 1997; Bakken et al 1992; Luger and Krauss 1990; Ang et al 2011; Busson et al 2012), and it is important that commercial laboratories utilise validated kits (CDC 2005; Klempner et al 2001). However, there has been very limited interassay standardisation, especially in the European market, and not unsurprisingly, different test methodologies can result in differences with respect to test quality. Indeed in Germany alone, at least 55 different companies provide a variety of diagnostic tests (Müller et al 2012) which can lead to a high number of both false negative and false positive results. There is an urgent need for improved interassay standardisation of commercially available test kits, and independent clinical evaluation of assays should be a legal requirement before they are marketed.

False-positive results of serological tests for Lyme disease can sometimes occur in the ELISA from cross-reactive antibodies from patients exposed to other spirochaetal infections, e.g., syphilis, leptospirosis or relapsing fever (Shapiro and Gerber 2000).

Whilst it would be ideal to have an exact diagnosis for epidemiological reasons, from a clinical perspective, the treatments for these diseases are virtually the same as for Lyme disease, since having similar aetiology, i.e. first line treatment is tetracycline (such as doxycycline). For persistent infections, addition of medications such as penicillin, ceftriaxone and azithromycin, are indicated for the above diseases, which are all part of the protocol for Chronic Lyme disease treatment.

It is also possible that antibodies directed at spirochaetes that are part of the normal oral flora may cross-react with *B. burgdorferi* (Shapiro and Gerber 2000). There have also been false positive reports in cases of recent primary infection with varicella-zoster virus (Feder et al 1991), Epstein-Barr virus (Beradi et al 1988; Goossens et al , 1999), cytomegalovirus (Goossens et al 1999), Herpes simplex type 2 virus (Strasfeld et al 2005), and *Rickettsia rickettsia* (Beradi et al 1988). In addition to these examples associated with other infectious diseases, a case of subacute granulomatous thyroiditis (de Quervain's thyroiditis) was positive for Lyme disease in a screening ELISA and the reflexed Western blot was IgG negative but IgM positive, but once the fever and thyroid function test had returned to normal several weeks later, the tests for Lyme disease were all negative (Garment and Demopoulos 2010). False positive *Borrelia* serology and facial paralysis due to anaplastic lymphoma mimicking Lyme disease was recently reported showing an aggressive lymphoma may present with both clinical and serological Lyme characteristics (Deeren and Deleu 2012).

Once again, this highlights the need for clinical expertise in determining the correct diagnosis. It is standard practice that a medical practitioner should always create a list of differential diagnoses, and prescribe a range of diagnostic testing to rule out other ailments. This is one of the main reasons Lyme patients suffer such a huge financial burden. Patients in our support group report spending large sums on specialist services and diagnostic testing, even once a diagnosis of Lyme disease and co-infections has been established, in order to continually rule out other conditions and causes. It is also well recognised by experienced Lyme-literate practitioners, that a disease such as Lyme that is extremely inflammatory to the body, and that also suppresses the immune system, can encourage, trigger or exacerbate other medical conditions.

Herzberg (2013) reported that statistics from one NATA accredited lab in Sydney (2010-2011) showed that most ELISA tests return a negative finding (IgM-89% and IgG-96%). Of the few positive ELISA tests that progressed to the Western blot, only 23% returned a positive result.

Rather than proving that Lyme Borreliosis does not exist in Australia, it is more likely that this testing method is not sensitive enough to detect Australian, or indeed most other overseas, *Borrelia* species that are infecting patients.

### **Current Australian Lyme Disease Testing**

**Again, the author is “cherry picking” the research on testing methodologies.**

In terms of testing for the disease, the NSW Health fact sheet states: “Tests for Lyme disease should only be done by laboratories that have current accreditation with National Association of Testing Authorities (NATA)” (NSW Health 2012a). As at May 2013, there are only two such accredited labs in Australia.

NSW Health also provides advice for clinicians testing for Lyme disease (NSW Health 2012b). In this, NSW Health directs clinicians to follow the USA Centers for Disease Control (CDC) guidelines for Lyme disease testing. (As already mentioned, the CDC guidelines are for surveillance purposes only, and NOT for diagnosis, nor to guide medical treatment.) This involves a two-tier testing procedure. Only if a positive result is returned from the first tier of testing does the second tier proceed.

The first tier of testing is the ELISA test (Enzyme-Linked Immunosorbent Assay) - a sensitive immunoassay that uses an enzyme linked to an antigen as a marker for the detection of a specific antibody. The antibody being sought in this test in Australia is specific to *Borrelia burgdorferi* sensu stricto. This test would not return a positive result if a patient is infected by other strains, such as the yet-to-be-studied Australian strains or strains more commonly found in Europe and Asia.

Thus, the two-tiered Lyme testing system in Australia, by its very concept, fails to detect individuals who may be positive to infection by other strains of *Borrelia*, and thus cannot be used to conclude that Lyme-like borreliosis is not present in Australia.

Another issue with relying on the ELISA test as a first screening is that Lyme disease bacteria and other bacteria associated with co-infections can suppress the immune system particularly in the chronic or late stage of infection (Herzberg et al. 2013). This means that there is a high chance of the ELISA test returning negative results from infected individuals.

A third issue is that *Borrelia* often resides in tissues (e.g. the nervous system and collagen) and may not always be present in the blood stream.

Overall, the ELISA test is not considered sensitive enough and hence its validity for use as a clinical diagnostic test has been questioned (Herzberg et al. 2013).

If an ELISA test is negative, NSW Health directs that the second tier of testing is not carried out. This second tier involves the Western blot test, a far more sensitive and specific test for Lyme disease. The Western blot test for Lyme disease often shows infection when an ELISA test does not.

One reason for this is that the immunoblot kits used in Australia, both in house and commercial, have either *Borrelia burgdorferi* sensu stricto and *B. afzelii*, or *B. burgdorferi* sensu stricto and *B. garinii*. That is, they are searching for strains other than just the strain found in the USA. *Borrelia afzelii* is known from Europe and *B. garinii* is a more widely distributed Eurasian strain.

However, it should be noted that these are the strains which have been long-known to science and do not cover the range of relatively new strains discovered from Australia or overseas.

Another issue is the timing of the test. If an ELISA or a Western blot test is undertaken on a person too soon after a tick bite, their body may not have produced the antibodies needed for the tests (CDC 2011). As mentioned above, if a person is immune-suppressed (such as most chronic Lyme sufferers), the ELISA test is not effective, as they have stopped producing the antibodies that this test detects.

