Analysis of ‘Lyme Borreliosis, the Australian Perspective’ (1995)

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Introduction

As a result of persistent patient campaigning, a Senate inquiry was conducted into the ‘Growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australian patients’ in 2015-2016. The Senate committee received submissions from over 1280 patients, carers, doctors and researchers.

Every submission was of interest to us, but one, by Dr Stuart King, particularly caught our attention. It included excerpts from the thesis of Michelle Wills, a document we were previously unaware of.

Dr Wills is well known in the Australian ‘Lyme-like illness’ community. As a recent university graduate, she and her supervisor Prof Richard Barry were the first to report Borrelia (a species of bacteria of which some strains cause Lyme disease) in Australian ticks, in 1991.

The pair then collaborated with other members of the Microbiology departments of Newcastle University and Sydney’s Royal North Shore Hospital, to examine the responses of patients with Lyme-like illness to Lyme disease tests.

After promising findings on this second project were published in 1994, the trail went cold; we couldn’t find any further documents by Wills and Barry.

However, also in 1994, a government-funded study (known as the ‘Russell and Doggett’ study1) concluded that there were no Borrelia in Australian ticks. They suggested that Wills and Barry’s 1991 discovery was simply disintegrating forms of other bacteria, that had the appearance of Borrelia.

The government accepted this conclusion, and no further research funding was made available. The Australian Government Department of Health has consistently used the Russell and Doggett study to support their “No Lyme here” stance ever since.

Subsequently, the previously unavailable excerpts from Dr Wills’ thesis were a revelation to us. The LDAA’s then-President Sharon Whiteman reached out to Dr Wills for more information. This resulted in a supplementary submission to the Senate inquiry, which included an interview with Dr Wills.2

In November 2017, the LDAA were pleased to announce that, in partnership with Dr Wills, we had ensured that the entire thesis was available online for the first time. In less than a month, the site housing the thesis has been visited on more than 1000 occasions, and the thesis has been downloaded over 200 times.3

The thesis is 319 pages in length, and, as one would expect, is at times highly technical. The LDAA have therefore produced this document to summarise the information that was previously not widely available to the media and patient community, and put it into context. It has been reviewed by Dr Wills to ensure accuracy – we thank her for her time.

We hope that this analysis will highlight the Australian government’s ongoing bias in relation to progressing our understanding of Lyme-like illness, and as a result, patient wellbeing.
Chapter 3 – Preliminary characteristics of Borrelia-like microorganisms found in Australian ticks (pages 99-174)

Our analysis begins in Chapter 3, which documents perhaps the most significant information in the thesis; the discovery of Borrelia in Australian ticks. The study involved collecting bacteria from the guts of 168 Australian ticks, and attempting to grow that bacteria in laboratory conditions. This process is known as ‘culturing.’

Wills and Barry had hoped to isolate and culture spirochaetes (a form of bacteria) like the Borrelia strains known to cause Lyme disease.

Most of the ticks were of the *Ixodes holocyclus* species, the type most likely to cause illness in humans. Many were collected from domestic livestock or pets. Those collected from pets were often removed by vets, who were treating the animals for tick paralysis.

In a 1991 letter to the Medical Journal of Australia, Wills and Barry reported their “preliminary results.” Forty two percent of the ticks were “culture positive for Borrelia-like spirochaetes.” The spirochaetes were described as “indistinguishable” in shape to *Borrelia burgdorferi sensu stricto*, the strain that causes American Lyme disease. They also used the same uncommon “irregular rotating movement.” Various methods of testing (ELISA, Western Blot and immunofluorescence) revealed that antibodies respond to the *Borrelia*-like spirochaetes in a similar way to those that cause Lyme.

These findings were dismissed by the Russell and Doggett study, which had involved similar activity. They also found what they called “spirochaete-like objects”, but suggested that both discoveries were actually “artefacts” (broken down parts of other types of bacteria), rather than dead spirochaetes.

However, Wills’ thesis documents a different outcome, via her study’s final results.

