Department of Health’s response to Prof. John Mackenzie’s Scoping Study

In August 2013, the department contracted an eminent microbiologist, Prof. John Mackenzie, to conduct a scoping study to identify the research needs for an investigation into whether a causative tick-borne microorganism for Lyme disease exists in Australia. The Scoping study to develop a research project(s) to investigate the presence or absence of Lyme disease in Australia (Scoping Study) raised 11 questions and recommended five research programmes which would assist in determining whether there is a Borrelia spp causing illness in humans in Australia.

Upon advice from the Clinical Advisory Committee on Lyme Disease in Australia (CACLD) the department decided to seek public comment on the Scoping Study requesting that comments be directed towards the research programmes. Twenty-four submissions were received, along with eight expressions of support for the Lyme Disease Association of Australia’s submission and four letters from people describing their personal situations (36 submissions in total).

The department acknowledges the time and effort taken to provide comments on the Scoping Study. All the submissions were considered individually, then collated and consolidated into overarching themes and recommendations specifically on the research that is required to investigate the presence or absence of Lyme disease or a Lyme disease-like syndrome in Australia.

Having taken into account the submissions on the Scoping Study—which will continue to stand alone—this response has modified the proposed research programmes to incorporate the feedback received.

Gaps in current knowledge

The scoping study raised 11 questions that identified the gaps in current knowledge and these were:

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. burgdorderi s.l.?
4. Can we find better diagnostic tools to search for Lyme borreliosis?
5. Is there an indigenous species of Borrelia in Australia able to infect humans and able to cause a Lyme disease-like syndrome?
6. Do other possible pathogens occurring in Australian ticks cause a Lyme disease-like syndrome?
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?
9. Is there an immune response to B. burgdorferi s.l. or to any other possible agent in the sera of patients presenting with a Lyme disease-like syndrome?
10. Are there any B. burgdorferi-specific IgG antibodies in the sera of patients with Lyme disease-like syndrome?
11. If there is evidence found to indicate the presence of Lyme borreliosis or a Lyme disease-like syndrome in Australia, what is the geographic spread of cases?
After considering the submissions received the following question would also be included:

12. Are there other potential vectors that could transmit *Borrelia* in Australia?

**Recommended research programmes from the Scoping Study**

The five research programmes recommended in the Scoping Study are provided below (in no particular order) with amendments that take into consideration the feedback received.

**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. Any recruitment of patients should be done with a clear, succinct case definition and criteria in place. Relevant case history and symptoms should also be collected including any treatment administered and long term outcomes of the patients.

The study seeking evidence of past infections with *B. burgdorferi* s.l. should be undertaken by recruiting patients with serological test results for IgG to *Borrelia* antigens. To provide a broad, strong result, this should be done with a 2-tier approach. Additional blood samples should be taken, so that PCR tests can be performed in parallel by three identified laboratories. Consideration should also be given, depending on the case definition, or testing for other strains of *Borrelia*.

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 experts that include: infectious disease physicians; neurologists; rheumatologists; cardiologists; epidemiologists; and general practitioners, both from within Australia and overseas.

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 disease-like syndrome symposium under the auspices of a local partner organisation, such as the biennial 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 Australasia’s annual scientific meeting. An acknowledged expert in Lyme diseases and *Borrelia* ecology could also be asked to give a series of public lectures.

The diagnostic testing methods used in any patient study should take into consideration any results/outcomes from the research programme *Do we have the best reagents for detecting novel Borrelia species, including B. miyamotoi, especially in clinical specimens?* In particular any standard method that may have been developed should be used.
The case histories and information should be analysed to determine if there are any trends or patterns that could imply infection with a common agent or identification of a common syndrome.

All research on humans should comply with the National Health and Medical Research Council’s *National Statement on Ethical Conduct in Research Involving Humans 2007*. The primary purpose of the Statement is the protection of the welfare and the rights of participants in research and to facilitate research that is or will be of benefit to the researchers’ community or to humankind.

**Clinical studies of patients presenting with symptoms suggestive of Lyme or Lyme disease-like syndrome.**

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 disease 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 and epidemiologists who have a specific interest in this area. Any recruitment of patients should be done with a clear, succinct case definition and criteria in place. Relevant case history and symptoms should also be collected.

There should be two major thrusts in this strand of the research programme: one is the collection and testing of biopsy material from EM rashes, and the other is the collection of paired sera from patients for assay of borrelial antigens using the two-tier protocol. It would be preferable if EM rash 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 48 hours after the bite of the tick, however if it is an allergic reaction to the tick bite, it should fully resolve within 24–48 hours.

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 (e.g., 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), and blood, 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*.

The diagnostic testing methods used in any patient study should take into consideration any results/outcomes from the research programme *Do we have the best reagents for detecting novel Borrelia species, including B. miyamotoi, especially in clinical specimens?* In particular any standard method that may have been developed should be used.
The possibility of person-to-person transmission of *Borrelia* has not been fully investigated and while such studies would be beneficial, they would not be of the highest priority until a causative organism has been established.

All research on humans should comply with the National Health and Medical Research Council’s *National Statement on Ethical Conduct in Research Involving Humans 2007*. The primary purpose of the Statement is the protection of the welfare and the rights of participants in research and to facilitate research that is or will be of benefit to the researchers’ community or to humankind.