Some medical practitioners now recognise that a negative blood test does not rule out Lyme disease, believing that the diagnosis of Lyme disease should be a clinical diagnosis based on medical history, various blood tests and clinical symptomology, including the individual's responses to pharmacological treatment, and not solely based on the ELISA and Western blot tests. As already mentioned, many commonly accepted illnesses are based on this type of diagnosis and do not receive the level of controversy that the diagnosis of Lyme disease does.

Such an approach is consistent with the recommendations of the Centers for Disease Control in the USA which states that the diagnostic blood tests were devised for surveillance purposes, recommending that they "*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...” (CDC 2011).*

Canada, like Australia, also uses this two-tier system for confirming Lyme disease. However, their statistics state that this system fails to detect up to 90% of cases and does not distinguish between acute, chronic, or resolved infection. Literature from the USA and Europe indicates that the ELISA test misses between 50 and 70% of Borreliosis in patients (Herzberg et al. 2013).

In Australia, the two NATA approved labs are both located in Sydney. Westmead Hospital uses the Western Blot test on *B. burgdorferi sensu stricto* and *B. afzelii*, and an in house – whole cell lysate process developed by David Dickeson – its OLD technology and part of the problem of borrelia not being identified in Australia.

The PALMS lab at Royal North Shore Hospital tests for *B. garinii*, *B. burgdorferi sensu stricto* and *B. afzelii*, and will do PCR testing on skin biopsy culture.

As the other 20 strains of *Borrelia* known to occur are relatively new and specific antibody tests are yet to be developed, PCR testing is the only way to test for their presence. With the exception of the PALMS lab, which only does PCR testing on skin biopsy culture, PCR testing is generally undertaken by a few private labs which lack NATA accreditation and are still in the developmental stages.

In contrast to the situation in Australia, screening tests in tick disease specialist labs overseas are more sensitive, and can pick up most tick borne infections. Those more region-specific strains are usually tested in the region of acquisition, e.g. in Japan tick-borne diseases are tested for using PCR.

Many Australians have resorted to sending their blood samples at considerable expense to overseas labs out of frustration with the apparent low level of accuracy found in Australia's two accredited labs. Many people clinically diagnosed with Lyme disease have had negative results from Australian labs but positive results from overseas labs. Please refer to LDAA Patient Survey 2012, which supports this statement.

This is due to the overseas labs conducting more specific Western blot testing, which is only offered in Australia when a positive ELISA test is returned. In addition, specialist tick borne disease labs often make their Western blots fresh from cultured bacteria and not from manufactured proteins so antibodies bind to them more accurately. The Western blot tests undertaken in Australia may contain either manufactured (recombinant) *Borrelia* proteins or natural proteins. Recombinant proteins may fold differently and give rise to inaccurate binding sites for patients' antibodies and lower sensitivity of the test. One of the labs used by desperate Australians is IGeneX, a lab in California which specialises in tick borne diseases.

Despite the continued controversy about locally acquired Borreliosis in Australia and the frustration from doctors and patients with the low level of detection from Australia's accredited labs, no public funding has been made available to research these issues in recent years. It has also been very difficult for advocates to get the attention of our political leaders to assist with research.

Rather than publicly funding research into Australia's own genospecies search for *Borrelia*, we are following overseas testing procedures that were developed specifically for epidemiological surveillance screening in North America.



Complicating this vexed issue is that there can be a 50% false negative/positive testing rate of error, making a clinical diagnosis critical in determining if a person has Borreliosis concurrent with medical history, symptomology and other tests.

Further, the USA CDC requires five positive bands in their Western blot test to be positive for Lyme disease. The CDC surveillance criteria were devised to track a narrow band of cases for epidemiologic purposes and were not intended for diagnostic purposes. The private USA tick borne disease reference lab IGeneX only requires two positive bands and are more aligned with clinical symptoms.

The CDC deliberately left out band 31 which is specific to Lyme disease, because this band was used to mark the success of a vaccination for Lyme disease. This vaccination later proved to be inadequate and is no longer available. As Lyme disease vaccinations were never available in Australia, the presence of band 31 in a Western blot test is an important marker for Lyme disease.

**This question deserves to be answered: Why does Australia NOT accept overseas testing for Lyme disease together with a clinical diagnosis?** Please ensure that the outcomes of this Scoping Study clearly address this major problem that has long prevented, and continues to prevent, accurate and timely treatment for people with Lyme disease and co-infections.

#### **(h) Co-transmission of tick-borne organisms.**

Ticks are hosts and vectors of a number of parasites, bacteria and viruses, and able to transmit more than one organism per blood meal. The main organisms which are transmitted by *Ixodes* spp. ticks, other than *Borrelia* species, are species of *Anaplasma*, *Babesia*, *Bartonella*, "*Candidatus Neorhlichia mikurensis*", *Ehrlichia*, *Francisella*, *Rickettsia*, *Theileria*, and various viruses (Lotric-Furlan et al 2001; Swanson et al 2006; Coipan et al 2013), as well as multiple *Borrelia* spp. (Floris et al 2007; Ružić-Sabljić et al 2005; Herrmann et al 2013). Recently, *Leptospira* spp. have also been found in *I. ricinus* ticks, but the incidence and relevance is yet to be determined (Wojcik-Fatla et al 2012). The following organisms are those most commonly associated with ticks and believed to be co-transmitted. Only information on Australian examples of these organisms is shown, unless the organism has yet to be reported in Australia.

**Anaplasma.** Two *Anaplasma* species occur in Australia, *A. platys* which causes canine anaplasmosis (Brown et al 2006; Jefferies 2006) and *A. marginale* which is one of the causes of bovine anaplasmosis or bovine tick fever in northern and eastern Australia and is transmitted by the cattle tick, *Rhipicephalus (Boophilus) microplus* (Rogers and Shiel 1979; Jonnson et al 2008), but neither are known to infect humans.