It recounts that some of the cultures were sent to Prof Alan Barbour and his colleague Dr Virgilio Bundoc in Texas. Prof Barbour’s input was crucial; he was one of the team who first identified, isolated and cultured *Borrelia burgdorferi sensu stricto.*

(Still recognised an international expert, Barbour has written two books on Lyme disease and is currently a Professor of Microbiology and Molecular Genetics at the University of California.)

It was confirmed in the Texas laboratory that the cultures did indeed contain spirochaetes, which were then identified as *Borrelia* species.

“The five tick isolates...were sent to the laboratory of Professor A Barbour and Dr V Bundoc at the University of Health Science Centre at San Antonio, Texas. On arrival the cultures were passaged into fresh BSK-II. After 5 days of incubation...limited growth was obtained and were examined by both of the above-mentioned scientists. They reported “intact (but apparently dead) spirochaetes from some of the culture tubes” and furthermore agreed “that they really are spirochaetes.””

“During a brief visit in May 1992 to the laboratory of Professor Barbour, isolate 51 was subcultured into BSK-II and, after 5 days incubation, was centrifuged and fixed onto a glass slide. An indirect immunofluorescence test was performed...which specifically reacts to the flagellin protein of Borrelia
sp. On examination by fluorescence microscopy, spiral shaped organisms were observed that fluoresced strongly, and were of identical morphology to the control B. burgdorferi."

Given that some spirochaetes appeared “dead,” the scientists killed a sample of genuine European *Borrelia afzelii* spirochaetes with antibiotics. They then compared the dead *B. afzelii* with the spiral-shaped, immobile bacteria they’d found. The two were identical.

“...the morphology of the antibiotic treated *B. afzelii* was indistinguishable from the spiral organisms isolated from the ticks...This finding supports the conclusion that the rigid spiral structures isolated from the Australian ticks could be ‘dead’ spirochaetes.”

About Russell and Doggett’s artefact theory, Wills stated “The data presented here supports an opposite conclusion, namely that spiral structures are ‘dead’ spirochaetes, that have maintained their spiral morphology, although having apparently lost their outer membrane, so that endoflagella are fully exposed and are disintegrating.”

Basic DNA testing identified that the Australian *Borrelia* were more closely related to European strains, particularly *Borrelia garinii*, than the American *Borrelia burgdorferi sensu stricto*.

“...the polypeptide profile of isolate 17 resembled more closely that of *B. garinii...than it did of either *B. afzelii* or *B. burgdorferi sensu stricto.*”

Wills concluded her chapter with a detailed rebuttal of the Russell and Doggett study, including the following points:

1. Their ‘artefacts’ theory was based on a 1992 Missouri study. The study came to that conclusion as it wasn’t thought that Missouri was Lyme-endemic, and the tick species involved weren’t considered capable of carrying *Borrelia*.

   However, one of the study’s authors reported in 1994 that Lyme is common in Missouri, and the ticks carry *Borrelia*.

   “The artefact hypothesis was generated because, at the time, Missouri was not considered to be an endemic LB [Lyme Borreliosis, or Lyme disease] area, and *Amblyomma* sp. ticks were thought to be unlikely borreliae vectors. Thus it was proposed that the bundles of flagella might be degenerative products of normal tick gut bacteria. One of the authors of the Missouri study subsequently reported that not only is LB common in Missouri but that *Amblyomma* sp carry and probably transmit *B. burgdorferi* (Masters et al, 1994). This retraction brings into question the artefact theory, and a more likely explanation...is that they are disintegrating forms of *B. burgdorferi.*”

2. All the gut contents she examined contained common species of bacteria. It is therefore a reasonable assumption that if the spirochaetes she had found were artefacts, all the guts should have contained similar artefacts. However, only 44% did.

   “Common to all cultures of the tick gut contents were particular bacterial species. It is reasonable to propose, if the spiral structures were artefacts, that all prolonged cultures in which the common gut bacteria are present should contain such structures. This however
was not found to be the case, since in this study only 74 of the 168 (44%) cultures contained spiral structures.\textsuperscript{13}

3. It is also a reasonable assumption that the artefacts would appear randomly in the cultures of all species of ticks. However, they were present in only four of the 12 species of tick examined by Russell and Doggett.

