Lyme Borreliosis, the Australian perspective

by

Michelle Catherine Wills

B. Ag. Sc. Hon 1 (University of Sydney)

A Thesis submitted for the degree of DOCTOR OF PHILOSOPHY

Faculty of Medicine, University of Newcastle.

Newcastle, New South Wales, Australia.

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution

Michelle Catherine Wills
In memory of Sally Therese Wilson.
24th July 1961 - 12th June 1970.

Be like a very small
happy child
living gloriously in the
ever present Now
without a single worry or concern
about even the next
moment of time.

Eileen Caddy
The Dawn of Change
ACKNOWLEDGMENTS

I would like to thank the following people for the support and encouragement they provided.

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To my family, especially my mother, thank you for the support you have given me. I love you all very much.

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flagellin (41 kDa)

Osp A and Osp B (31 kDa and 34 kDa respectively)

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### Abbreviations

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<tr>
<td>aa</td>
<td>amino acid</td>
</tr>
<tr>
<td>ACA</td>
<td>Acrodermatitis chronica atrophicans</td>
</tr>
<tr>
<td>BCIP</td>
<td>5-bromo-4-chloro-3-indolyl phosphate</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>BSK</td>
<td>Barbour-Stoenner-kelly</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECM</td>
<td>erythema chronicum migrans</td>
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<tr>
<td>EDTA</td>
<td>ethylene diamino tetraacetate</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
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<tr>
<td>EM</td>
<td>erythema migrans</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>fla</td>
<td>flagellin gene</td>
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<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>H.L.</td>
<td><em>Haemaphysalis longicornis</em> (alternate spelling <em>Haemaphysalis longicornis</em>)</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-2-hydroxyethylpiperazine-N(^1)-2-ethane-sulphonic acid</td>
</tr>
<tr>
<td>I.H.</td>
<td><em>Ixodes holocyclus</em></td>
</tr>
<tr>
<td>IFAT</td>
<td>immunofluorescent antibody titre</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase</td>
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<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>LB</td>
<td>Lyme borreliosis</td>
</tr>
<tr>
<td>M</td>
<td>mole (s), molar</td>
</tr>
<tr>
<td>mA</td>
<td>milliampere</td>
</tr>
<tr>
<td>MAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mM</td>
<td>millimole (s), millimolar</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>NBT</td>
<td>nitroblue tetrazolium</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>Osp</td>
<td>Outer surface protein</td>
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<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
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<td>PBS</td>
<td>phosphate buffered saline</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>rpm</td>
<td>revolutions per minute</td>
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<td>S.E.M.</td>
<td>scanning electron microscopy</td>
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<td>SDS</td>
<td>sodium dodecyl sulfate</td>
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<tr>
<td>Taq</td>
<td><em>Thermus aquaticus</em> DNA polymerase</td>
</tr>
<tr>
<td>T.E.M.</td>
<td>transmission electron microscopy</td>
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<tr>
<td>TEMED</td>
<td>N,N,N(^1),N(^1)-tetramethylethylenediamine</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>V</td>
<td>volts</td>
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<td>µg</td>
<td>microgram</td>
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<tr>
<td>µm</td>
<td>micrometre</td>
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<tr>
<td>X-Gal</td>
<td>5-bromo-4-chloro-3-indol-β-D-galactoside</td>
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SYNOPSIS

In 1989 when this study commenced, Lyme disease or Lyme Borreliosis (LB), was still a relatively recently identified disease entity. The earliest clinical description of some aspects of this disease are from Europe and extend back as far as 1883. These observations accumulated for nearly a century before the various manifestations were correlated and described as a single entity. Clinically, the description of LB is complex and comprises a diverse range of syndromes. Research established that distinct differences in the clinical presentations of patients occurred depending on the geographical locality in which they were infected.

Anecdotal reports that an illness not dissimilar to the Northern Hemisphere LB was common in the Manning Valley District of New South Wales (NSW) began circulating in the late 1980's. However, because of the geographical isolation of Australia from the Northern Hemisphere it seemed at that time only a remote possibility that such a disease could exist on this continent. If it did exist, it was questionable as to how likely it would be that it had the same cause as Northern Hemisphere LB.