**Babesia.** Bovine babesiosis is a significant disease of cattle in Australia, having been introduced as early as 1829 by cattle imported from Indonesia, It currently costs the industry as much as \$29 million each year in lost production. Two species of *Babesia* cause bovine tick fever, *B. bovis* and *B. bigemina*, and they are transmitted by *R. microplus*. The former is by far the most important causing 80% of outbreaks. Considerable work on *B. bovis* was undertaken by CSIRO scientists in the 1940s through to the 1960s, especially in livestock (J. Curnow, personal communication). The first report of locally-acquired case of human babesiosis, caused by *Babesia microti*, was in a 56 year old man who had never travelled and had no history of blood transfusions (Senanayake et al

2012). The origin of the aetiological agent is uncertain; it is most closely related to North American strains, and the patient was either bitten by an imported tick or a local tick might have transmitted an autochthonous infection, presumably originating from one or more species of introduced rodent. If it was a local tick, the most likely candidate would be *I. holocyclus* as *Ixodes* species are the usual vectors overseas. Canine babesiosis is a cause of anemia, thrombocytopenia, and a wide range of clinical signs ranging from mild to fatal infections in dogs, and three species of canine *Babesia* spp. occur in Australia, *B. canis*, *B. vogeli* and *B. gibsoni* (Brown et al 2006; Jefferies et al 2007; Irwin 2010; Mitrovic et al 2011). A *Babesia* species has been identified in the blood of wild captured woylies (*Bettongia penicillata ogilbyi*) in Western Australia (Paparini et al 2012), and a similar species has been found in ticks in eastern Australia.

A new species of *Babesia* has been recently identified in Australia. Please refer to the attached journal article: "Observation of a novel *Babesia* spp. In Eastern Grey Kangaroos (*Macropus giganteus*) in Australia" Dawood et al 2012, *International Journal for Parasitology: Parasites and Wildlife*. It should be considered in terms of transmissibility and pathogenicity to humans, with appropriate research undertaken.

***Bartonella*.** *Bartonella* species occur both in domestic and wild animals in Australia. *Bartonella henselae* and *B. clarridgeiae*, causative agents of cat scratch disease, have been reported in Australia, but are most frequently transmitted to humans by cat fleas rather than ticks (Flexman et al 1995; Barrs et al 2010). Several *Bartonella* species have been reported in ticks and fleas collected from marsupial hosts, including brush-tailed bettong or woylie (*Bettongia penicillata*), western barred bandicoots (*Perameles bougainville*), yellow-footed antechinus (*Antechinus flavipes*), and Eastern grey kangaroos (*Macropus giganteus*), as well as from various rodents (Fournier et al 2007; Saisongkorh et al 2009; Kaewmongkol et al 2011), and the most frequent tick vectors were *Ixodes* spp., including *I. australiensis*, *I. tasmani*, and *I. myrmecobii*, but it is uncertain whether these species of *Bartonella* can cause human disease, or whether their tick vectors bite humans. There has been no record of co-infection of *Bartonella* species with *B. borgdorferi* s.l. overseas.

The statement above, "There has been no record of co-infection of *Bartonella* species with *B. borgdorferi* s.l. overseas" is ABSOLUTELY INCORRECT and should be removed from this Scoping Study. Alarming, the author later references the opposite of this statement in the paragraph "Co-infection concerns". Please refer Meitz et al, "Occurrence of *Bartonella henselae* and *Borrelia burgdorferi* sensu lato co-infections in ticks collected in Germany. *Clin Microbiol Infect.* 2011; 17:918-920 – taken directly from the author's reference list in this Scoping Study. Please note, the author has mis-spelt "Meitz" which is in fact "Meitze" as cited on the original publication. There are many other studies available that show evidence of co-infection of *Bartonella* with *B. borgdorferi* overseas, for example: Holden et al (2006) Co-detection of *Bartonella henselae*, *Borrelia burgdorferi*, and *Anaplasma phagocytophilum* in *Ixodes pacificus* Ticks from California, USA *Vector-Borne and Zoonotic Diseases*. 6(1): 99-102

Again, this is another case of "cherry picking" by the author.

***Candidatus Neoehrlichia mikurensis*.** *Ca. Neoehrlichia mikurensis* is a newly recognised human pathogen. Small Gram-negative obligate intracellular cocci, they belong to the *Anaplasmataceae* and lack cross-reactivity with other genera in the family, such as *Anaplasma* and *Ehrlichia* (Kawahara et al 2004). First recognised as a human pathogen by Wellinder-Olsson et al (2010), they have been shown to cause human infection in China and found there in ticks and rodents (Li et al 2012), and co-



infection of *I. ricinus* ticks in Sweden (Andersson et al 2013), Denmark (Fertner et al 2012), Switzerland (Maurer et al 2013), and the cause of human disease in Germany (von Loewenich et al 2010). Interestingly it has been shown to exist widely in China where it exhibits significant genetic diversity.

**This organism has not been found in Australia, but it almost certainly hasn't been looked for at this stage.** Indeed, yet another example of "absence of evidence is not evidence of absence". Much more research is required into ALL tick-borne pathogens, not just *Borrelia*, especially for pathogens affecting our Asian neighbours, countries with which we have very close connections in terms of imports and immigration.

***Ehrlichia*.** *Ehrlichia* species have not been recognised in Australia, although *E. canis* is an infection found in dogs worldwide except Australia due to effective quarantine regulations (Irwin 2007), but it is not known whether any species occur in native wildlife.

Again, the author is INCORRECT. *Ehrlichia platys* has been detected in dogs in Australia. Please refer to this abstract from study conducted by Brown et al (2001):

*This study reports for the first time Ehrlichia carriage by dogs in Australia. It also indicates the usefulness of the PCR technique in rapidly and accurately identifying diseases that are otherwise difficult to detect. By using universal primers directed against bacterial 16S ribosomal DNA and sequencing analysis, the detection of potentially pathogenic Ehrlichia organisms that had not previously been found in Australia has been made possible.*

It is of concern that this research was done 13 years ago, yet has somehow eluded the author that has been entrusted with scoping the direction of tick-borne diseases management in Australia. This "cherry picking" of research is NOT ACCEPTABLE, and is clearly evident throughout this document.

***Francisella*.** The first evidence of a *Francisella tularensis* subsp. *novicida* in Australia was its identification from an environmentally-acquired foot infection sustained in the Northern Territory (Whipp et al 2003). This low pathogenicity subspecies of *Francisella tularensis* is relatively rare, and this represents the first time it has been found in the Southern Hemisphere. A second infection, a case of ulceroglandular tularemia due to *Francisella tularensis* subsp. *holarctica*, occurred in a woman bitten by a ringtail possum (*Pseudocheirus peregrinus*) in Tasmania, suggesting an ecological niche for this organism in the native forests of western Tasmania (Jackson et al 2012). The first evidence of *Francisella* species in ticks in Australia was obtained in the Northern Territory using DNA isolated from pools of *Amblyomma fimbriatum* hard ticks (Vilcins et al 2009). The 16S rRNA gene sequences obtained from the ticks indicated that the *Francisella* species grouped phylogenetically with *Francisella*-like endosymbionts in a cluster separate to pathogenic and free-living *Francisella* species. There is no evidence to suggest that these organisms are pathogenic for humans.