“...the studies...indicated that the spiral structures were found in only 4 of the 12 species of ticks that were cultivated...Such findings argue against the generation of artefacts from degenerating bacteria, because artefacts would be anticipated to occur randomly, in all cultures, from all tick species.”\textsuperscript{14}

4. She was able to culture some spirochaetes; this would not have been possible if they had been artefacts.

“5 tick derived spirochaete-like organisms were eventually purified, each was successfully subcultured and maintained in the absence of other bacteria. In light of this finding, the case for the origin of spiral structures as degenerative products of other bacteria is difficult to sustain.”\textsuperscript{15}

5. Regarding Russell and Doggett’s inability to culture their own 'spirochaete-like objects” — and her own difficulties with culturing - she noted that not all \textit{Borrelia} are easily cultured, and provided examples of relevant international studies.

“...not all \textit{B. burgdorferi} types grow readily in BSK. In the UK, infected ticks, as determined by PCR, are common and widespread. However Nuttall et al (1994) succeeded in obtaining only one isolate from 85 tick pools (representing 504 \textit{I. ricinus} nymphs and adults) collected in the UK. By contrast, using identical conditions, \textit{B. burgdorferi} was isolated from 1 of 7 tick pools (87 ticks) from Switzerland, and a single pool of 10 ticks from Slovakia.”\textsuperscript{16}
Chapter 4 - Serological and clinical evidence for the existence of Lyme Borreliosis in Australia (pages 175-223)

This chapter is well summarised in a letter published in the Journal of Spirochaetal and Tick-borne Diseases in 1994. However, a short overview is provided below, as it is relevant later in this document.

This study, involving members of the Microbiology departments of Newcastle University and Sydney’s Royal North Shore Hospital, focused on the development of a Western Blot test specifically for Australian patients.

Simply put, Western Blots are a method of identifying patient immune responses to specific parts of the Borrelia known to cause Lyme disease. They are still the most common laboratory test to confirm a Lyme diagnosis.

The two Borrelia parts the test sought a response to were OspA and flagellin.

Prior to the study, Australian patients with suspected Lyme disease were only tested for the American strain. However, given how little was known about Lyme-like illness, the two European strains were included in this study.

The blood of 171 volunteers were tested. They formed three groups:

1. Healthy people
2. People with connective tissue disorders
3. People likely to have Lyme disease acquired in Australia, based on a clinical assessment by Infectious Diseases Specialist Dr Bernie Hudson. His criteria included EM rashes, arthritis and nerve issues. Hudson’s involvement is important, as the letter acknowledges that he regularly treats “clinical cases of LB acquired in Australia.”

The results were significant. Fifty five percent of the 23 patients with Lyme-like illness had produced antibodies to both OspA and flagellin. While 55% is not an overwhelming majority, it is very impressive given that some patients never make antibodies to OspA.

By comparison, only 3.3% of the participants with connective disorders, and 2.2% of the healthy participants had OspA antibodies.

As with Wills’ earlier study, the presence of the European strains featured far more prominently than the American strain.

The remainder of this chapter covers seven patient case studies previously not known, on pages 201-218.
This study, included in Dr King’s Senate submission, was previously completely unknown to us.

It is a preliminary investigation into sero-epidemiology; that is, the identification of *Borrelia* antibodies in the blood of patients located in different regions.

Doctors located in tick-infested NSW coastal areas had contacted Newcastle University’s laboratory to ask about a blood test that might help them determine if their patients had Lyme-like illness, as opposed to other chronic musculo-skeletal conditions.

It was for that reason that the custom Western Blot test earlier mentioned was developed.

Doctors were invited to send patient blood samples for testing. Only samples with medium or strong OspA and flagellin reactions were deemed ‘positive.’

