Debilitating Symptom Complexes Attributed to Ticks (DSCATT)

Literature Review to support the DSCATT Clinical Pathway

December 2020

ALLEN+CLARKE CONSULTING

This original artwork in our Acknowledgement of Country was produced by Emma Walke. Emma is a Bundjalung Aboriginal woman from Northern New South Wales.

ABOUT *ALLEN + CLARKE*

Founded in 2000, *Allen + Clarke* is an established and respected consultancy with offices in Melbourne and Wellington. Our areas of work cover three core areas of practice including evaluation and research services; policy, regulatory, and business changes services; and governance, secretariat, and program support services. More information about our work can be found on our website at www.allenandclarke.com.au.

This publication was prepared for the Australian Department of Health by Dr Robyn Haisman-Welsh, Pauline Van, Dr Carolyn Hooper, and Paul Houliston from *Allen + Clarke*, and Catherine Marshall (Independent Guideline Adviser).

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LIST OF ABBREVIATIONS

AACODS	Authority, Accuracy, Coverage, Objectivity, Date, Significance
ACA	Acrodermatitis chronica atrophicans
ACIIDS	Australian Chronic Infectious and Inflammatory Disease Society
AHPRA	Australian Health Practitioner Regulation Agency
ALS	Amyotrophic lateral sclerosis
AMSTAR 2	**A M**ea**S**urement **T**ool to **A**ssess systematic **R**eviews, version 2
APP	Approved Pathology Practitioner
ASF	Australian Spotted Fever
ASID	Australasian Society for Infectious Diseases
CASP	Critical Skills Appraisal Programme
CDC	Centers for Disease Control and Prevention
CDNA	Communicable Diseases Network Australia
CFS	Chronic Fatigue Syndrome
CME	Continuing Medical Education
CMO	Chief Medical Officer
CMV	Cytomegalovirus
COREQ	**CO**nsolidated criteria for **RE**porting **Q**ualitative research
DNA	deoxyribonucleic acid
DSCATT	Debilitating Symptom Complexes Attributed to Ticks
EBV	Epstein-Barr virus
EIA or ELISA	Enzyme-linked immunosorbent assay
EM	Erythema Migrans. Bull's Eye Rash
ESCMID or ESGBOR	European Society of Clinical Microbiology and infectious Diseases
EUCLAB	European Concerted Action on Lyme Borreliosis
FDA	Food and Drug Administration
FISF	Flinders Island Spotted Fever
GP	General Practitioner
HIV	Human Immunodeficiency Virus
ID	Infectious Disease
IDSA	Infectious Diseases Society of America
IDSA/AAN/ACR	Infectious Diseases Society of America (IDSA)/American Academy of Neurology (AAN)/ American College of Rheumatology (ACR)
IFA	Indirect Immunofluorescence Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intramuscular
IV	Intravenous
KMF	Karl McManus Foundation
LA	Lyme Arthritis
LB	Lyme borreliosis
LDAA	Lyme Disease Association of Australia
LNB	Lyme neuroborreliosis
MBA	Medical Board of Australia

MDT	Multidisciplinary care team
ME	Myalgic Encephalitis
MMWR	Morbidity and Mortality Weekly Report
MUPS	Medically Unexplained Physical Syndrome
MUS	Medically Unexplained Symptoms
NATA	National Association of Testing Authorities, Australia
NICE	National Institute of Health and Care Excellence
NIH	National Institutes of Health
NHMRC	National Health and Medical Research Council
NPS	National Prescribing Service
NRL	National Serology Reference Laboratory
PANDAS	Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections
PBS	Pharmaceutical Benefits Scheme
PCR	Polymerase Chain Reaction
PTLDS	Post-Treatment Lyme Disease Syndrome
RACGP	Royal Australian College of General Practitioners
RCPA	Royal College of Pathologists of Australasia
RCT	Randomised controlled trial
REP	Reptile-associated
RF	Relapsing fever
RNA	ribonucleic acid
SLE	Systemic Lupus Erythematosus
spp.	species
TGA	Therapeutic Goods Administration
QTT	Queensland Tick Typhus
UK	United Kingdom
US	United States of America

GUIDANCE ON HOW TO READ THIS LITERATURE REVIEW

This report is a narrative literature review. It contains the following key parts:

- The **Executive Summary (page 1 onwards)** provides an overview of the purpose of the literature review, and the key findings by research question.
- **Sections 2-6 (page 13 onwards)** provides a summary of the findings of this literature review presented by the research questions. The literature reviewed and a high-level summary of the quality of the evidence for each research question is also included.
- The methodology of this literature review is described in **Appendix A: Methodology.**
- The critical appraisal templates for systematic reviews, randomised controlled trials (RCTs), and cohort studies are included in **Appendix B: Critical Appraisals.**
- The AGREE II overall results for guidelines are in **Appendix C: AGREE II**.
- The critical appraisal templates for grey literature are included in **Appendix D: AACODS Grey Literature Appraisals.**
- We have noted studies and publications cited by the authors of articles included in this literature review and provided these as in text citations. We feel this brings another layer of comprehensiveness to the literature review and several advantages to the reader. By identifying the cited authors in the text and providing the citation in the text, the reader has the opportunity to view at a glance the authors, the recency of the publication and also the consistency/comparability with which papers have been cited by authors to inform their conclusions/recommendations relevant to the various research questions. This approach also acknowledges literature not included in our literature review but published within the inclusion dates for the review. The addition of in text citation provides easy access for readers who may wish to explore an article further.

1. EXECUTIVE SUMMARY

ALLEN+CLARKE CONSULTING

1.1. Purpose and scope

The Australian Department of Health (the Department) commissioned Allen and Clarke Policy and Regulatory Specialists Limited (*Allen + Clarke*) to develop an evidence-based clinical pathway and multidisciplinary care model (the Clinical Pathway) for patients suffering from debilitating symptom complexes attributed to ticks (DSCATT) that can be flexibly applied in both private and public health settings.

The Clinical Pathway must be informed by relevant literature and key documents. As the Clinical Pathway was developed to support decision-making on differential diagnosis and referral pathways for patients presenting with either new onset or unresolved debilitating symptoms with or without a history of tick bites and that cannot be attributed to another condition (acute or chronic), this literature review focuses on peer-reviewed evidence and grey literature published since January 2008 that can inform an evidence base to underpin the development of the Clinical Pathway. Acknowledging the attribution to ticks in the term DSCATT, this literature review includes consideration of tick-borne diseases (overseas acquired Lyme disease and known Australian tick-borne diseases) and considerations, including approaches to management of care for patients for whom a diagnosis for their symptoms may not be established.

1.2. Debilitating symptom complexes attributed to ticks (DSCATT)

Debilitating symptom complexes attributed to ticks (DSCATT) is the term used by the Australian Government to describe the group of Australian patients suffering from the symptoms of a chronic debilitating illness, which many associate with a tick bite (Department of Health, 2018a), to appropriately acknowledge this patient group and the multifaceted illness they are experiencing and acknowledge that their illness is poorly understood. The Australian Government acknowledge that many of these patients experiencing debilitating symptom complexes are living in turmoil because their illness cannot be easily diagnosed and treated. With the causes of DSCATT as yet unknown, the Australian Government urges patients and health professionals to keep an open mind about the cause of a patient's symptoms.

In 2016 the Senate Community Affairs References Committee (the Senate Committee) released two reports, an interim and a final report of the *Inquiry into the growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australian patients* (the Senate Inquiry). The Australian Government responded to both Senate Committee reports.

DSCATT was also proposed as a name to move away from the stigma and controversy associated with the terms previously used to describe this patient group such as "*Lyme disease-like Illness*" and "*Chronic Lyme Disease*" (Department of Health, 2018a).

DSCATT is not clearly defined and is not formally reported. It has no diagnostic criteria, known cause or causes, no treatment and these symptoms may be the end point for several different disease processes. The symptom complexes to which the name DSCATT has been given incorporates a wide range of nonspecific symptoms. Some people may have a diagnosis that has not yet been identified that explains these symptoms while others may have a cluster of medically unexplained symptoms (MUS) that require management. MUS are defined as physical symptoms persisting for more than several weeks and for which adequate medical examination has not revealed a condition that adequately explains the symptoms. Patients with MUS may be very unwell and require complex care.

1.3. Methodology

A detailed description of the methodology, including research questions, search strategy, and a summary of inclusions is provided in Appendix A: Methodology. A total of 119 items were included in the literature review.

1.4. Key findings

1.4.1. Research Question 1: What is the clinical epidemiology of DSCATT?

There are no peer-reviewed published epidemiological or clinical studies about patients experiencing DSCATT in Australia.

As such, there is no reliable evidence on the clinical epidemiology of DSCATT in Australia, including on the prevalence, demographics and geographic distribution of patients experiencing symptoms associated with DSCATT or on the symptomology and clinical signs associated with DSCATT, that can reliably inform an evidence-based Clinical Pathway. Given that DSCATT is not clearly defined, has no case definition, has no known cause(s) and is not an identified diagnosable disease, the lack of studies is to be expected.

Available information on the prevalence, demographics and geographic distribution of patients experiencing DSCATT in Australia.

Two Australian Government reports and a published peer-reviewed review of evidence undertaken to advise the Chief Medical Officer (CMO) of the Department of Health whether there is a Lyme-like illness (DSCATT) in Australia indicate there is no reliable data on the prevalence or incidence of DSCATT in Australia at this time. The Senate Inquiry and The House of Representatives Standing Committee on Health – Case study on tick-borne and Lyme-like diseases specifically investigated prevalence and incidence of DSCATT and concluded that as DSCATT is not clearly defined and not formally reported, available statistics on its incidence across Australia are limited and are difficult to determine. The review for the CMO of over 500 cases of Lyme-like illness identified in Australia and published in the scientific literature concluded that the unreliability of the published case reports in their diagnostic methods means the evidence for Australian Lyme-like cases (DSCATT) remains quite unsubstantial and unconvincing and the cause of the illnesses remains unknown.

For geographic location, patients and patient advocacy groups told the Senate Inquiry of the location they believe they acquired their symptoms that they attribute to DSCATT, with New South Wales, Queensland, Western Australia or Victoria being the more frequently reported states.

Available information on the symptoms and clinical signs associated with DSCATT as reported by Australian patients and treating physicians.

Patients described the symptoms they have experienced and attribute to DSCATT to the the Senate Inquiry, DSCATT Patient Forum and DSCATT Think Tank, the latter held to inform the development of the DSCATT Clinical Pathway. An analysis of publicly available submissions to the Senate Inquiry from patients who identified as suffering from Lyme disease or DSCATT found the most common symptoms were: fatigue (62.6 per cent); disordered thinking (51.9 per cent); sensory disturbance (46.1 per cent); arthralgia (45.6 per cent); headache (44.5 per cent); followed by myalgia; rash; mood disturbance; visual disturbance; dizziness; pain; fever; nausea; palpitations; insomnia; seizures; diarrhoea; tremor; and personality change. Patients reported having experienced symptoms for a median of 10 years. Similarly, multiple symptoms and signs being attributed to DSCATT were identified by stakeholders who attended the Think Tank in May 2019, with neurological symptoms

(including brain fog, cognitive dysfunction, memory loss, fine motor impairment and reduced verbal fluency) and chronic fatigue being the most commonly identified symptoms and signs.

The available information on the symptomology and clinical signs associated with DSCATT, while of low quality, due to the information provided being self-reported or anecdotal can, however, provide some insights for medical professionals when patients present to primary care and question whether they may be experiencing the symptoms associated with DSCATT.

1.4.2. Research Question 2: What information is available on diseases or disorders patients experiencing DSCATT symptoms have been diagnosed with and what are the most likely differential diagnoses?

There are no peer-reviewed published epidemiological or clinical studies about patients experiencing DSCATT that include the investigation of the diseases and disorders they have been diagnosed with.

As such there is no reliable evidence on the diseases or disorders patients experiencing DSCATT have been diagnosed with that can reliably inform an evidence-based Clinical Pathway. Again, given that DSCATT is not clearly defined, has no case definition, has no known cause(s) and is not an identified diagnosable disease, the lack of studies is to be expected at this point in time.

The available information on the diseases and disorders experienced by patients with symptoms associated with DSCATT is based on self-reported or anecdotal information provided by patients, their treating doctors or patient advocacy groups. Patients and patient advocacy groups described the diseases and disorders they or their members have been diagnosed with to the Senate Inquiry, the DSCATT Patient Forum and the DSCATT Think Tank. In some cases patients reported having been diagnosed with other known tick-borne infections such as Q fever, Spotted Fever, Rickettsia, Queensland Tick Typhus (QTT) or allergy to tick toxin and received treatment, or been diagnosed with non-specific conditions including chronic fatigue syndrome, fibromyalgia, Epstein-Barr virus or a mental health condition such as depression. An analysis of submissions to the Senate Inquiry by patients who identified as having Lyme disease or DSCATT found one in ten patients reported being given another diagnosis that could explain their physical symptoms including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and motor neurone disease. In over half of the submissions analysed, patients reported having been diagnosed with co-infections [not further defined]. Almost 10 per cent of submitters self-diagnosed after being exposed to a media report of Australian Lyme disease.

The potential to misdiagnose potentially treatable illness as Lyme disease was a major cause of concern raised in the Senate Inquiry reports.

The available information, while of low quality, due to the information provided being self-reported or anecdotal can, however, provide some insights for medical professionals when patients present to primary care and question whether they may be experiencing the symptoms associated with DSCATT.

Acknowledging the attribution to ticks in the term DSCATT, the likely differential diagnoses considered include overseas acquired Lyme disease, known Australian tick-borne diseases (Queensland Tick Typhus (QTT), Flinders Island Spotted Fever (FISF), Australian Spotted Fever (ASF) and Q Fever), other diagnoses (infectious and non-infectious) and MUS.

There is robust evidence on Lyme disease, known Australian tick-borne diseases and MUS to inform a differential diagnosis in patients who present with either new onset or unresolved debilitating

symptoms with or without a history of tick bites and that cannot be attributed to another condition (acute or chronic). The evidence meets the scientific quality to underpin an evidence-based clinical pathway.

Overseas-acquired Lyme disease

In Australia, Lyme disease should be considered in the differential diagnosis for patients presenting with a travel history to Lyme disease endemic areas along with supporting symptoms and/or a known tick bite. Lyme disease is endemic in parts of the United States (US), Europe, including the United Kingdom (UK), and Asia. A person visiting a Lyme disease endemic area may become infected with *Borrelia burgdorferi* sensu lato through a tick bite and subsequently develop Lyme disease. Overseas travellers to Lyme disease endemic areas may return to their home country before becoming symptomatic and/or being diagnosed. Overseas-acquired Lyme disease is not a notifiable disease in Australia.