This dissertation has been directed towards obtaining conclusive evidence that LB exists in Australia. The objectives have been:

1. To determine whether Australian ticks carry and transmit spirochaetes related to *Borrelia burgdorferi*.

2. To develop a specific and sensitive sero-diagnostic test to assess whether or not there is a correlation between clinical illness and the presence of *Borrelia burgdorferi* specific antibodies in likely Australian LB candidates.

3. To access the distribution of LB along the East Coast of Australia.
This study was initiated in December 1989 and concluded in December 1994.

It began as an attempt to detect the presence of *B. burgdorferi* in Australian ticks and concentrated on the Manning Valley, where two main species of ticks are common; *Ixodes holocyclus* and *Haemophysalis longicornis*. Because these tick species are widely distributed, the study extended south to include the Hunter Valley and to the Sydney regions (Plate 1).

Plate 1  Map of Australia. Main locations of study are as indicated

Despite modest success in the isolation of fragile spiral shape organisms, using conventional Borrelia culture methods (Wills and Barry, 1991), a controversy
subsequently developed as to the true nature of these agents, because it was claimed that they were artefacts, probably aggregates of bacterial flagellae (Russell et al., 1994). From experiments based on improvements in culture conditions and examination of the ultrastructure of antibacterially treated cultures of *B. burgdorferi*, it was concluded that the spiral shaped organisms detected in this study were mostly dead spirochaetes. Subsequent studies using monoclonal antibodies directed against the major structural proteins, as well as polymerase chain amplification of microbial DNA, provided evidence that *B. burgdorferi* - like spirochaetes are likely to occur in Australian ticks.

A second approach was an attempt to correlate the presence of LB-like symptoms in patients with the presence of LB-specific antibody. This study commenced in 1992, in conjunction with Dr B. Hudson, Infectious Diseases Physician at Royal North Shore Hospital, Sydney. The objective of this phase of the work was to develop a suitable serological test. The three genospecies of *B. burgdorferi* were used to develop and evaluate the usefulness of an immunoblot procedure and interpretation of this test was based on recommendations made by the American Center for disease Control [Centers for Disease Control and Prevention. Case definition for public health surveillance. MMWR, 39(RR-13):19-21, 1990] (Appendix C). Using stringent criteria for the clinical diagnosis of LB, Dr Hudson subdivided candidate LB patients into three categories based on the decreasing likelihood of LB specific illness. A correlation was established between the likelihood of clinical illness and positive serology. An unexpected finding to emerge was that the diagnostic specificity of the immunoblot test varied according to which genospecies of *B. burgdorferi* was used as antigen. Sera from Australian patients were most likely to be reactive to Osp A of *B. garinii*, with reactivity to *B. afzelii* Osp A less common. They were least likely to be reactive to *B. burgdorferi* sensu stricto.

The clinical manifestations of LB, acquired in Australia, resembled more closely those described in Europe, rather than in the USA. This may correlate to the nature of Australian *B. burgdorferi*. Due to more frequent seroreactivity in Australian patients to
B. garinii compared with other strains, especially B. burgdorferi sensu stricto, suggests that Lyme borreliosis in Australia is more likely to be caused by an organism resembling B. garinii.

This dissertation supports the conclusion that LB exists indigenously in Australia 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.
FOREWORD

This dissertation is concerned with the biology, morphology and cultural characteristics of a *Borrelia burgdorferi*-like organism found in Australian ticks and with the clinical and serological features of a Lyme borreliosis like illness thought to be associated with tick bite.

The introduction to this dissertation examines the history of Lyme disease, including the early clinical presentations observed independently in both America and Europe, and the ultimate realisation that both disease syndromes are caused by the same organism, *B. burgdorferi*. The review continues to examine the complexity and fastidious nature of *B. burgdorferi*, its modes of transmission and the difficulties in the diagnosis of the various clinical syndromes with which it is associated. The introduction ends with a discussion of the epidemiology of the disease and its importance in non-human animals.

This review has taken into account all publications in print and available in Australia at the 30th July 1995.

Following the literature review (Chapter 1), there is a description of the experimental methods (Chapter 2). The experimental results have been subdivided into three chapters:

1. **Chapter 3**: Preliminary characterisation of Borrelia-like microorganisms found in Australian ticks.
2. **Chapter 4**: Clinical and immunological assessment of Australian LB patients.
3. **Chapter 5**: Epidemiology of Australian LB.

Each experimental chapter is a discrete unit, containing both the experimental findings and a discussion that is relevant to the topic under consideration.