***Rickettsia*.** Several rickettsial diseases occur in humans in Australia (Graves et al 2006), but not all are tick-borne. The tick-borne human pathogens are Queensland tick typhus (*Rickettsia australis*) transmitted by *I. holocyclus* and *I. tasmani*; Flinders Island spotted fever (*R. honei*) transmitted by the reptile tick *Aponema hydrosauri* and humans are probably accidental hosts (Stewart 1991); and a variant of the latter caused by *R. honei* strain *marmionii* or *R. marmionii* (Unsworth et al

2007) but the tick vectors of which remain to be determined, although one case in north Queensland was transmitted by *Haemophysalis novaeguineae*; and Q fever (*Coxiella burnetii*) which is carried by several tick species, but most human cases are acquired by aerosol. However, an early investigation demonstrated the presence of *C. burnetii* in *I. holocyclus* ticks (Smith 1942) collected from bandicoots (*Isodon macrourus*) in southeastern Queensland. This tick represents a potential vector for the transmission of *C. burnetii* from natural hosts to domestic animals, livestock, and humans. Another tick species of importance as a reservoir for *C. burnetii*, *Amblyomma triguttatum*, is primarily found on macropodids, but is also promiscuous in host species and has a wide distribution across Australia (McDiarmid et al. 2000). More recently, Cooper et al (2013) found *C. burnetii* DNA in *I. holocyclus* ticks collected from the common northern bandicoot (*Isodon macrourus*) and in *A. triguttatum* collected from the eastern grey kangaroo (*Macropus giganteus*). Thus although most human infections with Q fever are acquired by aerosol, the potential also exists for transmission from wildlife through a tick bite. Perhaps the most interesting of these tick-borne pathogens is *R. marmionii* which has an apparently wide distribution but may also be associated with occasional chronic diseases, including a chronic fatigue-like illness. Wildlife species harbour various Rickettsia, including *R. gravesii* sp. nov. BWI-1 transmitted in Western Australia by *Amblyomma triguttatum triguttatum* (Owen et al 2006; Li et al 2010), and rickettsial DNA most closely associated with *R. tamurae* from *Amblyomma fimbriatum* reptile ticks collected in the Northern Territory (Vilcins et al 2009).

**Viruses.** A number of viruses belonging to different families and genera are transmitted by ticks and are important human pathogens. Perhaps the most relevant for this discussion are the tick-borne flaviviruses including Tick-borne encephalitis virus, which is commonly found in *Ixodes ricinus* and other *Ixodes* spp. ticks in Eurasia from France to Japan (reviewed by Hubalek and Rudolf 2012), and frequently co-transmitted with *Borrelia* spp.; Powassan virus an occasional pathogen in the United States (Romero and Simonsen 2008), and Kyasanur forest disease virus in Karnataka State in India (Holbrook 2012).

Various viruses have been isolated from ticks in Australia and Australian territories, especially from seabird ticks, and from neighbouring countries of south-east Asia (Mackenzie and Williams 2009). Two flaviviruses, Gadgets Gully and Samaurez Reef, have been described in Australia and Australian territories. Gadgets Gully has been isolated from *Ixodes uriae* ticks collected on Macquarie Island (St George et al., 1985; Major et al 2009). Ticks were collected from areas inhabited by Royal penguins (*Eudyptes chrysolophus schlegeli*), but no disease association with seabirds has been established (St George et al 1985). Antibodies to Gadgets Gully virus have been reported in human sera from residents of the Great Barrier Reef (Humphery-Smith et al 1991). Samaurez Reef virus was isolated from *Ornithodoros capensis* seabird ticks collected from nests of various seabird species on coral cays off the east coast of Queensland, and from *Ixodes eudyptidis* ticks taken from two dead Silver gulls (*Larus novaehollandiae*) in northern Tasmania (St George et al 1977). This latter investigation was initiated following reports of febrile illness in meteorological workers operating on Saumarez Reef who had been bitten by ticks, but no association could be found. Experimental infection of Little blue penguins (*Eudyptula minor*) with Saumarez Reef virus resulted in a fatal infection (Morgan et al 1985). Thus neither of these two flaviviruses has been associated with human disease. St George et al (1985) also isolated a novel Bunyavirus in the Phlebovirus genus, Precarious Point virus. More recently, three other novel viruses have been

reported from *I. uriae* ticks on Macquarie Island, an Orbivirus and two Bunyaviruses from the Phlebovirus and Nairovirus genera. The novel Orbivirus was isolated from ticks collected from the King penguin colony and given the name of Sandy Bay virus; the novel Nairovirus was also obtained from ticks associated the King penguin colony, and named Finch Creek virus; and the novel Phlebovirus isolate was isolated from ticks associated with the Rockhopper penguins and named Catch-me-cave virus. This latter virus was found to be related to but distinct from Precarious Point virus (Major et al 2009). None of these viruses have been associated with illness in the penguins nor is there any evidence that they are infectious to humans.

Thus the role, if any, that these seabird-associated tick-borne viruses play in human disease is unknown, except for the antibodies to Gadgets Gully virus in some residents of Great Barrier Reef islands.

A number of Australians are also testing positive to *Brucella*, known to be transmitted by ticks. This bacterium also needs to be tested for as well as Q fever. Some have tested positive to most of the above listed pathogens. Both these organisms can become chronic in humans and have similar symptoms to Borreliosis. *Brucella* has NOT been eradicated from Australia.

**Co-infection concerns.** Co-infection between *B. burgdorferi* s.l. complex species and other tick-borne organisms may lead to different and varied clinical manifestations and different levels of disease severity (Belongia 2002; Swanson et al 2006; Moro et al 2006), and abnormal laboratory test results may be frequently observed (Swanson et al 2006). Indeed co-infections are very often under diagnosed, although they occur frequently. Concurrent infection should be considered in a patient with unusually severe or atypical features of Lyme disease (Marques 2010). Humans infected with Lyme disease and babesiosis appear to have more intense and prolonged symptoms than those with Lyme borreliosis alone (Swanson et al 2006). There are many examples of ticks carrying Lyme *Borrelia* together with one or more additional organisms, including *Anaplasma phagocytophilum* (Hildebrandt et al 2003; Nieto and Foley 2009; Soleng and Kjelland 2013), *Babesia microti* (Schulze et al 2013), *Bartonella henselae* (Mietz et al 2011) [please refer to comments regarding “Bartonella” above,](#)

*Ehrlichia* (Levin and Fish 2000; Stanczak et al 2002), *Babesia microti*, *Borrelia miyamoto*, and Powassan virus (Tokarz et al 2010). There are also examples of double infections with Lyme *Borrelia* and tick-borne encephalitis virus and other agents in patients (Arnez et al 2003; Broker 2012).