The difficulty in diagnosing Lyme disease, even in Lyme disease endemic areas, was highlighted in a systematic review for the Department of Health UK. The systematic review reported that clinicians find it challenging to diagnose Lyme disease accurately due to the wide variation in symptoms; the infrequency with which they see the disease in practice; their level of confidence about being able to diagnose correctly; the ambiguity they experience about diagnostic tools; and their beliefs and behaviour relating to atypical or recurring symptoms. International authority guidance highlights caution about diagnosing Lyme disease without a supportive history or positive serological testing because of the risk of missing an alternative diagnosis and providing inappropriate treatment.

In a country such as Australia, where Lyme disease is not endemic and is not commonly seen in clinical practice, there are additional challenges in diagnosing Lyme disease. Australian guidance stress that, due to the non-specific nature of many clinical signs and symptoms, the diagnosis of Lyme disease in non-endemic Australia cannot reliably be made on clinical signs and symptoms alone, as many other infectious and non-infectious diseases can have similar features to Lyme disease. Laboratory testing is essential.

The inclusion of a travel history as part of a clinical history is important, as despite multiple studies that have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australian ticks nor any other vector that could transmit the disease to humans. It is for this reason that the Australian medical profession does not support the diagnosis of locally acquired Lyme disease in Australia. While some Australians and healthcare providers believe that a form of 'chronic Lyme disease' exists, globally, 'chronic Lyme disease' is a disputed diagnosis which lacks sufficient supporting evidence.

Known Australian tick-borne diseases

Australian tick-borne diseases should be considered in a differential diagnosis in patients who have not travelled overseas to Lyme disease endemic areas AND who have or may have been bitten by a tick (recently or in the past) or who engage in bushwalking AND present with relevant acute or chronic symptoms.

Apart from the occasional local bacterial infection at the tick bite site (eschar), the only two systemic infections that are definitely known to be transmitted by tick bites in Australia are Rickettsial infections from infection with:

- *Rickettsia* spp. (QTT, FISF and ASF) and
- Q fever (*Coxiella burnetii*).

The signs and symptoms of Rickettsial infections in Australia include eschar, fatigue, fever, headache, myalgia and rash (macular, papular, vesicular) although the severity and duration of Rickettsial diseases vary considerably. QTT and ASF have similar core clinical manifestations with a range of other symptoms observed. Early clinical features are often non-specific, making diagnosis challenging. Additionally, symptoms may overlap with other infectious diseases including those that are transmitted by non-tick vectors as well as a number of chronic diseases.

QTT is an emerging public health threat and increasingly recognised as an important cause of community-acquired febrile illness in Eastern Australia. In Australia, Q fever *(Coxiella burnetii)* is a nationally notifiable disease, has a Q fever laboratory case definition and Communicable Diseases Network Australia (CDNA) National Guidelines for Public Health Units. While a disease attributed to ticks, the majority of cases of Q fever occur through inhalation of infected aerosols and dust, contaminated with birth fluids, faeces and urine from infected reservoir animals such as goats, sheep, cattle, kangaroos and domestic pets.

Alternative diagnoses

In patients who present with new onset (e.g. fever, rash) or persistent debilitating symptoms and tick-borne disease is not suspected, the following differential diagnosis should be considered: infectious; autoimmune; neoplastic; psychological; inflammatory; vascular; neurological; cardio-respiratory; life-style related.

Medically unexplained symptoms

In patients for whom a diagnosis cannot be established, MUS may be considered. MUS are defined as physical symptoms persisting for more than several weeks and for which adequate medical examination has not revealed a condition that adequately explains the symptoms. Patients with MUS may be very unwell and require complex care.

An analysis of patient submissions to the Senate Inquiry noted the unquestionable, real, and debilitating physical and social harm from illness reported in the submissions. The author's conclusion suggested that patients who identified as having DSCATT displayed a symptomology similar to MUS syndrome.

1.4.3. Research Question 3: What are the issues associated with diagnostic testing for Lyme disease both in Australia and by overseas laboratories?

Diagnostic tests are used to help identify those cases in which Lyme disease is the cause.

The symptoms of Lyme disease, other than erythema migrans (EM), such as facial palsy, joint pains or nerve pains can be seen in many other conditions. Diagnostic tests are used to help identify those cases in which Lyme disease is the cause, so that appropriate treatment can be given and ensure that other important diseases are not misdiagnosed as Lyme disease. It is important that the tests used have both the ability to identify infection with the Lyme disease bacteria and to discriminate this from other causes of infection or disease. Following the discovery of *B. burgdorferi* as the causative agent of Lyme disease, numerous tests were developed by clinical and private laboratories. As direct detection of *B. burgdorferi* by polymerase chain reaction (PCR) or culture has been challenging, most diagnostic test development has focussed on indirect detection of infection by assessing the antibody response of the patient. As such, indirect tests through serological assays for antibodies to *B. burgdorferi* s.l. are the mainstay of laboratory diagnosis, the most common diagnostic methodologies

employed, the prerequisite laboratories facilities are widely available and specimens are easy to obtain.

There is strong international consensus on the two-tier serology test protocol for diagnosing Lyme disease.

There is strong international consensus on the use of the two-tier serology testing protocol within the literature and by international authorities and guidelines. A 2019 review of European and US guidelines (16 guidelines from seven countries) indicated that the diagnosis of Lyme disease is currently based on a two-tier serology at all stages of infection except for the early localised dermatological presentation, EM.

The US Centers for Disease Control and Prevention (CDC) and other international guidelines recommend a two-tier serology approach to improve specificity, the two steps consisting of a sensitive enzyme-linked immunosorbent assay (EIA or ELISA), followed by immunoblotting (Western blot) of samples that are positive or indeterminate in the first step with strict interpretative criteria. The rationale for this approach is that the overall sensitivity and specificity are maximised when these tests are performed in sequence. The final result of serological testing is considered positive only when the ELISA is reactive (positive or equivocal) and the Western blot is also positive. Thus, the two-tiered system maximises the sensitivity and specificity of the assays and increases the likelihood of observing a seroconversion (from IgM to IgG) that is evident in most *bona fide B. burgdorferi* infections. Not following the two-tiered algorithm (e.g., performing a Western blot alone or after the ELISA is negative) can increase the frequency of false positive results, with false positive results potentially leading to possible misdiagnosis and unnecessary treatment.

Very recent (2019) international guidance from Infectious Diseases Society of America (IDSA)/American Academy of Neurology (AAN)/American College of Rheumatology (ACR) in the US advises that serologic (serum antibody) testing is highly sensitive in patients with non-cutaneous manifestations of Lyme disease, as these manifestations typically develop after weeks to months of infection; serologic testing is also highly specific when performed and interpreted according to current guidelines; predictive value is increased when results are correlated with clinical features, patient history and risk factors; and currently, the only Food and Drug Administration (FDA) cleared or approved diagnostic tests for Lyme disease are antibody tests.

There are recognised limitations of serology tests for Lyme disease and the usefulness of serological tests for Lyme disease depend on the pre-test probability and subsequent predicative values in the setting where the tests are being used.

Currently available tests for Lyme disease carry limitations. All diagnostic tests produce both false positive and false negative results. The frequency depends on the specificity and sensitivity of the test and the prevalence of the disease in the population. Four systematic reviews, some with meta-analysis, all found accuracy of serology tests increased with progression of the disease, with test sensitivity increasing with progression of *B. burgdorferi* infection from early to late. However, all reviews found marked variation and heterogeneity in study findings of sensitivity and specificity for each test technology, whether it be ELISA, Western blot, or two-tiered test methodology. The three reviews that assessed study quality all found the studies to be at high risk of bias.

While both National Institute for Health and Care Excellence (NICE) and IDSA/AAN/ACR, in their guidelines, identify the currently available protocol as reliable when used appropriately, both note limitations of the testing protocol. Limitations highlighted include: that false positive and false negative results can occur; and possible reduction of accuracy of the test can occur if testing is carried out too early (before antibodies have developed) or the person has reduced immunity, for example

in people on immunosuppressant treatments. Additionally, in a seropositive patient it can be difficult to determine whether antibody reactivity is due to past infection, active/current infection, or both.

The interpretation of serological assays in Lyme disease requires an understanding of the clinical indications and limitations of the tests, and the usefulness of serological tests for Lyme disease depends on the pre-test probability and subsequent predicative values in the setting where the tests are being used. The most common cause of poor performance in serological testing (as in other infectious diseases diagnosed by antibody testing) is their use in unselected patient populations with a low pre-test probability of Lyme disease. The most crucial factor governing pre-test probability for Lyme disease is exposure history.

Despite multiple studies which have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australia. In areas not endemic for Lyme disease [for example, Australia] the positive predictive value of the serology test will be low. Some people believe that they have acquired Lyme disease in Australia because the results of screening antibody tests to *B. burgdorferi* are positive. However, where a patient has not travelled overseas, these positives are all likely to be false positive test results. Even a highly specific test will produce some false positives, so that people who have never been exposed to *B. burgdorferi* can have reactive antibody results. Tests for Lyme disease should only be requested if there is well-founded suspicion of Lyme disease and not in situations of low pre-test probability, to minimise the risk of a false positive result. In an area of low Lyme disease incidence in the US, a study of Lyme disease testing showed an 80 per cent false positive rate, which puts patients at risk of incorrect Lyme disease diagnosis and adverse drug reactions from inappropriate treatment.

Noting the limitations of serology testing, there is significant international work being undertaken to improve laboratory diagnosis of Lyme disease, particularly by the National Institutes of Health in the US.

Diagnostic testing for Lyme disease in Australia follows international best practice.

In a country such as Australia where Lyme disease is not endemic and is not commonly seen in clinical practice, there are additional challenges in diagnosing Lyme disease. The current standard laboratory protocol for diagnosing Lyme disease in Australian diagnostic laboratories follows international best practice and uses a two-tier serology system. There is established Australian guidance for diagnostic testing for Lyme disease.

In Australia, laboratory diagnostic testing for Lyme disease is required for two reasons:

1. Unless the clinician is familiar with the pathognomonic EM rash, it is clinically safer to obtain supportive evidence of infection through diagnostic testing (culture or PCR of the tissue or more usually antibody testing on a convalescent sample).
2. Diagnostic laboratory support is preferred for patients presenting with non-specific signs and symptoms of a disease syndrome, notwithstanding the limitations of the tests.

Diagnostic testing for Lyme disease should only be initiated following advice from appropriate experts such as a consultant infectious disease (ID) physician or a specialist microbiologist and should only be undertaken in Australia in a pathology laboratory accredited by National Association of Testing Authorities, Australia (NATA) and Royal College of Pathologists of Australasia (RCPA) to conduct such testing.

NATA accreditation is highly regarded both nationally and internationally as a reliable indicator of technical competence. All pathology laboratories in Australia receiving funding via Medicare must be accredited by the NATA/RCPA Laboratory Accreditation Program. In Australia, the National Serology

Reference Laboratory (NRL) review of serological assays to diagnose Lyme disease determined the tests used by accredited laboratories to diagnose Lyme disease had equivalent reliability to tests used in overseas laboratories. This therefore means Australian NATA/RCPA accredited laboratories are able to confidently diagnose classical Lyme disease acquired in patients who have travelled to endemic areas and have contracted the infection more than four weeks prior to testing, noting that most patients seroconvert within four to eight weeks of infection. A follow up paper to the NRL report noted that in the known negative population, specificities of the immunoassays ranged between 87.7 per cent and 99.7 per cent, and in Australia's low prevalence population this would translate to a positive predictive value of <4 per cent.

There are commercially available laboratory testing methods to be avoided.

Unvalidated commercially available laboratory testing methods not recommended, based on evidence, by international authorities and guidelines such as the CDC and IDSA/AAN/ACR include: non-standard serology interpretation, urine antigen or deoxyribonucleic acid (DNA) testing, lymphocyte transformation tests, quantitative CD57 lymphocyte assays, culture, immunofluorescence staining, or cell wall-deficient or cystic forms of *B. burgdorferi*, 'Reverse Western Blots', in-house criteria for interpretation of immunoblots, measurements of antibodies in joint fluid (synovial fluid) and IgM or IgG tests without previous ELISA/EIA/IFA.

The quality of the evidence on the current issues associated with diagnostic testing for Lyme disease in Australia and in international laboratories is robust and meets the scientific quality to underpin an evidence-based clinical pathway.

1.4.4. Research Question 4: What are the treatment modalities that have been provided to patients (including subgroups of patients) with DSCATT in Australia and what is the evidence base to support these treatment modalities?

There are no published peer-reviewed publications of clinical studies on the treatment and treatment outcomes in patients experiencing the symptoms associated with DSCATT.

The available information on the treatment modalities that have been provided to patients experiencing DSCATT in Australia comes from self-reported information provided by patients, anecdotal information provided by patient advocacy groups or anecdotal information and medical opinion from treating medical professionals, as reported to the Senate Inquiry and The House of Representatives Standing Committee on Health.

A large number of patients [not further defined] who identified as suffering from Lyme disease or DSCATT told the Senate Inquiry that 'Lyme literate' practitioners often prescribe a course of treatment that may include antibiotics and/or natural therapies that are not supported by Medicare or the Pharmaceutical Benefits Scheme (PBS). Evidence from a 'Lyme literate' doctor to the Senate Inquiry confirmed that patients with Lyme-like illness are being provided with antibiotics, including long-term antibiotics and long-term intravenous (IV) antibiotic therapy. Self-reported evidence collected by the Lyme Disease Association of Australia (LDAA) (2012) and provided to the Senate Inquiry found treatment regimens reported by patients to LDAA included natural supplements, antibiotics, diet, salt and Vitamin C combination, adrenal treatment, hormone treatment and heavy metal chelation treatment.

An analysis of the patient submissions to the Senate Inquiry found 49.9 per cent of submitters reported having received antibiotics, with 45.7 per cent having received oral antibiotics and 16.6 per cent reporting having received IV/IM antibiotics. One in four submitters had seen a 'Lyme literate'

doctor for diagnosis and treatment and 17.2 per cent reported having been treated overseas. The author's conclusion suggested patients may have sought alternative and potentially non-evidence-based diagnoses and treatments.

The appropriateness of treatments provided to patients who experience DSCATT was a major concern raised to the Senate Inquiry and The House of Representatives Standing Committee on Health by Australian medical authorities and medical professional associations.

Overseas acquired Lyme disease

The majority of international guidelines recommend one course of antibiotics for all presentations of Lyme disease with approaches to therapy being generally similar on both sides of the Atlantic.