In 1993-1994, 1043 samples were received from five areas, categorised as Far North Coast, Mid North Coast, Hunter Valley, Central Coast and Sydney district. Twenty percent of the samples – 210 patients - received a positive result.

Of those patients, 87% reacted with the OspA of only one strain of *Borrelia*, usually *B. garinii*. The remaining 13% reacted to more than one OspA, usually both *B. garinii* and *B. afzelii* “and were considered to either be mixed infections or multiple infections.”

This, of course, further reinforces the 1991 findings that the Australian *Borrelia* is most closely related to the European strains.

The region with the highest percentage of positive results was the Mid North Coast, at 61% - this matches the data we’ve obtained from patient surveys more than 15 years later. The lowest percentage came from the Far North Coast, at 10%.

Of the Sydney results, Wills clarified “The positive specimens were not distributed randomly throughout the city but occur predominantly in the north-eastern suburban areas... the most positive subjects live on the fringe of a major national park (Kuringai/Broken Bay Bay); many reported a history of... tick-bite.”

Wills stated that the outcome supports “the conclusion that an indigenous LB-like illness, associated with tick bite...occurs annually throughout tick-infested areas. As such it becomes a matter of public health concern and deserves further detailed investigation.”
Recommendations disregarded

While the studies documented in Dr Wills’ thesis provide strong evidence of an Australian Lyme-like illness caused by Borrelia found in ticks, they are not definitive.

Dr Wills therefore called for additional research:

“This dissertation supports the conclusion that LB exists indigenously in Australian and provides a reasonable explanation for the controversy created by previous Australian studies. Further research is needed concerning several issues arising from this study, namely:

1. Development of suitable cultural conditions for the growth and maintenance of Australian B. burgdorferi
2. The molecular characteristics of Australian strains of B. burgdorferi so that a taxonomical comparison with existing genospecies can be obtained.
3. A more exact definition of the clinical manifestations of Australian Lyme disease and the immunological responses of patients.
4. Determination of epizootiology of LB in Australia, and the importance of LB in Australian wild and domestic animal populations.”

Dr Wills’ recommendations were not pursued. The government had accepted the findings of the Russell and Doggett study as conclusive, and did not fund any further research for another 20 years.

Dr Wills told us “there [are] a lot of questions unanswered and we identified them in my thesis and we said...these are areas that need to be investigated with some urgency and we did try to get funding after I submitted my thesis. But the politics at that time in Australia, there was no way you could continue with Lyme disease research. There was just...no one was willing to fund it.”

“What I don’t understand is, why the government didn’t want more research into it, ...[why] they didn’t accept the fact that people with this particular clinical picture responded to this treatment and they should be given the treatment if they present in that way... suddenly Lyme disease became a black hole, and nobody wanted to talk about it again.”

In 1997, Dr Russell himself acknowledged Dr Wills’ thesis, and some of the criticisms of his work, conceding “It is possible there is an indigenous form of Lyme disease in Australia, probably due to infection with a tick-borne spirochaete(s) unlikely to be Borrelia burgdorferi ss [sensu stricto] and that current laboratory methods require modification to culture the causative organisms from ticks or human specimens.”

He, too, was pessimistic about the possibility of future research. “Despite the greater incidence of tick-related problems, there is also little to be optimistic about with prospective tick research. The groups working with tick-problems...have all been disbanded as funding has disappeared...Notwithstanding, the discovery of a new infectious organism (eg. A spirochaete that can be shown to be responsible for the so-called Lyme disease syndrome in south-eastern Australia) may bring a renewal of interest and funding.”
**General disregard by the government and scientific community**

Despite the lack of interest in Lyme-like illness, a core group of doctors continued to diagnose and successfully treat patients. With the rise of the internet, patients found new ways to connect with each other. New online support groups began to form, the media began to pay attention, and the LDAA and Karl McManus Foundation (KMF) were established. In 2012, the first patient protest was held outside a NSW Health building in Sydney.

After decades of silence, the Department of Health faced increasing pressure to act, and the interest of the Australian scientific community was piqued.