Several studies have shown (both internationally and within Australia) that over 80% of people infected with *Borrelia* also have at least one other co-infection (McFadzean 2012, and LDAA 2012) contracted through tick bites. This is primarily because many other co-infections are not tested for in Australia, despite being found in previous Australian research and clinical studies and being commonly tested for and identified in overseas labs.

## MAJOR GAPS IN OUR KNOWLEDGE OF LYME-LIKE DISEASE IN AUSTRALIA.

There are a number of major gaps in our knowledge of Lyme disease in Australia which need to be investigated as a consequence of this scoping study. The essential questions can be enumerated as follows:

1. Does *Borrelia burgdorferi* s.l. occur in Australian ticks, and especially in *I. holocyclus*?
2. Do other Australian tick species transmit Lyme borreliosis?
3. Can Australian ticks be infected with, maintain, and transmit *B. burgdorferi* s.l.?
4. Can we find better diagnostic tools to search for Lyme borreliosis? We surely hope so. We would like to see Australian ticks and tissue samples from vectors sent to Europe and USA for further independent research in isolating pathogens.
5. Is there an indigenous species of *Borrelia* in Australia able to infect humans and to cause Lyme-like disease?
6. Do other possible pathogens occurring in Australian ticks cause Lyme-like disease? Yes- *Brucella* sp. (Brucellosis) and *Coxiella burnettii*, (Q Fever), especially in the chronic forms.  
In Europe and America 28% of *Ixodes* ticks have more than one bacterium, virus or protozoan that can cause disease in humans.
7. Are there any relapsing fever group *Borrelia* species in Australia?
8. Can *B. burgdorferi* s.l. be detected with any certainty in EM rashes following a tick bite, as demonstrated by PCR and/or culture of biopsy specimens? Why is the work of Dr Peter Mayne not being recognised? The comment "with any certainty" is indeed the epitome of disrespect to Dr Peter Mayne. His excellent research paper titled: "Investigation of *Borrelia burgdorferi* genotypes in Australia obtained from erythema migrans tissue" is included in the author's reference list. It would seem that the Russell et al (1994) study was not challenged in this way. Again, there is a tone of scepticism apparent in the work of the author, as noted repeatedly throughout this Scoping Study. Please ensure that professionalism and objectivity prevail.
9. Is there an immune response to *B. burgdorferi* s.l. or to any other possible agent in the sera of patients presenting with Lyme-like disease?
10. Are there any *B. burgdorferi*-specific IgG antibodies in the sera of patients with chronic Lyme borreliosis?
11. If there is evidence found to indicate the presence of Lyme borreliosis in Australia, what is the geographic spread of cases? The Lyme Disease Association of Australia (LDAA) and

Karl McManus Foundation (KMF) have collected this data. It should be considered as part of this Scoping Study.

12. Allow NATA labs to undertake Western blots regardless of ELISA test and compare these findings with those of overseas labs. Allow a lab in USA and Europe to undertake testing on the same blood draw from an Australian and compare the results.
13. Consider examining other vectors, such as: leeches, fleas, mites, lice, biting flies, mosquitoes, rodents – in fact, any organism that could potentially transmit pathogens from an animal to a human from bites/scratches etc. Patients in our support group report having severe reactions to leech bites as well as ticks, and one became chronically unwell after being bitten by a mouse.

Studies suggest sexual transmissibility of *borrelia* (Carmel 2014) and this needs to be considered in terms of prevention and early detection and treatment.

14.

The above topics are just some of the broad issues, and there are many additional queries that need to be addressed, but most will emerge naturally as the information on the major issues becomes clearer.

#### **RESEARCH PROGRAMMES TO DETECT/CONFIRM/DISPROVE THE PRESENCE OF LYME BORRELIOSIS IN AUSTRALIA.**

A number of areas need to be addressed to fill in the uncertainties and lack of evidence-based, scientific information about Lyme-like disease in Australia. In respect of this scoping study, it should be considered that ‘the Jury is out’ and it is in everyone’s best interests to come to an evidence-based answer which fulfils the criteria of Lyme disease or otherwise.

It cannot be over-emphasised that “evidence-based” includes CLINICAL evidence, as per international Lyme disease treatment protocols (ILADS). This illness should not be considered in any way differently from other well-recognised illnesses, such as MS, Parkinsons disease etc, that rely on clinical diagnostic criteria.

Two initial actions need to be stressed: all research carried out in the search for evidence of Lyme borreliosis, or with any other organism that may be associated with Lyme-like symptoms, must agree to (a) sharing of specimens that are believed to be positive for Lyme disease, whether the specimens are clinical material such as serum or whether they are ticks; and (b) with the permission of the patient and the attending physician, to undertake confirmatory testing of any positive clinical specimens using a NATA-accredited laboratory.

There is a large volume of test results held by KMF and LDAA from many Australians who have tested positive to Lyme disease and its co-infections. These results should be requested from Australians and some detailed analysis of the results summarised to give a clearer picture of the complexity and multi-systemic nature of the disease. It will be found

that they correlate with overseas clinical pictures for Borreliosis and co-infections. We are sure that Australians will offer up their results to an independent researcher. Both overseas and Australian testing needs to be considered equally without discrimination.

The sole use of NATA-accredited laboratories may simply reinforce the same problems that have plagued Lyme disease detection in Australia for decades. It is important that specimens are shared for the purposes of quality improvement and refinement of testing methods and protocols, BUT NOT FOR DIAGNOSTIC PURPOSES.

It is of vital importance that the criteria for a positive or negative result must not be based on current NSW Health guidelines that use out-dated USA CDC case-definition criteria never designed for diagnostic purposes. This continues to be a major barrier to correct diagnosis and must be redefined as a matter of urgency. This up-dated information needs to be received by medical practitioners in all levels of public and private health care.

The European experience may be the most useful in assessing the Australian situation. More pathogenic strains of *Borrelia* are found in Europe than in North America, and they have therefore extensive expertise in uncovering new *Borrelia* species. Greater involvement with European experts would be a valuable resource, and assistance should be sought through the European Centre for Disease Control (ECDC) in Stockholm. It would be helpful if a panel of reference sera and reference organisms could be obtained from an accredited European laboratory and kept by the major Australian NATA-accredited laboratories. It would also be preferable if an accredited European laboratory could undertake some confirmatory testing of putative positive specimens, at least in the short term.