Lyme disease is treated with antimicrobials from several classes with activity against *B. burgdorferi,* including doxycycline, penicillin, amoxicillin, cefuroxime, ceftriaxone and azithromycin, with the goals of treatment being the resolution of objective signs and symptoms of infection with prevention of relapsed active infection or new complications of infection. Under most circumstances, oral therapy is effective and preferred over intravenous therapy due to equivalent efficacies, tolerability, and cost.

Treatment recommendations, based on available randomised controlled trials (RCTs) published by US professional bodies such as the IDSA, the American Academy of Paediatrics and a variety of national and supranational associations in Europe (EUCALB) indicate that the approaches to therapy are generally similar on both sides of the Atlantic with some minor differences in the recommended dosage and treatment duration.

The majority of international guidelines, including IDSA, NICE and IDSA/AAN/ACR, recommend one course of antibiotic therapy for all presentations of Lyme disease.

There is a strong body of evidence and international authority recommendations that do not support ongoing and long-term treatment of Lyme disease with antibiotics.

Prolonged intravenous or oral antibiotic therapy for Lyme disease did not significantly improve outcomes in studies performed in North America and Europe and can be associated with significant adverse effects. A 2016 RCT on longer-term therapy for symptoms attributed to Lyme disease found longer-term antibiotic therapy did not provide additional benefit or better outcomes compared to shorter-term antibiotics.

There are therapeutic modalities not recommended for the treatment of Lyme disease.

Therapeutic modalities **not recommended** for treatment of patients with any manifestation of Lyme disease include combinations of antimicrobials, long-term antibiotic therapy, hyperbaric oxygen, fever therapy, intravenous immunoglobulin, ozone, cholestyramine, energy and radiation-based therapies, vitamins and nutritional managements, magnesium and bismuth injections, chelation and heavy metal therapies, and stem cell transplants. Lack of biological plausibility, lack of efficacy, absence of supporting data or the potential for harm underpin this advice.

Known Australian tick-borne diseases

There is official Australian guidance for the treatment of known Australian tick-borne diseases.

Treatment recommendations for known Australian tick-borne diseases are provided by Therapeutic Guidelines Ltd and, additionally for Q fever, by the CDNA Guidelines for Public Health Units. QTT, FISF, ASF, and Q fever are all treated with doxycycline.

For QTT, early initiation of doxycycline is considered critical, as a delay in appropriate antibiotic therapy is associated with increased likelihood of progression to severe disease and complications.

The Q fever CDNA guidelines specify that if Q fever is suspected clinically (in people with appropriate symptoms AND who are at high risk of contracting Q fever), empirical treatment should be commenced without waiting for laboratory tests.

1.4.5. Research Question 5: What current guidelines and approaches to investigation and ongoing syndromic management of symptoms associated with DSCATT have been found effective internationally?

Patients with MUS are at risk of potentially harmful additional testing and are often subjected to repeated diagnostic investigations, and unnecessary and costly referrals and interventions.

People experiencing debilitating symptoms attributed to ticks, without any definitive diagnosis, could be considered to fall within the definition of MUS. International evidence indicates patients with MUS are at risk of potentially harmful additional testing and are often subjected to repeated diagnostic investigations, and unnecessary and costly referrals and interventions. In managing MUS in general practice, balancing the iatrogenic risk of investigation with the therapeutic risk of missing something important, is a challenge for GPs.

An analysis of patient submissions to the Senate Inquiry noted that, in patients who identified as having Lyme disease or DSCATT, the non-specific symptoms, female preponderance and lack of confirmatory laboratory testing suggested patients were more likely to be experiencing a medically unexplained physical syndrome (MUPS) disorder (such as chronic fatigue syndrome (CFS)) than an active or latent infection. Additionally, they experience social and financial harms and are at risk of nosocomial harms and may also have sought alternative and potentially non-evidence-based diagnoses and treatments.

A 2017 review of MUS guidelines in Europe estimates that 3-11 per cent of patients visiting general practice repeatedly consult their GP for MUS. However, this finding might not be entirely applicable to Australia. MUS exist along a continuum ranging from self-limiting symptoms to recurrent and persistent symptoms through to symptom disorders.

Advice from the Royal Australian College of General Practitioners (RACGP) and a review of the international MUS guidelines summarising guidelines from the Netherlands, Denmark, UK and Germany is consistent: patients with MUS often feel stigmatised and not taken seriously.

To manage these concerns, all guidelines make the following recommendations:

- Highlighting the importance of paying attention to the doctor-patient relationship.
- Providing an individualised approach that recognises the patient's illness and taking the patient's symptoms seriously.
- Demonstrating empathy with consultations aiming to validate the patient's distress.
- Highlighting the importance of providing an explanation in the patient's language about the possible causes of their symptoms (patients benefit from an explanation that makes sense, removes blame from the patient, generates ideas on how to manage the symptoms. The 2011 UK guidance, published by the Royal College of General Practitioners in the UK, advises that GPs should be explicit about their thoughts, uncertainties, and expectations of referrals to specialist care).

- Caution that patients with persistent [MUS] suffer from their symptoms, are functionally impaired, and are at risk of potentially harmful additional testing and treatment.

The stepped care approach is recommended internationally to manage symptom severity in patients with MUS.

International and Australian guidelines on MUS recommend a stepped care approach to address three levels of symptom severity, which lack clear cut-off points. They also advise that it is important that one care provider, preferably the GP, keeps control and co-ordinates the care process.

The stepped care model of care is internationally recognised and familiar to and widely used by GPs in Australia in all aspects of patient care. Stepped care is an evidence-based, staged system comprising a hierarchy of interventions, from the least to the most intensive, matched to the individual's needs. Within a stepped care approach, an individual will be supported to transition up to higher intensity services or transition down to lower intensity services as their needs change.

In addition to being recommended as an approach for managing care for people with MUS, the stepped care service model has been shown in RCTs to be effective for the management of chronic pain, for the management of depression and anxiety, and in the assessment and management of anxiety and depression in adult cancer patients. Stepped care models are widely used in England, Scotland, US, New Zealand, and Australia.

2. RESEARCH QUESTION 1: WHAT IS THE CLINICAL EPIDEMIOLOGY OF DSCATT IN AUSTRALIA?

ALLEN+CLARKE CONSULTING

2.1. Overview and key findings

This section provides the findings of the literature reviewed to answer research question 1:

What is the clinical epidemiology of DSCATT in Australia?

Specifically, we have sought to answer, from peer-reviewed published literature:

- *What information is available on the prevalence, demographics and geographic distribution of patients experiencing DSCATT in Australia?*
- *What information is available on the symptoms and clinical signs associated with DSCATT as reported by Australian patients and treating physicians?*

2.1.1. Key findings

There are no peer-reviewed published epidemiological or clinical studies about patients experiencing DSCATT in Australia.

As such, there is no reliable evidence on the clinical epidemiology of DSCATT in Australia, including on the prevalence, demographics and geographic distribution of patients experiencing symptoms associated with DSCATT or on the symptomology and clinical signs associated with DSCATT, that can reliably inform an evidence-based Clinical Pathway. Given that DSCATT is not clearly defined, has no case definition, has no known cause(s) and is not an identified diagnosable disease, the lack of studies is to be expected.

Available information on the prevalence, demographics and geographic distribution of patients experiencing DSCATT in Australia.

Two Australian Government reports and a published peer-reviewed review of evidence undertaken to advise the Chief Medical Officer (CMO) of the Department of Health whether there is a Lyme-like illness (DSCATT) in Australia indicate there is no reliable data on the prevalence or incidence of DSCATT in Australia at this time. The Senate Inquiry and The House of Representatives Standing Committee on Health – Case study on tick-borne and Lyme-like diseases specifically investigated prevalence and incidence of DSCATT and concluded that as DSCATT is not clearly defined and not formally reported, available statistics on its incidence across Australia are limited and are difficult to determine. The review for the CMO of over 500 cases of Lyme-like illness identified in Australia and published in the scientific literature concluded that the unreliability of the published case reports in their diagnostic methods means the evidence for Australian Lyme-like cases (DSCATT) remains quite unsubstantial and unconvincing and the cause of the illnesses remain unknown.

For geographic location, patients and patient advocacy groups told the Senate Inquiry of the location they believe they acquired their symptoms that they attribute to DSCATT, with New South Wales, Queensland, Western Australia or Victoria being the more frequently reported states.

Available information on the symptoms and clinical signs associated with DSCATT as reported by Australian patients and treating physicians.

Patients described the symptoms they have experienced and attribute to DSCATT to the Senate Inquiry, DSCATT Patient Forum and DSCATT Think Tank, the latter held to inform the development of the DSCATT Clinical Pathway. An analysis of publicly available submissions to the Senate Inquiry from patients who identified as suffering from Lyme disease or DSCATT

found the most common symptoms were: fatigue (62.6 per cent); disordered thinking (51.9 per cent); sensory disturbance (46.1 per cent); arthralgia (45.6 per cent); headache (44.5 per cent); followed by myalgia; rash; mood disturbance; visual disturbance; dizziness; pain; fever; nausea; palpitations; insomnia; seizures; diarrhoea; tremor; and personality change. Patients reported having experienced symptoms for a median of 10 years. Similarly, multiple symptoms and signs being attributed to DSCATT were identified by stakeholders who attended the Think Tank in May 2019, with neurological symptoms (including brain fog, cognitive dysfunction, memory loss, fine motor impairment and reduced verbal fluency) and chronic fatigue being the most commonly identified symptoms and signs.

The available information on the symptomology and clinical signs associated with DSCATT, while of low quality, due to the information provided being self-reported or anecdotal can, however, provide some insights for medical professionals when patients present to primary care and question whether they may be experiencing the symptoms associated with DSCATT.

2.1.2. Literature reviewed

Australian Government reports	(House of Representatives Standing Committee on Health, 2016; Senate Community Affairs References Committee, 2016a, 2016b)
Australian Department of Health reports, reports to, and guidance	(Allen + Clarke, 2019; TMS Consulting Pty Ltd, 2018b)
(Inter)national authority and intergovernmental reports, evidence reviews, guidelines and guidance	
Guidelines and guidance (International and Australian) by clinical and professional bodies	
Systematic Reviews (with/without meta-analysis)	
Narrative literature reviews and reviews	(Chalada et al., 2016)
Randomised controlled trials	
Prospective cohort studies	
Observational studies	(Brown, 2018)
Other	

2.1.3. Quality of the evidence

The quality of available information on the prevalence, demographics and geographic distribution of DSCATT in Australia is overall low. Similarly, the quality of evidence on the symptoms and clinical signs associated with DSCATT is overall low and unreliable.

Two peer-reviewed, published articles were relevant to this research question. These were a review by Chalada et al. (2016), where the authors reviewed scientific reports of cases of Lyme-like illness in Australia, and an analysis of patient submissions made to the Senate

Inquiry by Brown (2018). The latter is an observational study, based on self-reported information and as such is necessarily classified as low-quality evidence.

Other available information of relevance to this research question was found in three Australian Government reports (i.e. the Senate Community Affairs References Committee Interim and Final Reports (2016a, 2016b) and House of Commons Standing Committee on Health (2016), and two reports prepared for the Department of Health (i.e. DSCATT Patient Forum (2018b) and DSCATT Think Tank (2019). The quality of these reports was generally assessed as 'high' overall using the AACODS[1] checklist.

However, the information and estimates pertaining to the prevalence, demographics, geographical distribution, symptoms and clinical signs associated with DSCATT in Australia within these reports was provided by patients, patient advocacy groups, researchers or Lyme-literate doctors and was self-reported, anecdotal or opinion. While the veracity of this information about prevalence, demographics, geographic distribution and symptomology associated with DSCATT is not being questioned in this literature review, the self-reported and anecdotal nature of the information means it is academically assessed as of low reliability and at high risk of bias. As such the available evidence does not meet the scientific quality required to underpin an evidence-based clinical pathway.

However, the information, particularly about symptomology, can still be useful to inform the DSCATT Clinical Pathway by providing insights for primary care medical professionals when patients present questioning whether they may be experiencing the symptoms associated with DSCATT. Indeed, Brown noted that while Senate submissions fall short of the standards required of a systematic survey of patients definitively to describe symptoms and epidemiology, the promotion of the inquiry by Australian Lyme disease advocacy groups, the large number of responses and the use of a standardised format by many respondents means that broadly useful conclusions may still be drawn (Brown, 2018).

[1] The AACODS checklist is a tool for assessing grey literature. The acronym stands for Authority, Accuracy, Coverage, Objectivity, Date, Significance.

2.2. What information is available on the prevalence, demographics and geographic distribution of DSCATT in Australia?

This section presents the findings from the available information on the prevalence, demographics and geographic distribution of DSCATT in Australia.

Two Australian Government reports, one review and one observational study reported information of relevance to the prevalence, demographics and geographic distribution of DSCATT in Australia.

2.2.1. Prevalence of DSCATT in Australia

There are no epidemiological studies on the prevalence of patients experiencing DSCATT in Australia.

As DSCATT is not clearly defined, is not formally reported, has no diagnostic criteria, or known cause or causes, and is not a diagnosable disease, the prevalence of DSCATT in Australia cannot be established at this time.

Patients, patient advocacy groups and their treating doctors have provided submissions to two Government inquiries that sought to establish the prevalence and incidence of DSCATT in Australia. The relevant reports of these inquiries were:

- The Senate Community Affairs References Committee Interim Report (Senate Community Affairs References Committee, 2016a); and
- The House of Representatives Standing Committee on Health – Case study on tick-borne and Lyme-like diseases (House of Representatives Standing Committee on Health, 2016).

Additionally, two peer-reviewed published papers of relevance to this research question were identified. These were:

- a review of evidence undertaken to advise the Chief Medical Officer of the Department of Health whether there is a Lyme-like illness in Australia (Chalada et al., 2016), and
- an analysis of submissions to the Australian Senate Inquiry to describe 'Australian Lyme disease' epidemiology and impact (Brown, 2018).

The terms of reference for the Senate Inquiry 'Growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australian patients' included calls for submissions regarding the prevalence of Lyme-like illness in Australia (Senate Community Affairs References Committee, 2016a, p. 4). While the committee received over 1100 submissions, the majority (not further defined in the interim report) of which were from or on behalf of Australians suffering from chronic debilitating symptoms, (Senate Community Affairs References Committee, 2016a) the number of submissions cannot be considered a reliable indication of the prevalence of DSCATT in Australia. The number of submissions does, however, provide an indication that several hundred Australians identified as suffering from Lyme disease or DSCATT.

The findings of the Senate Committee regarding prevalence included the following:

- As DSCATT is not clearly defined and not formally reported on, available statistics on its incidence across Australia are limited.
- While medical authorities had stated that without a clear and agreed definition the prevalence of DSCATT cannot be accurately estimated, patient advocacy groups

stated that DSCATT should be made a notifiable disease and that the CDNA decision not to add Lyme disease to the National Notifiable Diseases List should be reviewed in light of the increasing number of patients being diagnosed with the condition.