We are currently aware of four prominent documents that review the scientific evidence of a Lyme-like illness in Australia.

Below, we list those documents, and their disregard for the information in Dr Wills’ thesis.

1. **Scoping Study to develop a research project(s) to investigate the presence or absence of Lyme disease in Australia, 2013**

   Microbiologist Prof John Mackenzie was contracted by the Australian government to write this document, a pivotal part of the government’s Clinical Advisory Committee into Lyme Disease (CACLD).

   Although he mentions the preliminary 1991 results of Wills and Barry’s initial study, he does not mention its final results. Nor does he reference any part of the thesis, including the studies outlined in Chapters 4 and 5.

2. **Does Lyme disease exist in Australia? 2016**

   This article was co-authored by Dr Gary Lum, an employee of the Department of Health, and a key figure of the CACLD. Alarmingly, it was published while the Senate inquiry was underway, leading Senator Lambie to question his impartiality and respect for patients.

   Like his colleague Prof Mackenzie, the only mention of Dr Wills’ research that Dr Lum makes is in relation to the initial findings of the 1991 study.

3. **Lyme disease: why the controversy? 2016**

   The author of this article, Prof Miles Beaman, is an Infectious Diseases Specialist, and pathologist specialising in microbiology. He is known for his strong scepticism in relation to Lyme-like illness, telling the media that “30 years of research showed no evidence of Lyme bacteria in patients who had not left the country.”

   Prof Beaman doesn’t acknowledge any of the studies Wills was involved in.
4. Is there a Lyme-like disease in Australia? Summary of the findings to date 2016

This article was co-authored by scientists from Central Queensland University and a pathology laboratory directed by Prof Stephen Graves, a consistently strong sceptic of Lyme-like illness.

It not only mentions the 1991 findings, but acknowledges that “Although this investigation was conducted as a part of the author’s (Wills’) PhD, no follow-up report to these preliminary findings was ever published in the scientific literature.”

No further mention of Dr Wills’ work is made, so it is a safe assumption that no effort was made to obtain a copy of the thesis.

In fact, the document goes on to repeat the suggestions of Russell and Doggett, before stating that their conclusion “therefore seems more plausible than the conclusions of Wills and Barry.”

We acknowledge that we were unaware of the thesis. However, Dr Wills was a driving force in Lyme research, who had clearly identified her initial findings as “preliminary.” Although not all of her work was published in the general domain, we believe that it’s a reasonable expectation that the authors of the reviews conduct them with diligence. This includes seeking out Dr Wills’ thesis, which not only includes the findings of an international expert, but was substantiated by the three specialists who reviewed it. All three passed it with only minor grammatical corrections.

At the time of the thesis’ completion, the highly charged toxic atmosphere in the scientific community relating to anyone who disagreed with the government-endorsed Russell and Doggett study was notorious. Dr Wills has confided that she found this deeply upsetting, and a very real threat to the start of her hard-earned career. As a result, the young scientist didn’t seek publication in journals, instead focussing on other priorities.

That said, the thesis was certainly not inaccessible; it was always available at the University of Newcastle, who shared a copy with the University of Technology Sydney, at their request. It was also mentioned in a 2012 book.

The debate about Lyme-like illness has been raging for decades, devastating thousands of lives; a thorough and complete review of all the evidence is certainly warranted.
Impact to the patient community

Dr Wills’ thesis had the genuine potential to significantly progress our understanding of Lyme-like illness decades ago. Indeed, it could be argued that Brazil formally identified their own Lyme-like illness, Baggio-Yoshinari Syndrome,\textsuperscript{34} using less information than is available in Dr Wills’ thesis.

Instead, more than 20 years later, chronically ill patients continue to appeal to the government for an objective and genuinely committed approach to the issue. In fact, not long after our supplementary submission to the Senate inquiry detailing Dr Wills’ work, NSW Health fact sheet’s statement “there is little evidence that Lyme disease is caused by Australian ticks” was updated to read that there is no evidence.\textsuperscript{35}

Some of the opportunities denied to patients are listed below:

1. **The chance to commercialise the effective Western Blot test developed by Dr Wills and her colleagues**

   Dr Wills told us “It was a laboratory based test, in that it wasn’t set up for a commercial facility. It was very time consuming and labour intensive, but we knew it worked. To do the next step as a commercial project, we needed support... unfortunately we had this test that showed people had Lyme disease, and then suddenly the Australian medical community said “No, there's no Lyme disease.”