In addition, it would seem to be eminently sensible to ensure that specified laboratories are selected to be reference laboratories for Lyme borreliosis, and the obvious two initially would be the Institute for Clinical Pathology and Medical Research at Westmead and Royal North Shore Hospital. In addition, there is a strong case for a reputable, independent private laboratory and the most relevant would be the Australian Rickettsial Reference Laboratory, Geelong. If the panel of reference sera and organisms are obtained from overseas, they should be distributed to the three reference laboratories. It might also be that a further reference laboratory be established in Western Australia at PathWest.

Australian Biologics is currently in the process of obtaining NATA accreditation and should be added to this list. However, our position is that the government should state the qualities they require in a lab, then allow those labs to come forward to be nominated as reference labs.

We again emphasise that research needs to be done on the efficacy of Australian NATA accredited labs in regards to Lyme disease testing and compare their findings with overseas specialists labs to ensure they are using ALL available testing methodologies. Clinical diagnosis is also NOT to be dismissed.

Independent private laboratories should also be allowed into this “exclusive club” to test for *Borrelia*, not just Rickettsia! There is enough concern in Australia about the Australian government not being open and transparent, and deliberately hiding and stifling research and acknowledgement of Lyme disease.



It is also of great concern to members of the Lyme community in Australia that researchers, Stephen Doggett and David Dickeson from the Russell et al (1994) study (also commonly known as “The Westmead Study”) are still very much involved in the NSW Health system in regard to tick-borne illness issues (with Richard Russell only retiring in 2012). It is understood that these two gentlemen hold positions of considerable influence and are representatives on various steering committees, with Stephen Doggett being a representative on the CACLD. Earlier in this Scoping Study, David Dickeson, a current employee of Westmead Hospital (home of the “Westmead Study”), indicated in his personal communication with the author, that even a positive test result from a NATA lab on a patient who has never been overseas, cannot be considered positive!! There could very easily be a conflict of interest and lack of objectivity prevailing (whether intentional or not) in this institution when researchers such as David Dickeson are long-standing employees with considerable influence. It is entirely feasible that it is NOT in the interests of this researcher/employee, nor this institution where the research was conducted, to find *borrelia* when there is a study that *didn't* find it to uphold! This is worthy of further investigation by high-level, independent authorities to ensure that there is no chance of bias nor corruption in our health system in relation to Lyme disease detection.

Patients justifiably feel betrayed by Australia's current laboratory testing methods and the institutions that have controlled these for too long. It is time to expand away from current, unreliable systems and paradigms. A “free-market” approach is now required to encourage more scrutiny, transparency and innovation.

The most obvious question about Lyme disease in Australia is whether or not *B. burgdorferi* exists in Australia, either endemically or epidemically, and if the latter, whether it needs to be re-introduced to cause sporadic infections,

DNA studies in the USA have confirmed that re-infection by *borrelia* is possible.

or is there a novel indigenous species of *Borrelia* which causes Lyme-like disease with occasional instances of relapsing disease-like symptoms.

Or, in fact, both may be the case!

All other questions relevant to the occurrence of Lyme-like disease or the development of chronic Lyme disease are dependent on this initial question. Thus it follows that most, but not all, research efforts should be directed towards providing an answer to this. It should be noted, however, that it is always much harder to prove a negative!

The above statement “it is always much harder to prove a negative” seems to imply that even if positive research results are obtained, then it should still be considered that they could actually be negative! Such comments are extremely disturbing to Lyme patients and is yet another example of the underlying tone of denial detected throughout this Scoping Study. **THIS STUDY MUST BE OBJECTIVE IN EVERY WAY.**

The major research programmes required to accomplish the terms of reference of this scoping study are enumerated below.

**1. Experimental programme to determine whether there is a *Borrelia* species in ticks in Australia causing Lyme-like disease, or whether another tick-borne pathogen is involved in human Lyme-like disease.**

There is no confirmed agent of Lyme borreliosis in Australia at this time, and although there have been positive and negative reports of *B. burgdorferi* s.l. strains in Australia, confirmed and sustainable isolates remain elusive. A broad and detailed investigation of ticks for *Borrelia* spp. and other pathogens needs to be the major initial focus area for research, and should be conducted in more than one laboratory. The closest potential vector in Australia is *I. holocyclus*, the paralysis tick, which is the most common tick found biting humans in the coastal fringe of eastern Australia, but in a single report was found not to be able to support and transmit a North American strain of *B. burgdorferi* s.s., although this does not preclude this species being a transmitter of other *Borrelia* species. In Western Australia where cases of Lyme-like disease have also been reported, *I. holocyclus* does not occur, but several other ticks commonly bite humans and need to be investigated. Thus the single most important issue to be addressed is whether *Borrelia* strains exist in Australia which can cause Lyme disease, or whether other pathogenic organisms are responsible, including *B. miyamotoi* which can cause EM in some patients and relapsing episodes in others.

In North America and Europe, ticks infected with *B. burgdorferi* s.l. are full of spirochaetes which can readily be detected and/or visualised. This does not appear to be the case with Australian ticks, and it will be important to address the question of spirochaete carriage using more sensitive detection techniques, such as nested PCR or next generation sequencing, for example, 454 high throughput sequencing. Ticks should be collected from NSW in coastal regions and from the south-west of Western Australia where Lyme-like disease has been reported, and obtained by various means from different sources including veterinary clinics (for ticks taken from dogs);

Ticks should be collected from all regions in Australia where ticks can be found. Reliance on reporting of Lyme-disease is completely useless in a country where it is not only a non-notifiable disease, but it is widely believed not to exist! The number of undiagnosed cases of Lyme disease in this country is enormous! Reports of Lyme disease are only occurring because of the education of certain medical practitioners and members of various communities, NOT because of actual cases.