- The Lyme Disease Association of Australia (LDAA) had stated in its submission that based on data collected through online patient surveys, 1051 Australians had been diagnosed with DSCATT since 2012, with LDAA estimating these figures were the 'tip of the iceberg when it comes to the real incidence of Lyme-like illness in Australia' (LDAA, 2016, p. 13). However, in contrast, some state and territory governments had challenged the notion that there is an 'epidemic' in Australia, with the Western Australian Department of Health noting that the incidence of DSCATT is probably overstated and reflects frustration with the Australian health system (Senate Community Affairs References Committee, 2016a).

As in the Senate Inquiry interim report above, advice and opinions on the incidence of tick-borne or Lyme-like diseases in Australia by submitters to the 2016 House of Representatives Standing Committee on Health 'Case study on tick-borne and Lyme-like diseases' varied widely.

The House of Representatives Standing Committee on Health noted, from submissions, the incidence of tick-borne or Lyme-like diseases in Australia is difficult to determine (House of Representatives Standing Committee on Health, 2016). The House of Representatives Standing Committee on Health reported the following:

- The Department of Health had stated that tick-borne or Lyme-like disease had been previously assessed and was not added to the list of nationally notifiable diseases due to a lack of a good case definition and consensus about the cause, and that a case definition would need to be developed.
- The Karl McManus Foundation (KMF) were of the view that part of the difficulty of determining the incidence of tick-borne or Lyme-like illness was due to the non-specific symptoms and unreliable diagnostics of these diseases.
- The RACGP had indicated that it could not know how widespread tick-borne or Lyme-like illness is as no research had been undertaken into the disease in the Australian context.
- Dr Schloeffel was reported as having identified a 'tentative projection of 102,000 [people] in Australia with chronic borrelial infection'. Furthermore, Dr Schloeffel's view was 'we have no idea how many people may have symptoms that fits this category' and that he currently has '400 patients with borreliosis or related illnesses' (House of Representatives Standing Committee on Health, 2016, p. 142).

In 2018, Brown reviewed and analysed responses of all public, first-person published submissions (n=698) made to the Australian Senate Inquiry (Senate Community Affairs References Committee, 2016a, 2016b) to describe the epidemiology, symptoms and outcomes of published submissions from Australian people who identified as suffering from Lyme disease or Lyme-like illness (Brown, 2018). This number cannot be interpreted as an indication of prevalence of DSCATT in Australia as patients self-identified as having Lyme-like illness, self-selected to provide submissions, and the Senate Inquiry was promoted by Lyme disease advocacy groups. Additionally, Brown noted 'a bias towards more politically active, more literate and more severe symptoms is expected' (Brown, 2018, p. 425).

Chalada and colleagues (2016) undertook a review to assess the current situation of the 'controversial Lyme or Lyme-like illness reported by some to be present in Australia' in order

to advise the Australian Government Chief Medical Officer (Chalada et al., 2016, p. 43). Their literature review search to review the evidence on Lyme-like cases in Australia included only Academic Journals. They identified 10 papers published between 1982 and 2015 in which at least 525 human cases of Lyme-like illness have been mentioned in the scientific literature. The studies reviewed by Chalada et al. (2016) are outlined in the table below for completeness, noting that several of them are outside the timeframe for this literature review. While they have not been reviewed again for the purpose of this report, they are important to recognise.

Table 1: Studies reviewed by Chalada et al. (2016)

Date	Title	Bibliographic entry, from Chalada et al. (2016)
2015	Clinical determinants of Lyme Borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort.	P.J. Mayne, Clinical determinants of Lyme Borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort, Int. J. Gen. Med. 8 (2015) 15.
2014	Evidence for Ixodes holocyclus (Acarina: Ixodidae) as a vector for human Lyme Borreliosis infection in Australia.	P. Mayne, S. Song, R. Shao, J. Burke, Y. Wang, T. Roberts, Evidence for Ixodesholocyclus(Acarina: Ixodidae) as a vector for human Lyme Borreliosis infection in Australia, J. Insect Sci. 14 (2014) 271.
2013	Neuropsychiatric presentation of Lyme disease in Australia.	C. Maud, M. Berk, Neuropsychiatric presentation of Lyme disease in Australia, Aust.N. Z. J. Psychiatry 4 (2013) 397–398.
2012	Investigation of Borrelia burgdorferi genotypes in Australia obtained from erythema migrans tissue.	P.J. Mayne, Investigation of Borrelia burgdorferi genotypes in Australia obtained from erythema migrans tissue, Clin. Cosmet. Investig. Dermatol. 5 (2012) 69.
2011	Emerging incidence of Lyme Borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia.	P.J. Mayne, Emerging incidence of Lyme Borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia, Int. J. Gen. Med. 4 (2011) 845.
1998	Culture-positive Lyme Borreliosis.	B.J. Hudson, M. Stewart, V.A. Lennox, M. Fukunaga, M. Yabuki, H. Macorison, et al.,Culture-positive Lyme Borreliosis, Med. J. Aust. 168 (1998) 500–503.
1987	Lyme Borreliosis — A Case Report for Queensland.	N. Stallman, Lyme Borreliosis—A Case Report for Queensland, 21CDI, 1987 8–9.
1986	Lyme disease on the NSW central coast.	R. Lawrence, R. Bradbury, J. Cullen, Lyme disease on the NSW central coast, Med. J.Aust. 145 (1986) 364.
1986	Lyme disease on the NSW south coast.	I. McCrossin, Lyme disease on the NSW south coast, Med. J. Aust. 144 (1986) 724.
1982	Lyme arthritis in the Hunter Valley.	A. Stewart, J. Glass, A. Patel, G. Watt, A. Cripps, R. Clancy, Lyme arthritis in the Hunter Valley, Med. J. Aust. 1 (1982) 139.

Of the literature they reviewed, and of relevance to the question on prevalence of DSCATT in Australia, Chalada et al. (2016) made the following two statements:

> *In the last twenty-five years there have been over 500 reports of an Australian Lyme-like syndrome in the scientific literature. However, the diagnoses of Lyme Borreliosis made in these cases have been primarily by clinical presentation and laboratory findings of tentative reliability and the true cause of these illnesses is unknown (Chalada et al., 2016, p. 42).*
>
> and
>
> *Unreliability of the published case reports in their diagnostic methods means the evidence for Australian Lyme-like cases remains quite unsubstantial and unconvincing (Chalada et al., 2016, p. 48)*.

The findings of Chalada et al. (2016) regarding Lyme disease and Lyme-like illness in Australia are further discussed in 3.5.7 Situation in Australia in considering a differential diagnosis of Lyme disease.

2.2.2. Demographics of DSCATT in Australia

There are no epidemiological studies on the demographics of patients experiencing DSCATT in Australia. Investigating the age and sex of Australians who identified as having Lyme-like illness was not in the terms of reference of the Senate Inquiry 'Growing evidence of an emerging tick-borne disease that causes Lyme-like illness for many Australian patients and was therefore not reported upon in their interim (Senate Community Affairs References Committee, 2016a) or final (Senate Community Affairs References Committee, 2016b) reports.

Age

The available information on the age of patients experiencing DSCATT is very limited and unreliable. Brown's analysis of the 698 published submissions made to the Senate Inquiry (Senate Community Affairs References Committee, 2016a, 2016b) from patients who attributed their symptoms to DSCATT, revealed a minority provided their age and sex with less than half of patients (259, 37.2 percent) reporting data about their age (Brown, 2018). Of those who did, ages ranged from 15 to 84 years with the median age of patients being 44 years (Brown, 2018).

Chalada et al. (2016), in their review, noted that the largest study of Lyme-like illness in Australia by Mayne in 2015, which examined the clinical presentation of Lyme borreliosis, babesiosis, bartonellosis, anaplasmosis and ehrlichiosis from 500 patient records across all states in Australia over the course of five years, reported the average age of onset was in the mid-thirties and average age at presentation was 41 years. However, as Chalada et al. concluded the cause of the illness in the cases in that study were unknown, the findings cannot be reliably attributed to DSCATT.

Sex

Similarly, the availability of information on the sex of patients experiencing the symptoms associated with DSCATT is limited and unreliable. Brown's analysis found the majority of submissions to the Senate Inquiry (Senate Community Affairs References Committee, 2016a, 2016b) were from females (Brown, 2018). Just over half of all submissions (381, 54.6 percent) were from females, 13.3 percent (93) were from males and about one third (32.1 percent) were from patients who did not disclose their sex. Where data on sex was reported,

most (381, 80.4 percent) submissions were from females, with only 19.6 percent (n=93) being from males (Brown, 2018, p. 423).

Regarding the high proportion of Australian females represented in the submissions to the Senate Inquiry, Brown (2018) cited evidence published in 2013 and 2015, that indicated Lyme disease has a slight male preponderance in endemic areas, with Brown noting this was most likely attributed to males being more likely to engage in at risk occupations or hobbies (Nelson et al. (2015), and Strle et al. (2013) in Brown, 2018, p. 424). Brown also noted that Lyme advocacy groups requested sufferers make submissions and provided standardised templates. This may have impacted on the sex distribution of submissions from patients identifying as suffering from Lyme disease or Lyme-like illness, but it is impossible to determine if this was the case.

Of the 2015 paper by Mayne described above, Chalada et al. (2016) noted the majority of patients were female (62 percent) (Mayne 2015 in Chalada et al., 2016). However, as they concluded that as the cause of the illness in the cases in this study were unknown, the findings of this study cannot be reliably attributed to DSCATT (Chalada et al., 2016).

2.2.3. Geographic distribution of DSCATT in Australia

There are no epidemiological studies on the geographic distribution of patients experiencing DSCATT in Australia.

Geographic distribution of Lyme-like illness in Australia was included in the terms of reference for the Senate Inquiry (Senate Community Affairs References Committee, 2016a). Patients and patient advocacy groups told the Senate Committee (Senate Community Affairs References Committee, 2016a, 2016b) the location they believed they or members of their organisations had acquired DSCATT. The majority of submitters stated that they acquired their illness in Australia and the majority of submissions from patients who were experiencing chronic debilitating symptoms came from New South Wales, Queensland, Victoria and Western Australia (Senate Community Affairs References Committee, 2016a).

In Brown's analysis of patient submissions made to the the Senate Inquiry, New South Wales, Queensland, Western Australian and Victoria had the highest reported prevalence for acquisition of DSCATT at 38.3 per cent, 22.2 per cent, 15.9 per cent and 11.8 per cent respectively, while only 9.5 per cent of patients reported they had acquired their Lyme disease or DSCATT overseas (Brown, 2018). Two patients (0.3 per cent) reported they had acquired their Lyme disease or DSCATT congenitally (Brown, 2018).

Chalada and colleagues (2016), in their review of the literature on Lyme-like cases reported in Australia, described earlier, provided a map of locations of Lyme-like cases reported in the scientific literature. Figure 1 is reproduced from Chalada et al. (2016). The authors noted that only the Lyme-like cases with specified locations were included in the diagram. The majority of cases were in New South Wales.

Figure 1: Locations of Australian Lyme-like cases published in the scientific literature

- Specific location based on town, suburb or GPS coordinates.

■ Approximate location based on broad location description, e.g. 'rural Victoria' or 'Hunter Valley'

Source: Figure 1 Page 145 Chalada et al. 2016

While Chalada et al. (2016) identified that the majority of cases of Lyme-like illness reported in the literature were from New South Wales, their overarching conclusion was that the unreliability of the published case reports in their diagnostic methods meant the evidence for Australian Lyme-like cases remains quite unsubstantial and unconvincing (Chalada et al., 2016). This means the geographical location reported in these studies is unreliable evidence regarding the geographical distribution of DSCATT in Australia.

2.3. What information is available on the symptoms and clinical signs associated with DSCATT as reported by Australian patients and treating physicians?

This section reports on findings from available information on the symptoms and clinical signs associated with DSCATT as reported by Australian patients and treating physicians.

There are no clinical studies in the published peer-reviewed literature on the signs and clinical symptoms associated with DSCATT. As such, there is no reliable evidence on the signs and clinical symptoms associated with DSCATT to inform the development of the DSCATT Clinical Pathway.

The only available information on the symptoms and clinical signs associated with DSCATT as reported by Australian patients and treating physicians comes from submissions made to the Senate Inquiry (Senate Community Affairs References Committee, 2016a, 2016b), the DSCATT Patient Forum (TMS Consulting Pty Ltd, 2018b), the Think Tank (Allen + Clarke, 2019) and from an analysis by Brown (2018) of publicly available submissions to the Senate Inquiry from patients who identified as suffering from Lyme disease or DSCATT.

2.3.1. Review of available information

Patients and treating clinicians have told of the symptoms and clinical signs they have experienced, seen and attribute to DSCATT to the Senate Committee (Senate Community Affairs References Committee, 2016a, 2016b), DSCATT Patient Forum (TMS Consulting Pty Ltd, 2018b) and Think Tank (Allen + Clarke, 2019).

Some [not further defined] submitters told the Senate Inquiry that they became ill immediately following a tick bite in Australia, with symptoms described by these submitters including a rash around the bite and a range of symptoms including fatigue, arthritis and chronic pain (Senate Community Affairs References Committee, 2016a).

Brown's analysis of the 698 published submissions made to the Senate Inquiry from patients who self-identified as having Lyme disease or DSCATT in Australia revealed 656 patients reported having at least one symptom (Brown, 2018). Of those patients who reported at least one symptom, Brown identified nineteen symptoms**,** as described by the patients. The most common symptoms described by patients experiencing symptoms they attribute to Lyme disease or DSCATT to the Senate Forum were: fatigue (62.6 per cent); disordered thinking (51.9 per cent); sensory disturbance (46.1 per cent); arthralgia (45.6 per cent;) headache (44.5 per cent); followed by myalgia; rash; mood disturbance; visual disturbance; dizziness; pain; fever; nausea; palpitations; insomnia; seizures; diarrhoea; tremor; and personality change (Brown, 2018).

Patients reported having experienced symptoms for a median of 10 years and having seen a median of 13 doctors for diagnosis and treatment of their illness (Brown, 2018).

Similarly, multiple symptoms and signs being attributed to DSCATT were identified by stakeholders who attended the Think Tank in May 2019, with neurological symptoms (including brain fog, cognitive dysfunction, memory loss, fine motor impairment and reduced verbal fluency) and chronic fatigue being the most commonly identified symptoms and signs (Allen + Clarke, 2019).

In their literature review, Chalada et al. (2016) reported the symptoms described, where available, in the peer-reviewed studies regarding the evidence on Lyme-like cases in Australia.