   “And so, there was no market for a test for Lyme disease...Professor Barry tried to continue to get funding to develop it further but, you know... It’s not commercially viable.”\textsuperscript{36}

   Had this test become widely available. It is likely that many more patients would have been promptly diagnosed, allowing for treatment prior to their illness becoming chronic.

2. **Accurate testing from government laboratories**

   We are aware that at least one government laboratory that is accredited by the National Association of Testing Authorities, did not test patients for *B. garinii* for at least the period 1994-2012.\textsuperscript{37}

   This is even though the *Borrelia* found in Australian ticks was deemed by an international expert to be more closely related to *B. garinii* than the other strains that cause Lyme.

   That finding was supported by the outcomes reported in Chapters 4 and 5 of Dr Wills’ thesis.

3. **Possible suicide avoidance**

   Our patient surveys indicate that it takes patients an average of 10 years to be diagnosed with Lyme-like illness. They are then confronted with a significant stigma, very little support, poor access to effective treatment and significant expenses.
The patient community has suffered many suicides. Anecdotally, these are attributed to despair of ever being treated with respect and dignity, as much as they are the effects of the illness itself.38

Patients of Lyme-like illness – and indeed, all Australians – have a right to expect thorough, unbiased analysis of public health issues, and prompt resulting action. Our government is failing to meet these basis expectations.
Endnotes


2 Lyme Disease Association of Australia, Growing Evidence of an Emerging Tick-borne Disease that causes a Lyme-like illness for many Australian Patients, Senate Submission 528.1, http://www.aph.gov.au/Parliamentary_Business/Committees/Senate/Community_Affairs/Lymelikeillness45/Submissions

3 Wills, Lyme Borreliosis, the Australian Perspective, 1995, http://hdl.handle.net/1959.13/1350473


7 Wills, Lyme Borreliosis, the Australian Perspective, p 123-124

8 Wills, Lyme Borreliosis, the Australian Perspective, p 124

9 Wills, Lyme Borreliosis, the Australian Perspective, p 120

10 Wills, Lyme Borreliosis, the Australian Perspective, p 131

11 Wills, Lyme Borreliosis, the Australian Perspective, p 169

12 Wills, Lyme Borreliosis, the Australian Perspective, p 164-165

13 Wills, Lyme Borreliosis, the Australian Perspective, p 165

14 Wills, Lyme Borreliosis, the Australian Perspective, p 165-166

15 Wills, Lyme Borreliosis, the Australian Perspective, p 166

16 Wills, Lyme Borreliosis, the Australian Perspective, p 166


18 Wills, Lyme Borreliosis, the Australian Perspective, p 200

19 Wills, Lyme Borreliosis, the Australian Perspective, p 230-231

20 Wills, Lyme Borreliosis, the Australian Perspective, p 239

21 Wills, Lyme Borreliosis, the Australian Perspective, p 239-240

22 Wills, Lyme Borreliosis, the Australian Perspective, p 4

23 Lyme Disease Association of Australia, Growing Evidence of an Emerging Tick-borne Disease that causes a Lyme-like illness for many Australian Patients, Senate Submission 528.1, p52
Lyme Disease Association of Australia, Growing Evidence of an Emerging Tick-borne Disease that causes a Lyme-like illness for many Australian Patients, Senate Submission 528.1, p54


Lyme Disease Association of Australia, Growing Evidence of an Emerging Tick-borne Disease that causes a Lyme-like illness for many Australian Patients, Senate Submission 528.1, p55-56 http://www.aph.gov.au/Parliamentary_Business/Committees/Senate/Community_Affairs/Lymelikeillness45/Submissions