*Ixodes holocyclus* is the logical vector candidate for Lyme disease, and it is found along Australia's eastern coastline from the north of the continent just above Cooktown in Queensland to Lakes Entrance in Victoria at the very south of the continent and a small pocket in north eastern Tasmania (Dept of Medical Entomology 2003). More recent research by Song et al has demonstrated the presence of *I. holocyclus* is more extensive across central Victoria and Tasmania than previously believed (Song et al 2011). Ticks must be collected extensively throughout all regions of Australia in order that accurate results may be obtained.

general practice clinics where ticks have been removed from patients; blanket sweeps for collecting ticks in suitable habitats; from small animals/wildlife, especially rodents and bandicoots (the probable natural host species), with assistance from ecologists and zoologists (using on-going small animal collection studies where possible), archival sources (various museums, Commonwealth Scientific and Industrial Research Organisation (CSIRO), and entomology groups at Australian



universities). Although the primary tick focus should remain *I. holocyclus* in eastern Australia, other tick species should be considered including *Amblyomma* species and *Ornithodoros* species, whereas in Western Australia the focus should be on *I. australiensis*, and *A. triguttum* ticks. It is envisaged that several groups would explore ticks for possible spirochaetes, but as mentioned above, it's essential that potentially positive material should be shared between the groups as *Borrelia* species are often difficult to isolate and maintain in culture.

Reservoir hosts using blood and tissue samples need to be looked at concurrent with the testing of ticks for *borrelia* and other tick borne pathogens thought to be pathogenic to humans.

If an indigenous *Borrelia* species exists in Australia and is responsible for the Lyme-like disease, it is quite possible that current methods, primers, and antigens will not pick up the novel genospecies if it is significantly different from other members of *B. burgdorferi* s.l., and it is essential that new, techniques be developed to detect *Borrelia* species using a variety of genomic methodologies. These may include a relatively simple approach using broader and less stringent primers designed to bind to highly conserved sequences, or primers for the *flaB* and *gyrB* (Takano et al 2010), PCR-restriction fragment length polymorphism based on the *flaB* gene (Wodecka 2011), or it might include more sophisticated high throughput sequencing (454 and/or MySeq) of pooled tick DNA following quantitative PCR for *Borrelia* 16S rRNA. This latter approach is currently being developed at Murdoch University (P Irwin, personal communication). Other new *Borrelia* species have recently been described (Takano et al 2010), and any new techniques should incorporate this new species.

While the initial search is for *Borrelia* species, it is essential that other pathogens are not neglected and *Anaplasma*, *Babesia*, *Bartonella*, *Ehrlichia*, *Francisella*, *Neoehrlichia*, *Rickettsia*, and viruses should be considered and included in the detection process, both as individual pathogens and as examples of increased pathogenesis in co-transmission. Some of these may be less likely as pathogens as they are not normally found in Australia (eg. *Ehrlichia*), some have not been looked for previously (eg. *Neoehrlichia*), and some have not been found as in co-transmission with *Borrelia*, but are pathogens in their own right (eg. *Bartonella*).

Again, this is last statement is INCORRECT. *Bartonella* has been well established as a co-infection with *Borrelia*, as the author clearly references – Mietz et al 2011. This is a discrepancy that has occurred twice in this document, and requires immediate correction. Also, please refer to feedback comments regarding discovery of *Ehrlichia platys* in Australia by Brown et al (2001). These “oversights” by the researcher, if not addressed, will have ongoing implications for the management of tick-borne diseases in Australia. As already mentioned, it appears that the author has been “cherry picking” the research that is available, and has also not properly read the work he has referenced.

The viruses are in a different category. No tick-borne viral pathogens have been reported previously, and the only viruses from ticks collected in Australia or Australian territories are the two flaviviruses Samaurez Reef and Gadgets Gully from *I. uriae* on seabirds. Gadgets Gully is able to infect humans although no disease symptoms have been recognised (Humphery-Smith et al 1991). Only one other flavivirus found occasionally in ticks of relevance to Australia is West Nile virus, although the Kunjin clade of West Nile has not been reported in ticks. Of other virus groups, some Orbiviruses are found in ticks from Macquarie Island including Nugget virus, a member of the Kemorovo group (Gorman et

al 1984), a Bunyavirus from the Nairovirus genus, Taggart virus, a member of the Sakelín virus group (Doherty et al 1975), as well as two recent isolates, Sandy Bay and Finch Creek viruses which are related to Nugget virus and Taggart virus respectively (Major et al 2009). Other Orbiviruses have been isolated from mosquitoes and *Culicoides* in Australia, such as Wallal, Warrego and Wongurr viruses. Tick-borne virus isolates belonging to the Bunyavirus family, *Phlebovirus* genus, have also been found in ticks from Macquarie Island. Thus the potential of finding a virus in the ticks is relatively high.

It is noted that the references in the above paragraph are very old. Further research into tick-borne viruses is recommended.

The transmission time of disease-causing pathogens from ticks is not known in Australia. Therefore, the testing of reservoir hosts concurrent with ticks is imperative.

Karen Vanderhoof-Forschner says:

*“One of the least studied and most intriguing features of ticks is that some are systemically infected with pathogens found both in the mid gut (stomach) and salivary glands, while others are infected locally, with the agent present only in the mid gut. The distinction is very important. With Lyme disease, for example, a systemically infected tick may be able to transmit a pathogen in only a few hours whereas a tick with a localised infection may take 24-48 hours to move the bacteria into its salivary glands so that transmission can occur.”*

Transmission of pathogens while feeding can be very fast, different species of ticks can transmit infection at different rates. The bacterial load of each tick can vary depending on the previous reservoir animal on which they have been feeding.

## **2. Are Australian ticks competent to maintain and transmit *B.burgdorferi* s.l. genospecies, or other *Borrelia* species associated with relapsing fever?**

It would be important to determine whether common Australian tick species known to bite humans are able to be infected with, maintain, and transmit *Borrelia* genospecies. Early work had demonstrated that *I. holocyclus* ticks were unable to transmit a specific North American strain of *B. burgdorferi* s.s. (Piesman and Stone 1991), but there is no information of the competence of this species of tick to transmit European *Borrelia* genospecies, particularly *B. garinii* which has been found in the Southern Hemisphere, nor of the competence of other important Australian tick species to transmit *Borrelia* species. Thus vector competence studies should be carried out with some urgency to investigate whether *I. holocyclus* is able to transmit a wide spectrum of *Borrelia burgdorferi* s.l. genospecies, starting with *B. garinii*, and whether other Australian ticks of the *Ixodes*, *Haemaphysalis*, *Ornithodoros* and *Amblyomma* genera are competent to transmit examples of the major *B. burgdorferi* s.l. genospecies, and the relapsing fever species, including the species transmitted by soft ticks, *B. duttoni*, *B. crocidurae*, *B. hermsii*, and *B. hispanica*, and those transmitted by *Ixodes* species, particularly *B. miyamotoi*.

### 3. Do we have the best reagents for detecting novel *Borrelia* species, including *B. miyamotoi*, especially in clinical specimens?