As can be seen in the table below, the range of symptoms are diverse. For several of the studies where specific symptoms were reported, the EM rash is common, alongside headache, arthralgias and myalgias, lethargy and malaise, while two patients only had the EM rash and no systemic illness. From the studies where the symptoms are described more generically as 'Lyme-like presentation', it is not possible to comment further on individual symptoms.

As noted previously, Chalada et al. (2016) concluded that the unreliability of the published case reports in their diagnostic methods meant the evidence for Australian Lyme-like cases (DSCATT) remains quite unsubstantial and unconvincing. As such, the symptoms described in the studies as presented below cannot be reliably attributed to DSCATT.

Table 2: Geographic distribution of Australian Lyme-like cases from peer-reviewed scientific literature

Location	Travel history	Symptoms	Bibliographic entry, from Chalada et al. (2016)
Lower Hunter Valley, NSW	No data	Insect bite followed by EM with secondary lesions, relapsing arthritis with swelling and pain in the knee and left hip, behavioural change, headaches, memory loss, urinary retention, tachycardia	A. Stewart, J. Glass, A. Patel, G. Watt, A. Cripps, R. Clancy, Lyme arthritis in the Hunter Valley, Med. J. Aust. 1 (1982) 139.
Guerilla Bay near Moruya, NSW	No data	Insect bite followed by EM. Weeks after treatment, EM recurred.	I. McCrossin, Lyme disease on the NSW south coast, Med. J. Aust. 144 (1986) 724.
North Bendalong (between Nowra and Ulladulla), NSW	No data	One-month EM, lassitude, polyarthralgia, headaches	I. McCrossin, Lyme disease on the NSW south coast, Med. J. Aust. 144 (1986) 724.
Gorokan, NSW	No data	3 weeks of increasing lethargy, malaise, intermittent fevers, multiple EM, severe occipital headache, sore throat	R. Lawrence, R. Bradbury, J. Cullen, Lyme disease on the NSW central coast, Med. J.Aust. 145 (1986) 364.
Pittwater Shire, Sydney, NSW	17 months prior to tick bite, visited 3 countries in Europe known to be endemic for Lyme. Did not recall any tick bites or exposure to ticks. EM appeared at the site of the Australian tick bite.	EM at tick bite. Mild headache, malaise, and low-grade fever, non-pruritic rash, insomnia, generalised arthralgias, myalgias, insomnia, difficulty with memory and 'thinking clearly', secondary EM lesions. Duration > 18 months.	B.J. Hudson, M. Stewart, V.A. Lennox, M. Fukunaga, M. Yabuki, H. Macorison, et al.,Culture-positive Lyme Borreliosis, Med. J. Aust. 168 (1998) 500–503.
152.8E,31.66S	Yes	EM, no systemic illness	P.J. Mayne, Investigation of Borrelia burgdorferi genotypes in Australia obtained from erythema migrans tissue, Clin. Cosmet. Investig. Dermatol. 5 (2012) 69.

Location	Travel history	Symptoms	Bibliographic entry, from Chalada et al. (2016)
152.7E 31.73S	Never left Australia	EM, systemic illness	P.J. Mayne, Investigation of Borrelia burgdorferi genotypes in Australia obtained from erythema migrans tissue, Clin. Cosmet. Investig. Dermatol. 5 (2012) 69.
151.3E, 33.74S	Yes	EM, fever, menigism, severe headache worse with coughing and shaking of head, photophobia and retro-orbital pain	P.J. Mayne, Investigation of Borrelia burgdorferi genotypes in Australia obtained from erythema migrans tissue, Clin. Cosmet. Investig. Dermatol. 5 (2012) 69.
152.8E, 31.32S	Never left Australia	EM, no systemic illness	P.J. Mayne, Investigation of Borrelia burgdorferi genotypes in Australia obtained from erythema migrans tissue, Clin. Cosmet. Investig. Dermatol. 5 (2012) 69.
Rural Victoria	No data	Fever, regular presumed viral illness, chronic fatigue syndrome, severe arthritis in hands, auditory hypercussis, poor concentration, irritability and emotional lability, episodic sleep disturbances, two episodes of severe generalised body pain without cause, one episode of auditory hallucinations and paranoid ideas. Duration: 8 years.	C. Maud, M. Berk, Neuropsychiatric presentation of Lyme disease in Australia, Aust.N. Z. J. Psychiatry 4 (2013) 397–398.
Mid-north coast of NSW	Travelled from Byron Bay NSW to Eastlakes Victoria. No overseas travel.	Lyme-like presentation	P.J. Mayne, Emerging incidence of Lyme Borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia, Int. J. Gen. Med. 4 (2011) 845.
QLD	Travelled to northern NSW and Sydney, NSW; Melbourne, Victoria; Hobart Tasmania. No overseas travel.	Lyme-like presentation	P.J. Mayne, Emerging incidence of Lyme Borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia, Int. J. Gen. Med. 4 (2011) 845.
Armstrong beach, QLD	Karratha, WA. No travel.	Lyme-like presentation	P.J. Mayne, Emerging incidence of Lyme Borreliosis, babesiosis, bartonellosis, and granulocytic

Location	Travel history	Symptoms	Bibliographic entry, from Chalada et al. (2016)
			ehrlichiosis in Australia, Int. J. Gen. Med. 4 (2011) 845.
NSW	Victoria, Queensland, South Australia. No overseas travel.	Lyme-like presentation	P.J. Mayne, Emerging incidence of Lyme Borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia, Int. J. Gen. Med. 4 (2011) 845.

Source: Table 1 page 49 Chalada et al. 2016

3. RESEARCH QUESTION 2: WHAT INFORMATION IS AVAILABLE ON DISEASES OR DISORDERS PATIENTS EXPERIENCING DSCATT SYMPTOMS HAVE BEEN DIAGNOSED WITH AND WHAT ARE THE MOST LIKELY DIFFERENTIAL DIAGNOSES?

3.1. Overview and key findings

This section provides the findings of the literature reviewed to answer research question 2:

What information is available on diseases or disorders Australian patients experiencing DSCATT symptoms have been diagnosed with? and What are the most likely differential diagnoses?

3.1.1. Key findings

There are no peer-reviewed published epidemiological or clinical studies about patients experiencing DSCATT that include the investigation of the diseases and disorders they have been diagnosed with.

As such there is no reliable evidence on the diseases or disorders patients experiencing DSCATT have been diagnosed with that can reliably inform an evidence-based Clinical Pathway. Again, given that DSCATT is not clearly defined, has no case definition, has no known cause(s) and is not an identified diagnosable disease, the lack of studies is to be expected at this point in time.

The available information on the diseases and disorders experienced by patients with symptoms associated with DSCATT is based on self-reported or anecdotal information provided by patients, their treating doctors or patient advocacy groups. Patients and patient advocacy groups described the diseases and disorders they or their members have been diagnosed with to the Senate Inquiry, the DSCATT Patient Forum and the DSCATT Think Tank. In some cases patients reported having been diagnosed with other known tick-borne infections such as Q fever, Spotted Fever, Rickettsia, QTT, or allergy to tick toxin and received treatment, or been diagnosed with non-specific conditions including chronic fatigue syndrome, fibromyalgia, Epstein-Barr virus or a mental health condition such as depression. An analysis of submissions to the Senate Inquiry by patients who identified as having Lyme disease or DSCATT found one in ten patients reported being given another diagnosis that could explain their physical symptoms including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and motor neurone disease. In over half of the submissions analysed, patients reported having been diagnosed with co-infections [not further defined]. Almost 10 per cent of submitters self-diagnosed after being exposed to a media report of Australian Lyme disease.

The potential to misdiagnose potentially treatable illness as Lyme disease was a major cause of concern raised in the Senate Inquiry reports.

The available information, while of low quality, due to the information provided being self-reported or anecdotal can, however, provide some insights for medical professionals when patients present to primary care and question whether they may be experiencing the symptoms associated with DSCATT.

Acknowledging the attribution to ticks in the term DSCATT, the likely differential diagnoses considered include overseas-acquired Lyme disease, known Australian tick-borne diseases (QTT, FISF, ASF and Q fever), other diagnoses (infectious and non-infectious) and MUS.

There is robust evidence on Lyme disease, known Australian tick-borne diseases and MUS to inform a differential diagnosis in patients who present with either new onset or unresolved debilitating symptoms with or without a history of tick bites and that cannot be attributed to

another condition (acute or chronic). The evidence meets the scientific quality to underpin an evidence-based clinical pathway.

Overseas-acquired Lyme disease

In Australia, Lyme disease should be considered in the differential diagnosis for patients presenting with a travel history to Lyme disease endemic areas along with supporting symptoms and/or a known tick bite. Lyme disease is endemic in parts of the US, Europe and Asia. A person visiting a Lyme disease endemic area may become infected with *Borrelia burgdorferi* sensu lato through a tick bite and subsequently develop Lyme disease. Overseas travellers to Lyme disease endemic areas may return to their home country before becoming symptomatic and/or being diagnosed. Overseas-acquired Lyme disease is not a notifiable disease in Australia.

The difficulty in diagnosing Lyme disease, even in Lyme disease endemic areas, was highlighted in a systematic review for the Department of Health UK. The systematic review reported that clinicians find it challenging to diagnose Lyme disease accurately due to the wide variation in symptoms; the infrequency with which they see the disease in practice; their level of confidence about being able to diagnose correctly; the ambiguity they experience about diagnostic tools; and their beliefs and behaviour relating to atypical or recurring symptoms. International authority guidance highlights caution about diagnosing Lyme disease without a supportive history or positive serological testing because of the risk of missing an alternative diagnosis and providing inappropriate treatment.

In a country such as Australia, where Lyme disease is not endemic and is not commonly seen in clinical practice, there are additional challenges in diagnosing Lyme disease. Australian guidance stress that, due to the non-specific nature of many clinical signs and symptoms, the diagnosis of Lyme disease in non-endemic Australia cannot reliably be made on clinical signs and symptoms alone, as many other infectious and non-infectious diseases can have similar features to Lyme disease. Laboratory testing is essential.

The inclusion of a travel history as part of a clinical history is important, as despite multiple studies that have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australian ticks nor any other vector that could transmit the disease to humans. It is for this reason that the Australian medical profession does not support the diagnosis of locally acquired Lyme disease in Australia. While some Australians and healthcare providers believe that a form of 'chronic Lyme disease' exists, globally, 'chronic Lyme disease' is a disputed diagnosis which lacks sufficient supporting evidence.

Known Australian tick-borne diseases

Australian tick-borne diseases should be considered in a differential diagnosis in patients who have not travelled overseas to Lyme disease endemic areas AND who have or may have been bitten by a tick (recently or in the past) or who engage in bushwalking AND present with relevant acute or chronic symptoms.

Apart from the occasional local bacterial infection at the tick bite site (eschar), the only two systemic infections that are definitely known to be transmitted by tick bites in Australia are Rickettsial infections from infection with:

- *Rickettsia* spp. (QTT, FISF and ASF) and
- Q fever (*Coxiella burnetii*).

The signs and symptoms of Rickettsial infections in Australia include eschar, fatigue, fever, headache, myalgia and rash (macular, papular, vesicular) although the severity and duration of Rickettsial diseases vary considerably. QTT and ASF have similar core clinical manifestations with a range of other symptoms observed. Early clinical features are often non-specific, making diagnosis challenging. Additionally, symptoms may overlap with other infectious diseases including those that are transmitted by non-tick vectors as well as a number of chronic diseases.

QTT is an emerging public health threat and increasingly recognised as an important cause of community-acquired febrile illness in Eastern Australia. In Australia, Q fever *(Coxiella burnetii)* is a nationally notifiable disease, has a Q fever laboratory case definition and CDNA National Guidelines for Public Health Units. While a disease attributed to ticks, the majority of cases of Q fever occur through inhalation of infected aerosols and dust, contaminated with birth fluids, faeces and urine from infected reservoir animals such as goats, sheep, cattle, kangaroos and domestic pets.

Alternative diagnoses

In patients who present with new onset (e.g. fever, rash) or persistent debilitating symptoms and tick-borne disease is not suspected, the following differential diagnosis should be considered: infectious; autoimmune; neoplastic; psychological; inflammatory; vascular; neurological; cardio-respiratory; life-style related.

Medically unexplained symptoms

In patients for whom a diagnosis cannot be established, MUS may be considered. MUS are defined as physical symptoms persisting for more than several weeks and for which adequate medical examination has not revealed a condition that adequately explains the symptoms.

An analysis of patient submissions to the Senate Inquiry noted the unquestionable, real, and debilitating physical and social harm from illness reported in the submissions. The author's conclusion suggested that patients who identified as having DSCATT displayed a symptomology similar to MUS syndrome.

3.1.2. Literature reviewed

Australian Government reports	(House of Representatives Standing Committee on Health, 2016; Senate Community Affairs References Committee, 2016a, 2016b)
Australian Department of Health reports, reports to, and guidance	(Allen + Clarke, 2019; Communicable Diseases Network Australia, 2018; Department of Health, 2018b, 2018a; Lum et al., 2015; Mackenzie, 2013; TMS Consulting Pty Ltd, 2018a, 2018b)
(Inter)national authority and intergovernmental reports, evidence reviews, guidelines and guidance	(Brunton et al., 2017; Marzec et al., 2017; National Institute for Health and Care Excellence, 2018i, 2018h, 2018j; Public Health England, 2018)

Guidelines (International and Australian) by clinical and professional bodies	(Lantos et al., 2010, 2019; Murtagh, 2003; National Institute for Health and Care Excellence, 2018j; Royal Australasian College of Physicians, 2002; Royal College of General Practitioners, 2020; Royal College of Pathologists of Australasia, 2019; Royal College of Psychiatrists, 2017; Wormser et al., 2006)
Systematic Reviews (with/without meta-analysis)	(Lantos & Wormser, 2014; Waddell et al., 2016)
Narrative literature reviews and reviews	(Auwaerter et al., 2011; Banks & Hughes, 2012; Beaman, 2016; Borchers et al., 2015; Chalada et al., 2016; Collignon et al., 2016; Dehhaghi et al., 2019; Eastwood et al., 2018; Graves & Stenos, 2017; Lindsay et al., 2014; Olde Hartman et al., 2017; Stewart et al., 2017)
Randomised controlled trials	
Prospective cohort studies	(Nigrovic et al., 2019)
Observational studies	(Brown, 2018; Graves et al., 2016; Kobayashi et al., 2019; Lantos, Branda, et al., 2015)
Australian case reports	(Doolan et al., 2019; Senanayake et al., 2012; Subedi et al., 2015; Thomas & Wu, 2018)
Australian animal studies	(Dawood et al., 2013; Gofton, Doggett, et al., 2015; Gofton et al., 2018; Gofton, Oskam, et al., 2015; Greay et al., 2016; Harvey et al., 2019; Irwin et al., 2017; Kwak, 2018; Loh et al., 2016, 2017)
Other Australian resources and guidance	(Graves, n.d.)