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

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 disease-like syndrome 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 a broad geographical area (across the country) and include ticks from farmers, national park rangers, wildlife veterinarians, wildlife carers, zoo-based wildlife hospitals as well as where any Lyme disease-like syndrome has been reported (particularly NSW in coastal regions and from the south-west of Western Australia). Ticks should be obtained by various means from different sources including veterinary clinics (for ticks taken from dogs); 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 ongoing small animal collection studies where possible); the Invasive Animal Cooperative Research Centre; 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 *Ambylomma* species, *Ornithodoros* species and *Argasidae* species, whereas in Western Australia the focus should be on *I. australiensis*, and *A. triguttam* 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. In addition, it is important for any
research group to ensure that all collection and testing of ticks takes into account the appropriate sample size to ensure the results are significant.

Consideration should also be given to investigating all haematophagous arthropods, in particular mites and March flies.

If an indigenous *Borrelia* species exists in Australia and is responsible for a Lyme disease-like syndrome, 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 *Anaplasm*, *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 (e.g., *Ehrlichia*), some have not been looked for previously (e.g., *Neoehrlichia*), and some have not been found as in co-transmission with *Borrelia*, but are pathogens in their own right (e.g., *Bartonella*). 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, *viz.*, 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 viral 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 Nairobi virus genus, Taggert virus, a member of the Sakelin 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 Taggert 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.

**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. When developing any new methods, it would be beneficial to consult with other countries that have detected novel *Borrelia* strains to learn from their experience.

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 hospitals.

Other specific projects that should be considered include:

- Conducting a formal review to elucidate the testing procedures currently used in public health laboratories to diagnose Lyme disease.
- Inviting relevant laboratories, both in Australian and overseas, to participate in a collaboration and comparison study to evaluate the current methodologies used. It may be possible to develop standard Australian diagnostic criteria as a result of this work.

**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, Argasidae* 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*.

Consideration should also be given to investigating the competency of other haematophagous arthropods to transmit *Borrelia*, in particular mites and March flies. However, prioritisation of the vector competence studies should be undertaken prior to beginning the research.
Additional research programmes identified that would assist in determining whether there is a *Borrelia* spp causing illness in humans in Australia are provided below.

**Epidemiological research**

A baseline quantification of Australians diagnosed with Lyme disease after travelling to endemic areas should be conducted. Information collected should include: prior travel history, bite history, disease duration, symptoms, test results and treatment. Cases included in this study should be determined by a clear and succinct case definition.

A study should be conducted on the patients in Australia that have been diagnosed with a Lyme disease-like syndrome looking specifically at the geographical location where exposure occurred, detailed symptoms and compilation of any test results. This study could be conducted in conjunction with the research programmes *Clinical studies of patients presenting with symptoms suggestive of Lyme or Lyme disease-like syndrome* and *Retrospective investigation of chronic cases of Lyme borreliosis*. Due to the non-specific nature of the Lyme disease-like syndrome a very clear case definition would need to be developed that would identify the relevant patients. While, the working case definition may exclude potential cases this could be revisited once analysis of the collated symptoms of the first cohort was completed.

**Examine parallels with other countries that have detected a novel Borrelia**

In recent years, new species of *Borrelia* have been identified or known *Borrelia* species have moved into new geographical areas. A comprehensive literature review should be conducted to detail these experience and techniques used. This should be coupled with liaison with the relevant researchers. In particular, the experience in Brazil and the Baggio-Yoshinari Syndrome situation should be examined.

**Other issues that were raised in the submissions that were out of scope for the Scoping Study**

**Treatment options or guidelines**

Treatment options for Lyme disease or Lyme disease-like illness was out of scope for the Scoping Study. A number of the submissions received noted the absence of any discussion of treatment. The Department of Health acknowledges the importance of considering treatment options and organised a Lyme Disease Treatment Round Table meeting that was held on 27 May 2014. Outcomes from this meeting included research projects examining treatment options.

**Lyme Disease Association of Australia proposed patient focused strategic approach to the Lyme problem**

The Lyme Disease Association of Australia (LDAA) provided a Patient focused strategic approach to the Lyme disease problem as part of its submission in response to the Scoping Study. This document identified the patients’ key issues and objectives and suggested a number of strategies and actions to be completed. While this information is important and needs to be addressed, it is out of scope of the Scoping Study which was to identify the research required to investigate the presence of absence of Lyme disease in Australia. The Department of Health will be considering the patient focused strategic approach provided by the LDAA on behalf of the patients separately.
Research funding

A number of research programmes have been identified in the Scoping Study and amended here in the department’s response. Potential researchers are asked to note that the Department of Health is not a research funding agency.

The majority of Australian Government health and medical research funding is administered by the National Health and Medical Research Council (NHMRC). The Australian Research Council (ARC) has funded some Special Research Initiatives in the health and medical areas however the ARC does not generally fund medical research. The ARC Medical Research Policy is available on the [ARC web site](http://arc.gov.au). Researchers may also seek other avenues for funding including the higher education sector, business sector or the private non-profit sector.