It is possible that the PCR primers and other commonly used reagents cannot detect an indigenous strain or genospecies of *Borrelia* either in the tick or in clinical material. The former were briefly discussed above for detection in ticks, but the alternative route to investigate the presence of novel *Borrelia* species would be in biopsy material. If current PCR primers are ineffective with novel species, new methods will have to be developed. This might include a variety of methods, including a nested PCR using a broadly based, low stringency initial primer followed by more specific second round primer pairs based on common genetic sequences from known genospecies, perhaps in the rRNA gene or the flagellin gene, or some other conserved genetic element. Primer sets are also needed to detect and identify relapsing fever *Borrelia* species and the hard tick-transmitted relapsing fever-like species such as *B. miyamotoi*. Biopsy material might also be examined by immunofluorescent antibodies to expressed flagellin protein *flaB*, and to *ospA*, or C6 peptide.

In addition to new PCR primers, it is also important to develop and verify novel serological techniques to ensure highly specific, sensitive yet broadly based IgG and IgM antibody detection systems using expressed antigens for ELISA and other assay systems for detecting specific antibody, and immunological methods for detecting *Borrelia* species in biopsy material as an alternative to genomic methods, such as immunofluorescent antibodies to expressed flagellin protein, *ospA*, or C6 peptide. Archival biopsy specimens are available at Royal North Shore Hospital, and sera and other specimens are at Royal North Shore and Westmead.

There are other sources of specimens available that must be considered, such as those taken during autopsy from people with Lyme disease who have died. Please contact the Karl McManus Foundation for more information.

### 4. Clinical studies of patients presenting with symptoms suggestive of Lyme or Lyme-like disease.

The second strand of the research should be a prospective study directed at detecting *Borrelia* spp. or other pathogens in human cases presenting with Lyme-like symptoms. This would need to be undertaken with the consent, support and assistance of General Practitioners who see many of the relevant patients, as well as the patients themselves, and undertaken as a collaborative study with infectious disease/clinical microbiologists who have a specific interest in this area. There should be two major thrusts in this strand of the research programme – one is the collection and testing of biopsy material from EM, and the other is collection of paired sera from patients for assay of borrelial antigens using the two-tier protocol.

Please refer to the feedback comments regarding the unreliability of the two-tier protocol, mentioned already in this study.

It would be preferable if EM biopsy specimens could be taken from both the central bite region (Mayne 2012) and from the periphery or leading edge (Berger et al 1992). Tissue would then be tested by real-time PCR and culture (Aguero-Rosenfeld et al 2005; Ivacic et al 2007; O'Rourke et al 2013), and possibly other tests such as specific immunofluorescence using reference antisera as determined by the clinician.

There seems little doubt that some tick bites result in skin eruptions at the site of the bite which look like a form of EM and that this may progress in some instances to disease symptoms that may be reminiscent of Lyme borreliosis. Bites from *I. holocyclus* ticks can result in an allergic response (Gauci et al 1989), and the site of the bite can be erythemic and sometimes mimic EM. If the EM is indeed caused by *Borrelia* species, it will develop about 48hr after the bite of the tick, however if it is an allergic reaction to the tick bite, it should fully resolve within 24-48hr.

Patients with later symptoms suspected of being possibly due to disseminated Lyme borreliosis such as arthritis or neuroborreliosis, some of whom may not have had EM or instead had an atypical rash, should be tested using standard techniques, including culture, immunodiagnosis, and/or PCR of synovial fluids (eg. Nocton et al 1994; Priem et al 1998; Ivacic et al 2007; Li et al 2011) and for CSF (Skogman 2008; Cerar et al 2010), within the accepted guidelines (Mygland et al 2010). If patients present with repetitive episodes of sudden fever, myalgia, headache and nausea, relapsing fever should be considered, and although there is no evidence of relapsing fever group *Borrelia* species in Australia, the possibility of their actual presence should not be ignored both with respect to the normal relapsing species of *Borrelia*, but also *B. miyamotoi*.

## 5. Retrospective investigation of chronic cases of Lyme borreliosis.

As described in the Background review, this scoping study suggests that ‘the jury is out’ when considering the contentious issue of chronic Lyme borreliosis. However, it is in everyone’s interest to attempt to verify the diagnosis of Lyme borreliosis in these cases, not least for the patients themselves, and thus retrospective studies are recommended. It is suggested that this be done in two distinct series of studies; the first seeking evidence of past infection with *B. burgdorferi* s.l., and the other reviewing the clinical case histories of selected cases to gain greater insight into the diagnoses. In both instances, it is essential that patients are willing to be included and fully aware of rationale of the studies, that General Practitioners caring for the patients are comfortable with the study protocols and agree to be part of the study team, and that the studies meet all human ethical requirements.

The study seeking evidence of past infections with *B. burgdorferi* s.l. should be undertaken by serological tests for IgG to *Borrelia* antigens. To provide a broad, strong result, this should be done with a 2-tier approach.

Please refer to the feedback comments regarding the unreliability of the two-tier protocol, mentioned already in this study. It should be considered that a Western Blot test be administered regardless of whether the ELISA is negative, to give a comprehensive analysis. This is currently NOT happening, and perhaps this is one of the major problems with the testing methods in Australia.

For the study seeking a better understanding of the background diagnoses, it is recommended that clinical case history notes be assembled anonymously and reviewed by a panel of infectious disease experts from within Australia and overseas.

Lyme-literate medical practitioners must also be involved on such a panel! They are “at the coal-face” of the Lyme disease and co-infections issue in Australia, and have more

experience with and understanding of Lyme disease than any “infectious diseases expert” in Australia (which has been the frustrating experience of Lyme patients who have consulted same).

An invitation to bring an acknowledged international expert to Australia would be an extremely useful avenue to assist in assessing projects in topics recommended above, but more importantly could be part of an international Lyme and Lyme-like diseases symposium under the auspices of a local partner organisation, such as the annual Communicable Diseases Conference, or with the Australian Society for Infectious Diseases (ASID), the Australian Society for Microbiology (ASM), or the Royal College of Pathologists of Australia annual meeting. An acknowledged expert in Lyme diseases and *Borrelia* ecology could also be asked to give a series of public lectures.

6. Lyme disease and co-infections needs to be a “notifiable disease” in Australia, to enable accurate data collection and surveillance, to assist authorities to determine endemic regions so that suitable prevention, treatment and medical education strategies can be implemented. A “top down” approach is required if this issue is ever to be properly addressed.

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**It is noteworthy, and rather disturbing, that no Lyme Literate medical practitioners appear to have been consulted in the development of this Scoping Study.**