3.1.3. Quality of the evidence

The quality of the evidence on the diseases and disorders with which patients experiencing DSCATT symptoms have been diagnosed in Australia is overall very low, for the same reasons identified in research question 1.

As for research question 1, published peer-reviewed evidence comes from the review by Chalada et al. (2016), and the analysis of patient submissions to the Senate Inquiry by Brown (2018), which is based on self-reported information and is necessarily classified as low quality evidence. Again, while the quality of the Senate Inquiry Interim Report (2016a), House of Representatives Standing Committee on Health (2016), DSCATT Forum (TMS Consulting Pty Ltd, 2018a) and DSCATT Patient Forum (TMS Consulting Pty Ltd, 2018b) were generally assessed as 'high' overall using the AACODS tool, the information pertaining to the diseases and disorders patients experiencing DSCATT have been diagnosed within these reports was provided by patients, patient advocacy groups, researchers and treating doctors and was self-reported, anecdotal or opinion.

While the veracity of this information about the diseases and disorders patients experiencing DSCATT have been diagnosed with is not being questioned in this literature review, the self-reported and anecdotal nature of the information means it is academically assessed as of low reliability and at high risk of bias. As such, the available evidence does not meet the scientific quality required to underpin an evidence-based clinical pathway.

The evidence base used in this literature review to support the likely differential diagnoses is robust and meets the scientific quality to underpin an evidence-based clinical pathway. For Lyme disease, the evidence base is extensive and robust. The majority of the advice and recommendations when considering Lyme disease as a diagnosis in returning travellers came from the Australian Government Department of Health, international authorities, and guidelines from international and Australian professional clinical associations. We drew heavily on two recent guidelines on Lyme disease, both of which were underpinned by evidence-based reviews of the literature. The evidence about whether Lyme disease exists in Australia included reviews and Australian animal research studies by acknowledged experts and researchers in their fields. For known Australian tick-borne diseases, the evidence is more limited but robust and included high-level Australian national guidance from CDNA, professional clinical authority guidance and reviews. For MUS, evidence is more limited but robust and included international guidelines and Australian Government entity and medical professional association guidance.

3.2. Diseases or disorders patients experiencing DSCATT symptoms have been diagnosed with

There are no peer-reviewed and published epidemiological or clinical studies about patients experiencing DSCATT.

The only available information on the diseases and disorders patients experiencing DSCATT symptoms have been diagnosed with has been provided by patients and their treating medical professionals. While the veracity of information provided by patients and their treating doctors is not questioned in this literature review, the self-reported and anecdotal nature of the information means it is of low reliability and at high risk of bias and therefore does not meet the scientific quality required to underpin an evidence-based clinical pathway.

3.2.1. Review of available information

Patients and patient advocacy groups described diseases or disorders they or their members have been diagnosed with to the Senate Inquiry (Senate Community Affairs References Committee, 2016a, 2016b), DSCATT Patient Forum (TMS Consulting Pty Ltd, 2018b) and Think Tank (Allen + Clarke, 2019).

The majority of submitters to the Senate Inquiry stated they had acquired their illness in Australia, with many submitters stating they had had no history of travel to an endemic area for classical Lyme disease (Senate Community Affairs References Committee, 2016a).

Among submitters who had become ill following a tick bite, the Senate Inquiry noted that:

- in some cases, submitters were diagnosed with other known tick-borne infections, such as Q fever, Spotted Fever, Rickettsia, **QTT,** or allergy to tick toxin, and received treatment; and
- in most cases, submitters stated that medical practitioners were not able to identify or diagnose the illness or offer any effective treatment (Senate Community Affairs References Committee, 2016a).

In some cases submitters stated they received a diagnosis for a range of non-specific conditions including chronic fatigue syndrome, fibromyalgia, Epstein-Barr virus or a mental health condition such as depression, while in other cases submitters stated they were referred to multiple specialists and other practitioners who were not able to determine the cause of their illness (Senate Community Affairs References Committee, 2016a).

The largest group of submitters were reported to be those who had experienced a long-term chronic illness. In many cases, the submitters could not recall being bitten by a tick; where submitters could recall a tick bite, according to the Interim Report, this may have predated the onset of their symptoms by a number of years (Senate Community Affairs References Committee, 2016a). However, the LDAA told the Senate Inquiry that most patients are suffering from 'chronic' stage symptoms of Lyme disease (Senate Community Affairs References Committee, 2016a).

Additionally, the Senate Inquiry heard from many submitters who reported they had acquired their illness in Australia, that when their blood samples had been sent to an accredited Australian laboratory to test for *Borrelia* bacteria, the results had come back negative, but when these same submitters consulted a 'Lyme literate' practitioner, and their samples had been sent to either a non-accredited laboratory in Australia or laboratories in the US or Germany, on the recommendation of such practitioners, tests results from these laboratories had returned a positive result for *Borrelia* often with a number of coinfections

such as *Bartonella* and *Babesia.* The 'Lyme literate' practitioners subsequently used these test results to confirm their clinical diagnosis. The Senate Inquiry also heard that patient advocacy groups use the term 'Lyme-like illness' (DSCATT) to describe the diagnosis by 'Lyme-literate' practitioners of a range of infections that include *Borrelia* and coinfections such as *Babesia, Bartonella, Ehrlichia, Anaplasma,* and *Mycoplasma pneumoniae*, with the LDAA stating that they use the terms 'Lyme disease', 'Lyme-like illness' or simple 'Lyme' interchangeably to describe this diagnosis (Senate Community Affairs References Committee, 2016a).

The potential to misdiagnose potentially treatable illness while diagnosing Australian patients with debilitating symptom complexes as having Lyme disease was a major cause of concern raised in the Senate Inquiry reports (Senate Community Affairs References Committee, 2016a, 2016b), including by the Medical Board of Australia (MBA), Australian Health Practitioner Regulation Agency (AHPRA) and Medical Council of New South Wales. Concerns raised included the use of unconventional diagnostic techniques such as kinesiology to diagnose DSCATT, the reliance on non-accredited laboratories to diagnose Lyme disease, not referring patients with complex diagnoses to specialists where this would have been appropriate, not managing other co-existing medical conditions, and misdiagnosing cancers as DSCATT.

Brown's (2018) analysis of 698 first person submissions to the Senate Inquiry from Australian people who identified as suffering from Lyme disease or Lyme-like illness found respondents had seen a median of 13 (range 1-100) doctors for diagnosis and treatment of their illness, 41.7 per cent (n= 291) had seen a 'Lyme literate' doctor and 17.2 per cent reported have been treated overseas.

In his analysis of submissions by patients to the Senate Inquiry, Brown (2018) also reported on diagnosis, including the diagnostic testing laboratory, and other methods of diagnosis. The majority (58.8 per cent) of submissions did not comment on a tick bite, but where submitters did comment, the majority (257, 89.5 per cent) reported a positive history. Nearly one in ten patient submissions (68, 9.8 per cent) reported having self-diagnosed with Lyme disease after media reports, with a similar proportion (67, 9.8 per cent) reported having self-diagnosed with Lyme disease by research or on the internet. Two submissions (0.3 per cent) reported Lyme disease was acquired congenitally.

Regarding the diagnostic testing laboratory that had supported submitters diagnoses, Brown (2018) reported that of the 137 submissions that disclosed a NATA/RCPA-accredited diagnostic pathology test, only 14 (10.2 per cent) reported positive serology, which represented 2.8 per cent of all submissions that reported pathology and 2.0 per cent of all submissions. Of the 14 who reported positive serology, ten patients had travelled overseas while the four other patients who had either not travelled overseas or did not mention travel did not report the result of confirmatory (Western blot) serological testing. Additionally, two patients reported they had contracted Lyme disease overseas (US and France) and another two patients who reported travel also reported explicitly that only first-tier testing was positive. Brown commented only a small proportion of patients reported a positive Lyme disease serology test from a NATA/RCPA accredited laboratory and that a proportion of these may be positives from overseas exposure unrelated to their current illness.

Table 3: Diagnostic information reported in submissions

Diagnostic method	Number (per cent) of all patients	Number (per cent) of patients who reported data
Diagnostic laboratory testing		
Any	508 (72.8 per cent)	508 (100 per cent)
Pos NATA/RCPA	14 (2.0 per cent)	14 (2.8 per cent)
Neg NATA/RCPA	123 (17.6 per cent)	123 (24.2 per cent)
Pos non-NATA/RCPA	454 (65.0 per cent)	454 (89.4 per cent)
Neg non-NATA/RCPA	27 (3.9 per cent)	27 (5.3 per cent)
Neg NATA/RCPA, Pos non-NATA/RCPA	83 (11.9 per cent)	83 (16.4 per cent)

Source: Brown, 2018

Of these 698 submitters who self-identified to the Senate Inquiry as having Lyme disease or DSCATT in Australia, one in ten (73, 10.5 per cent) reported being given another diagnosis that could explain their physical symptoms (Brown, 2018). The diagnoses included: multiple sclerosis (23 patients); rheumatoid arthritis (19 patients); systemic lupus erythematosus (10 patients); Crohn's disease (seven patients); motor neurone disease (four patients); 'Other' (14 patients). Four patients reported more than one diagnosis.

Additionally, of these 698 submitters who self-identified as having Lyme disease or DSCATT in Australia, just over half (357; 51.1 per cent) stated to the Senate Inquiry that they had been diagnosed with coinfections [not further defined].

The House of Representatives Standing Committee on Health also reported on coinfections in the case study on tick-borne or Lyme-like diseases, noting the LDAA had stated 55 per cent of patients with tick-borne or Lyme-like diseases reported being diagnosed with at least one coinfection (and that this was a much higher rate than in the US). The report also noted Dr Schloeffel had submitted evidence to this inquiry and had listed 10 groups of coinfections associated with tick-borne or Lyme-like illness including relapsing fever, *Rickettsias,* and chronic viral infections including Human Immunodeficiency Virus (HIV) (House of Representatives Standing Committee on Health, 2016)).

Dr Schloeffel additionally told the DSCATT Forum (TMS Consulting Pty Ltd, 2018a) that infective organisms found in vector-borne disease patients included:

- *Borrelia* including relapsing fever,
- Rickettsias,
- *Bartonella,*
- Ehrlichiosis,
- Anaplasmosis,
- *Babesia*,
- *Coxiella burnetti,*
- *Mycoplasma*, and

- viruses.

In contrast to the evidence of Dr Schleoffel on infective organisms found in Australian patients with vector-borne disease, Professor Graves from Austin Health, University of Melbourne, reported, after extensive investigation of more than 50 patients with Lyme-like illness in the Austin Health ID Program, no evidence of babesiosis or rickettsiosis, based on laboratory evidence or failure to respond to medical therapy that is usually effective against these two diseases (TMS Consulting Pty Ltd, 2018a).

Amongst stakeholders who attended the Think Tank in May 2019 (Allen + Clarke, 2019), there was no consensus on the diseases and disorders most commonly experienced by adult patients, child patients and pregnant women, apart from those identified in the questions on signs and symptoms, including: cluster headaches; myocarditis; Lyme carditis; EM; Bell's palsy; encephalitis; multiple sclerosis; amyotrophic latera sclerosis (ALS); Lyme psychosis, osteomyelitis; atypical seronegative autoimmune disease; cherry angiomas; Borrelia Lymphocytoma; acrodermatitis chronica atrophicans (ACA); and autism.

3.3. What are the most likely differential diagnoses?

Acknowledging the attribution to ticks in the term 'DSCATT', and to consider likely differential diagnoses to inform the development of the Clinical Pathway, we reviewed the evidence on tick-borne diseases, specifically overseas-acquired Lyme disease and known Australian tick-borne diseases. Additionally, the symptom complexes to which the term 'DSCATT' has been given incorporates a wide range of non-specific symptoms. Some people may have a diagnosis that has not yet been identified that explains these symptoms while others may have a cluster of MUS that require management.

3.4. The importance of taking a detailed travel history and clinical history to support differential diagnosis

The inclusion of a travel history as part of the clinical history is important, as the organisms that cause Lyme disease have not yet been identified in Australia, but are endemic in parts of the US, Europe and Asia. Not all persons with Lyme disease recall having had a tick bite or notice a tick bite, thus a history of travel or exposure in a known endemic area for Lyme disease should be sought from possible cases (Royal College of Pathologists of Australasia, 2019). Lack of a tick bite history was found to not reliably exclude Lyme disease in a very recent prospective study involving children in the US (a Lyme disease endemic area); only a minority (18.5 per cent) of children diagnosed with Lyme disease had a recognised tick bite as recalled by the child or their parents within the year prior to the child's emergency department evaluation for Lyme disease (Nigrovic et al., 2019). If a person presents with symptoms that suggest the possibility of Lyme disease, NICE advises to explore how long the person has had the symptoms and their history of possible tick exposure, and to ask about activities that might have exposed them to ticks, and travel to areas where Lyme disease is known to be highly prevalent (National Institute for Health and Care Excellence, 2018j).

Similar to the guidance of NICE, the need for a detailed travel and clinical history prior to considering testing for Lyme disease was highlighted in a review on diagnostic testing for Lyme disease in Canada, a country where Lyme disease was reported to be on the rise due to the recent range expansion of the primary tick vector, *Ixodes scapularis* (Lindsay et al., 2014).

Lindsay et al. (2014) advised the following information is required prior to testing:

- a detailed travel history and date of onset of symptoms. This information should be included on the laboratory requisition, as it helps the diagnostic laboratory apply the most appropriate test platform. There are different tests to identify Lyme disease acquired in Europe/Asia versus North America and different tests are used for early infections versus infections that may have been present for some time.
- a history of antibiotic treatment. This can dampen the immune response to infection and may complicate the interpretation of serological tests.
- other infections and pre-existing conditions. Infection with other related pathogens (e.g. syphilis) and autoimmune disorders may cause false positive results.
- prior history of laboratory-confirmed Lyme disease. This is important as there is no pattern of serological response that can differentiate re-infection from an initial infection with *B. burgdorferi.*

Australian advice is concordant with international advice: epidemiological context is important. Determining a travel history and tick exposure prone activities are essential. The likelihood of Lyme disease increases as the probability of tick bite increases in a geographically endemic area (particularly wooded, brushy or grassy habitats). Endemic areas can be defined as those with established populations of vector ticks and evidence of enzootic transmission of relevant *Borrelia* species between the tick and resident animal population (Lum et al., 2015).

In patients who have not travelled internationally and present with symptoms suspicious for an Australian tick-borne disease, knowledge of where the patient has travelled in Australia will assist with differential diagnosis. Mosquito-borne diseases may present in the acute phase very similarly and a person who is at risk of tick bites is also likely to be at risk of mosquito bites which can appear very similar if the tick is not actually stuck on the skin,

particularly if the wound is inflamed and scratched. It is recommended that medical practitioners keep an open mind when patients speak of symptoms associated with tick bites as while the patient may have other underlying medical issues brought to light at the time of the tick bite, a considered investigation of the whole clinical history is indicated (Graves & Stenos, 2017).

3.5. Lyme disease

This section covers the identified evidence and international recommendations and advice on transmission and clinical presentation of Lyme disease, the challenges and other presentations and considerations in diagnosing Lyme disease, including the potential to misdiagnose potentially treatable conditions as Lyme disease. It also covers the evidence, guidance and considerations in considering a differential diagnosis of Lyme disease in Australia, the specific requirements for a confirmed diagnosis of Lyme disease in Australia and examples of confirmed cases of Lyme disease diagnosed in travellers returning to Australia.

3.5.1. Transmission and geographical distribution of Lyme disease

Lyme disease is endemic in parts of the US, Europe and Asia. A person visiting a Lyme disease endemic area may become infected with *Borrelia burgdorferi* sensu lato through a tick bite and subsequently develop Lyme disease. Overseas travellers to Lyme disease endemic areas may return to their home country before becoming symptomatic and/or being diagnosed.

Lyme disease is an infectious disease that can be transmitted to humans who are bitten by a tick carrying different species of *Borrelia* bacteria (spirochaetes) collectively known as *Borrelia burgdorferi* sensu lato (Department of Health, 2018b; Mackenzie, 2013; National Institute for Health and Care Excellence, 2018j; Royal College of Pathologists of Australasia, 2019).

In Lyme disease endemic areas, the risk of *Borrelia* infection after the bite of an infected tick is low at only 1 per cent and 3 per cent in the US (Sood et al. (1997), Shapiro et al. (1992) and Costello et al. (1989) in Borchers et al., 2015), and 3-12 per cent in Europe (Maiwald & Oehme (1998), Huegli et al. (2011), Fryland et al. (2011), Nahimana et al. (2004) and Korenberg et al. (1996) in Borchers et al., 2015). The duration of tick attachment is one of the most important predictors of subsequent Lyme disease, with infection more likely the longer a tick is attached to the skin (Borchers et al., 2015; Lantos et al., 2019; Mackenzie, 2013; National Institute for Health and Care Excellence, 2018j).

The duration of tick attachment is among the most important predictors of subsequent Lyme disease, with unengorged ticks not posing a significant risk for *B. burgdorferi* infection (Lantos et al., 2019). The likelihood of transmission increases with duration of attachment on both laboratory mice and patients, with the majority of transmission occurring after 36 hours of attachment; however, time required for infection to occur appears to depend on the target host species and the infecting strain of *B. burgdorferi* (Borchers et al., 2015; Mackenzie, 2013). In experiments with animals infection rarely occurs within 24 hours of tick attachment, with peak infection rates not reached until 48-72 hours of attachment (Piesman et al. (1987), Piesman et al. (1991), Piesman et al. (1993), des Vignes et al. (2001), Ohnishi et al. 2001) and Hojgaard et al. (2008) in Borchers et al., 2015).

In the US, the risk of infection for residents in endemic areas was minimal if ticks stayed attached for < 72 hours but increased significantly with longer attachment (Sood et al. (1997) and Nadelman et al. (2001) in Borchers et al., 2015). The risk of subsequent Lyme disease may exceed 20 per cent when a tick has been attached for ≥72 hours (Lantos et al., 2019). The incubation period is typically seven to fourteen days, but may be shorter, or longer (up to 30 days) (Mackenzie, 2013).

More than 18 spirochaete species comprise the *B. burgdorferi* s.l. complex. Four species are found only in North America, eleven species occur in and are restricted to Eurasia and three species occur in North America and Europe (Mackenzie, 2013).

The main species within this group include:

- *Borrelia burgdorferi* sensu stricto (North America, Europe);
- *Borrelia afzelii* (in Europe, China); and
- *Borrelia garinii* (in Europe, Asia) (Mackenzie, 2013; Royal College of Pathologists of Australasia, 2019).

Of the three main genospecies *B. garinii* and *B. afzelii* are antigenically distinct from *B. burgdorferi* s.s. which may account for the variation in clinical presentation in different geographic regions (Mackenzie, 2013).

Less-common species known to cause Lyme borreliosis include *B. bavariensis* (in Europe), *B. bissetiae* (US, Europe), *B. lusitaniae* (Europe), *B. mayonii* (in mid-west US), *B. spielmanii* (Europe), *B. valaisiana* (Europe, Asia) (Royal College of Pathologists of Australasia, 2019).

Lyme disease is found in high rates in endemic areas, mainly the north east of the US, some areas of Europe including the UK and some parts of Asia (Department of Health, 2018b). Almost all confirmed cases of Lyme disease have occurred in the Northern Hemisphere (Borchers et al., 2015). The majority of cases come from the US and Europe (including the European part of Russia), with far fewer cases from Asia and some from North Africa (Borchers et al., 2015).

In the US, the Northeast, the mid-Atlantic region and the upper Midwest are the prime areas of endemicity and ten states (Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island and Wisconsin) account for ≥93 per cent of annual cases (Borchers et al., 2015).

In most of Europe, while Lyme disease is not a reportable disease and available data are less reliable, Lyme disease is highly endemic. The highest incidence is reported from southern Sweden, Lithuania, Germany, Austria, and Slovenia with the total number of annual cases in Europe estimated to be about three-fold higher [i.e. three-times higher] than the number of cases reported to the CDC (Borchers et al., 2015). Infected ticks are found throughout the UK and Ireland, with particularly high risk areas being the South of England and the Scottish highlands (National Institute for Health and Care Excellence, 2018j).

3.5.2. Transmission in pregnancy, sexual contact or blood products

An evidence-based review of person-to-person transmission of Lyme disease to inform the 2018 NICE Lyme disease guideline (National Institute for Health and Care Excellence, 2018i) acknowledged that mother-to-baby transmission of Lyme disease is possible in theory. However, while there was an absence of evidence, the risk appears to be very low. NICE also found no evidence for transmission of Lyme disease through sexual contact or blood products.

In the earlier section on clinical epidemiology, two patients (0.3 per cent), in their submissions to the Senate Inquiry and analysed by Brown, had reported that they had acquired their Lyme disease or DSCATT congenitally (Brown, 2018).

The Australian Government Department of Health advises that because there is no person-to-person transmission of classical Lyme disease, the risk to Australia and Australians is low (Department of Health, 2020).

An evidence-based review (National Institute for Health and Care Excellence, 2018i) of person-to-person transmission of Lyme disease to inform the 2018 NICE guideline noted the possibility of person-to person spread of Lyme disease has been raised and developing Lyme disease during pregnancy is of concern to women who are pregnant. The committee therefore included person-to-person transmission in the scope of the guideline to assess what evidence was available. NICE acknowledged that mother-to-baby transmission of Lyme disease is possible in theory. However, while there was an absence of evidence, the risk appears to be very low (National Institute for Health and Care Excellence, 2018i). The evidence-based review included eight cohort studies, two case-control studies and two case series that reported outcomes related to vertical transmission (transmission of the pathogen directly from the mother to an embryo, fetus, or baby during pregnancy or childbirth).

NICE made the following Clinical Evidence Statements regarding person-to-person transmission of Lyme disease:

> *In relation to vertical transmission, no studies reporting incidence or prevalence figures were identified. Cohort studies reported adverse pregnancy outcome rates ranging from 11.4 per cent to 35.7 per cent with no direct evidence of a causal link with maternal Lyme disease. Evidence from 2 cohort studies comparing the rates of adverse pregnancy outcomes in women with and without Lyme disease suggested a trend towards an increased risk of adverse outcomes but the data was not adjusted for confounding factors.*
>
> *Evidence from 2 case-control studies was conflicting. Direct evidence of vertical transmission came from 1 retrospective analysis of autopsies and from 2 small case series showing cultivation of spirochetes and detection by immunofluorescence of autopsied tissue and placentas of stillborn fetuses, but the studies did not provide an incidence or prevalence estimate of Lyme disease through vertical transmission. All studies were at high risk of bias due to issues with the study populations, case definitions and methods of data collection. (National Institute for Health and Care Excellence, 2018i, p. 25)*

Overall, the NICE guideline committee considered the evidence inconclusive in terms of identifying a risk of vertical transmission of Lyme disease and emphasised there is a lack of good quality evidence in the area, but the risk appears to be very low. While the committee considered that vertical transmission is not impossible, no strong causal link between a maternal Lyme disease infection and adverse pregnancy outcomes could be found. The committee also found no evidence that a maternal infection resulted in a transmission of *Borrelia spirochaete* to the child. As such, the guideline committee recommended that women diagnosed with Lyme disease during pregnancy follow the same clinical pathway as the rest of the population, except for choice of antibiotic treatment (using amoxicillin as first line rather than doxycycline) and an individualised discussion about the potential risk of vertical transmission.

Additionally, NICE found no evidence for transmission of Lyme disease through sexual contact or blood products. As such, this review did not identify any evidence for sexual transmission of Lyme disease or transmission of Lyme disease through blood products.

3.5.3. Clinical presentation of Lyme disease

Many people may not notice or remember a tick bite. A recent infection with *B. burgdorferi* s.l. can sometimes go unremarked, with mild symptoms that are ignored by the person. When symptoms occur, this is called Lyme disease.

A tick bite can be followed by an EM rash, a circular target-like rash which is considered pathognomonic for Lyme disease but can sometimes be mistaken for cellulitis or ringworm, delaying effective treatment. While the prevalence of EM is seen in about 70 per cent of the cases reported to the CDC, ≥90 per cent in cohorts of paediatric and adult US patients and in 70-95 per cent in European epidemiological studies, central clearing of EM is seen only in 19 per cent of US patients compared to almost 80 per cent of European patients (Borchers et al., 2015), thus illustrating the variation in clinical manifestation according to where the infection was acquired and, therefore the need to take a travel history.

If there is no EM rash or it is unnoticed, diagnosis can be difficult as the same symptoms may be caused by many other conditions as well as Lyme disease (National Institute for Health and Care Excellence, 2018j). Subjective complaints and symptoms that are usually more prominent early in the infection include fatigue, arthralgia, headache, stiff neck, and impaired concentration; symptoms that are common in many infectious and non-infectious diseases (Auwaerter et al., 2011).

Lyme disease is customarily divided into three stages, with clinical manifestations varying in their occurrence and incidence depending on the infecting species and whether the infection was acquired in Eurasia or North America (Royal College of Pathologists of Australasia, 2019). Approximately 4-8 per cent of patients develop cardiac findings, 11 per cent develop neurologic findings and 40–60 per cent of patients manifest arthritis (Borchers et al., 2015), although surveillance data over the past 15 years documents a much lower annual incidence of 30 per cent for Lyme arthritis in patients with untreated EM (Lantos et al., 2019).

The three customary stages of Lyme disease are: Early stage (Stage I), early dissemination (Stage II) and late infection or late disseminated stage (Stage III), with clinical manifestations varying in their occurrence and incidence depending on the infecting species and whether the infection was acquired in Eurasia or North America (Borchers et al., 2015; Royal College of Pathologists of Australasia, 2019).

Early stage (Stage I)

The 'primary lesion' or early localised stage occurs at the site of the *Borrelia* inoculation. The infection manifests as EM: an erythematous papule, usually around 7-14 days post-infected tick bite either as a single expanding area, or a central spot surrounded by clear skin that is in turn encircled by an expanding red rash ('bull's-eye'), and centred on the tick bite is considered the typical and characteristic sign of early infection in about 80 per cent of patients (Royal College of Pathologists of Australasia, 2019). While the prevalence of EM is seen in about 70 per cent of the cases reported to the CDC, ≥ 90 per cent in cohorts of paediatric and adult US patients and in 70-95 per cent in European epidemiological studies, central clearing of EM is seen only in 19 per cent of US patients compared to almost 80 per cent of European patients (Borchers et al., 2015), thus illustrating the variation in clinical manifestation according to the where the infection was acquired and, therefore the need to

take a travel history. The skin lesion may have localised pain or pruritis (Borchers et al., 2015). Again highlighting the difference between US and European manifestations, the CDC case definition states that for surveillance purposes, a solitary lesion must reach at least 5cm in size, whereas European case definitions indicate lesions smaller than 5cm can be diagnosed as EM by experienced physicians (Borchers et al., 2015).

In this early stage, EM is frequently accompanied or sometimes preceded by constitutional systemic signs and symptoms including fatigue, headache, myalgia, arthralgia and fever, although the frequency of systemic symptoms overall and most of the individual symptoms is lower in European patients with EM (caused mostly by *B. afzelii* and *B. garinii*) compared to EM caused by *B. burgdorferi* in the US (Borchers et al., 2015; Royal College of Pathologists of Australasia, 2019). Regional lymphadenopathy may be observed in 13-22 per cent of patients (Borchers et al., 2015). In about 20 per cent of untreated people, this early stage with EM remains the only manifestation of Lyme disease (Borchers et al., 2015).

Of importance to diagnosing clinicians, a rash, which is not EM, can develop as a reaction to a tick bite. This rash usually develops and recedes during 48 hours from the time of the tick bite, is more likely than EM to be hot, itchy or painful, may be caused by an inflammatory reaction, or infection with a common skin pathogen (National Institute for Health and Care Excellence, 2018j). Borchers and colleagues concurred noting that the development of erythematous lesions during tick attachment or within 24 hours of removal of a tick most likely represent hypersensitivity reactions (2015). NICE advised other common causes of rashes that can be mistaken for EM include reaction to an insect bite, cellulitis, tinea corporis (ringworm), granuloma annulare, erythema multiforme (if multiple lesions) and nummular eczema (National Institute for Health and Care Excellence, 2018j) while Borchers and colleagues cited a review (Tibbles and Edlow (2007) in Borchers et al., 2015) that covered the features that help distinguish rashes for EM, noting a variety of rashes can mimic EM.

Early Dissemination (Stage II)

This stage is associated with early haematogenous dissemination to other sites in untreated patients. Manifestations include multiple EM lesions in about 20 per cent of patients (Royal College of Pathologists of Australasia, 2019), these being widespread erythematous plaques that are smaller but morphologically similar to the initial lesion (Borchers et al., 2015).

Nervous system involvement as Lyme neuroborreliosis (LNB) occurs in about 15 per cent of patients (Borchers et al., 2015; Royal College of Pathologists of Australasia, 2019). LNB most commonly develops within a few days to three months after infection (Borchers et al., 2015) with the most common manifestations of LNB typically having an abrupt onset (Lantos et al., 2019). Nervous system manifestations include headache, lymphocytic meningitis, mild neck stiffness, and facial palsy (Royal College of Pathologists of Australasia, 2019).

All of the available epidemiological data demonstrate the highest rates of LNB are in children, followed by adults ≥50 years of age, with a very consistent finding of male to female ratio of 1.5:1 among European LNB patients (Borchers et al., 2015). The classical triad of neurological manifestations in LNB consists of lymphocytic meningitis, cranial neuritis and painful radiculoneuritis alone or in various combinations and when in combination with a well-documented EM makes the diagnosis of LNB highly likely (Borchers et al., 2015).

In the evidence-based review by NICE of person-to-person transmission of Lyme disease (National Institute for Health and Care Excellence, 2018i), detailed above in 3.5.2 Transmission in pregnancy, sexual contact or blood products, NICE advised, the symptoms of Lyme disease in infants are not known and the review found no specific cluster of adverse pregnancy outcomes that was consistent across the studies reviewed. NICE recommended that babies born to mothers who have been treated for symptomatic Lyme disease during pregnancy be clinically assessed and discussed with a paediatric infectious diseases specialist (National Institute for Health and Care Excellence, 2018i).

In children, the characteristics of LNB differ from adults with less frequent and less severe radicular pain, more frequent headache and clinical signs of meningitis while the frequency of facial palsy is often higher but bilateral facial palsy is rare. Therefore, facial palsy and meningitis are the most frequent clinical manifestations of LNB in children (Borchers et al., 2015).

Lyme carditis is also a manifestation of early disseminated infection. Borchers and colleagues reported Lyme carditis may present within days and up to three months after the onset of EM or other signs and symptoms whereas the IDSA/AAN/ACR (Lantos et al., 2019) noted it typically occurs within several days to seven months, with an average of 21 days, following initial infection. Early studies suggested either four to eight per cent or four to ten per cent of untreated patients developed cardiac abnormalities, more recent studies have indicated the incidence may be much lower (Borchers et al., 2015; Lantos et al., 2019). While *B. burgdorferi* infection can affect all parts of the heart, it typically presents as acute onset atrioventricular conduction defects (atrioventricular nodal block) with a rapidly fluctuating complete heart block (Borchers et al., 2015; Lantos et al., 2019; Royal College of Pathologists of Australasia, 2019). Other manifestations include atrial and ventricular arrhythmias, myocarditis, pericarditis, endocarditis (Borchers et al., 2015; Lantos et al., 2019; Royal College of Pathologists of Australasia, 2019).

Joint involvement may also occur with brief attacks of large joint oligoarthritis (Royal College of Pathologists of Australasia, 2019).

Late Dissemination (Stage III)

This stage occurs after months to several years of untreated infection.

While several pieces of literature we reviewed noted that historically about 60 per cent of patients with untreated EM developed rheumatologic involvement as Lyme arthritis (LA) (Borchers et al., 2015; Lantos et al., 2019; Royal College of Pathologists of Australasia, 2019), the IDSA/AAN/ACR noted surveillance data over the past 15 years documents a much lower incidence of only 30 per cent, a figure also noted by Borchers and colleagues from CDC data (Borchers et al., 2015; Lantos et al., 2019). While LA can develop within days or weeks after EM or other manifestations of early Lyme disease, it is usually a late manifestation occurring months or years after infection in untreated patients. LA occurs more frequently in males in both the US and Europe with up to 75 per cent of adults and 70 per cent of children being male.

About 5 per cent of patients present with neuroborreliosis, peripheral neuropathy, spinal radicular pain, distal paraesthesias, encephalopathy leading to subtle cognitive disturbances, intrathecal antibody production and, rarely, cerebrospinal fluid pleocytosis (Royal College of Pathologists of Australasia, 2019).

ACA is a late cutaneous manifestation of Eurasian Lyme disease, primarily affecting middle-aged and elderly women with *B. afzelli* infection (Borchers et al., 2015; Lantos et al., 2019). It occurs in 1-7 per cent of European patients with Lyme disease, with only rare cases described in the US (Borchers et al., 2015). As such, patients evaluated in the US for ACA will most often have acquired their infection in Europe or in Lyme disease endemic areas of Central or East Asia (Lantos et al., 2019). It is a rare skin condition not seen in North American Lyme disease (Royal College of Pathologists of Australasia, 2019).

Table 4: Stages of Lyme disease in patients who have travelled to Lyme disease endemic countries

Early stage (Stage I)
• Constitutional (flu-like) signs and symptoms including headache, myalgia, arthralgia and fever may be present (Royal College of Pathologists of Australasia, 2019). • EM (usually around 7-14 days post-infected tick bite) either as a single expanding area, or a central spot surrounded by clear skin that is in turn encircled by an expanding red rash ('bull's-eye') which is centred on the tick bite is the characteristic sign of early infection in ~80 per cent of patients (Royal College of Pathologists of Australasia, 2019). • A rash, which is not EM, can develop as a reaction to a tick bite (National Institute for Health and Care Excellence, 2018j). This rash: - usually develops and recedes during 48 hours from the time of the tick bite - is more likely than EM to be hot, itchy or painful, and - may be caused by an inflammatory reaction, or infection with a common skin pathogen. Other common causes of rashes that can be mistaken for EM include: • reaction to an insect bite • cellulitis • tinea corporis (ringworm) • granuloma annulare • erythema multiforme (if multiple lesions), and • nummular eczema (Public Health England, 2018).
Early Dissemination (Stage II)
• Early haematogenous dissemination to other sites • Multiple EM lesions, (~20 per cent) • Nervous system involvement (~15 per cent) - headache, lymphocytic meningitis, mild neck stiffness, facial palsy • Cardiac involvement (~5 per cent) - acute onset of high-grade atrioventricular conduction defects, myopericarditis, and • Joint involvement – a large joint oligoarthritis with brief attacks (Royal College of Pathologists of Australasia, 2019).
Late Dissemination (Stage III)
This stage can potentially occur after months to several years following the initial infection though the pathologic mechanism is unclear. It is hypothesised that any ongoing symptoms are more immune related which may or may not be a consequence to the initial infection. Ongoing infection is regarded a debatable diagnosis by the medical profession globally.
• ~60 per cent present with rheumatologic involvement, intermittent attacks of joint swelling and pain in large joints, infiltration of mononuclear cells. • ~5 per cent present with neuroborreliosis, peripheral neuropathy, spinal radicular pain, distal paresthesia, encephalopathy leading to subtle cognitive disturbances, intrathecal antibody production and, rarely, cerebrospinal fluid pleocytosis. • Acrodermatitis chronica atrophicans - a rare skin condition not seen in North American Lyme disease (Royal College of Pathologists of Australasia, 2019).

3.5.4. Other presentations and considerations in diagnosing Lyme disease

NICE (National Institute for Health and Care Excellence, 2018j) provides the following recommendations and advice on other presentations and important considerations when considering the possibility of Lyme disease.

Table 5: Other signs and symptoms of Lyme disease (National Institute for Health and Care Excellence, 2018j)

Signs and symptoms of Lyme disease
In a patient with a history of travel to a Lyme disease endemic area, consider the possibility of Lyme disease in a patient presenting with several of the following symptoms as Lyme disease is a **possible but uncommon cause** of fever and sweats, swollen glands, malaise, fatigue, neck pain or stiffness, migratory joint or muscle aches and pains, cognitive impairment, such as memory problems and difficulty concentrating ('brain fog'), headache and paraesthesia.
In a patient with a history of travel to a Lyme disease endemic area, consider the possibility of Lyme disease in a patient presenting with symptoms and signs relating to one or more organ symptoms (focal symptoms) as Lyme disease is a **possible but uncommon cause** of • neurological symptoms (facial palsy, or other unexplained cranial nerve palsies, meningitis, mononeuritis multiplex or other unexplained radiculopathy) or rarely encephalitis, neuropsychiatric presentations or unexplained white matter changes on brain imaging) • inflammatory arthritis affecting one or more joints that may be fluctuating and migratory • cardiac problems such as heart block or pericarditis • eye symptoms such as uveitis or keratitis • skin rashes such as acrodermatitis chronica atrophicans or lymphocytoma.
Do not rule out the possibility of Lyme disease in people with symptoms but no clear history of tick bite.
Do not diagnose Lyme disease in people without symptoms, even if they have had a tick bite.
Be cautious about diagnosing Lyme disease in people without a supportive history or positive serological testing because of the risk of: • missing an alternative diagnosis • providing inappropriate management.

The RCPA position statement also highlights the need for caution to prevent misdiagnosis particularly when a patient presents with symptoms resembling Lyme disease but with no history of overseas travel (Royal College of Pathologists of Australasia, 2019).

Children with developmental, behavioural or psychiatric disorders for Lyme disease

The most recent international guideline on Lyme disease - the IDSA/AAN/ACR 2019 Draft Lyme Disease Guidelines (Lantos et al., 2019), suggested against routinely testing for Lyme disease in children presenting with developmental, behavioural or psychiatric disorders (weak recommendation; low quality evidence). In their systematic review to inform the draft Lyme disease guidelines the IDSA/AAN/ACR advised there are no data to support a causal link between tick-borne infections and childhood developmental delay or behavioural disorders (such as attention deficit hyperactivity disorder, autistic spectrum disorders, Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS), learning disabilities, or psychiatric disorders). The IDSA/AAN/ACR noted that as with many acute medical illnesses, Lyme disease could worsen behavioural or psychiatric

symptoms in children who are predisposed to these conditions. In addition, the IDSA/AAN/ACR also cautioned that because there is a low pre-test probability (prevalence) of Lyme disease in this population, broadly testing all such children will lead to a high proportion of false positive results, with misattribution of symptoms of Lyme disease leading to delays in care and unnecessary antibiotic exposure (Lantos et al., 2019).

Adult patients with psychiatric illnesses

IDSA/AAN/ACR recommends against testing for Lyme disease in patients with psychiatric illness ('strong recommendation, low quality evidence'). The rationale for the recommendation included that while Lyme disease can co-occur with psychiatric illness, there is no strong systematic evidence supporting a causal relationship that would warrant routine Lyme disease screening of patients with either ongoing or newly diagnosed psychiatric illness. Given the lack of an association between Lyme disease and specific psychiatric disorders, testing should be limited to patients with a reasonable *a priori* likelihood of Lyme disease based on exposure and clinical compatibility of their illness as indiscriminate testing may result in misattribution of symptoms to Lyme disease with potential delays in appropriate care and unnecessary antibiotic exposure (Lantos et al., 2019).

3.5.5. Diagnosing Lyme disease is challenging

The difficulty in diagnosing Lyme disease, even in Lyme disease endemic areas, was highlighted in a systematic review (Brunton et al., 2017) which reported that clinicians find it challenging to diagnose accurately due to the wide variation in symptoms; the infrequency with which they see the disease in practice; their level of confidence about being able to diagnose correctly; the ambiguity they experience about diagnostic tools; and their beliefs and behaviour relating to atypical or recurring symptoms.

While a review (Eldin et al., 2019) of 16 American and European guidelines from seven countries (where Lyme disease is endemic) for the diagnosis of Lyme borreliosis found all guidelines indicated the diagnosis is currently based on laboratory testing (two-tier serology) at all stages of the infection except for the early localised dermatological presentation EM, in a country such as Australia, where Lyme disease is not endemic and is not commonly seen in clinical practice, there are additional challenges in diagnosing Lyme disease. The Royal College of Pathologists of Australasia (Royal College of Pathologists of Australasia, 2019) has published guidance on the diagnosis of Lyme disease specific to the Australian context. These guidance documents and the 2013 report by Mackenzie (2013) stress that due to the non-specific nature of many clinical signs and symptoms the diagnosis of Lyme disease in non-endemic Australia cannot reliably be made on clinical signs and symptoms alone, as many other infectious and non-infectious diseases can have similar features to Lyme disease. Laboratory testing is essential. A diagnosis of Lyme disease requires:

- a careful medical history;
- a history of overseas travel to areas where Lyme disease is endemic: a patient must have been exposed to ticks; however, a history of documented tick bite is not essential as many tick bites go unnoticed;
- objective clinical findings; and
- appropriate in vitro diagnostic tests undertaken by NATA/RCPA accredited laboratories (Royal College of Pathologists of Australasia, 2019).

If Lyme disease is being considered, patients should be referred for Lyme disease serology to the GPs' regular Approved Pathology Practitioner (APP).

3.5.6. Potential to misdiagnose potentially treatable illnesses as Lyme disease

The potential to misdiagnose potentially treatable illness while diagnosing Australian patients with debilitating symptom complexes as having Lyme disease was a major cause of concern raised in the Senate Inquiry reports (Senate Community Affairs References Committee, 2016a, 2016b) including by the Medical Board of Australia (MBA), Australian Health Practitioner Regulation Agency (AHPRA) and Medical Council of New South Wales. Concerns raised included the use of unconventional diagnostic techniques such as kinesiology to diagnose DSCATT, the reliance on non-accredited laboratories to diagnose DSCATT, not referring patients with complex diagnoses to specialists where this would have been appropriate, not managing other co-existing medical conditions, and misdiagnosing cancers as DSCATT.

As noted above in 3.5.5 Diagnosing Lyme disease is challenging, diagnosis can be difficult because the same symptoms may be caused by many other conditions, infectious and non-infectious; this is true for all stages of Lyme disease all of which have features that mimic other medical conditions (Royal College of Pathologists of Australasia, 2019). The importance of not erroneously diagnosing Lyme disease in a patient presenting in Australia with symptoms resembling Lyme disease and no history of overseas exposure when they could have other potentially treatable conditions was highlighted by the RCPA. Such treatable conditions could include chronic pain syndromes, including fibromyalgia; complex neurodegenerative disorders such as motor neurone disease; or psychiatric illness such as major depression with somatisation (Royal College of Pathologists of Australasia, 2019).

The Royal College of General Practitioners in the UK advises that for patients being assessed for Lyme disease the possibility of alternative diagnoses must be fully evaluated to prevent misdiagnosis. Examples of alternative diagnoses include: anaemia, hypothyroidism, B12 deficiency, Vitamin D deficiency, neurological, rheumatological of cardiac conditions, gastrointestinal disorders, psychiatric/psychological disorders, malignancy, other causes including myalgic encephalitis (ME)/chronic fatigue syndrome (CFS), fibromyalgia, postural orthostatic tachycardia syndrome, and other multisystemic illnesses such as systemic lupus erythematosus (SLE) or Ehlers-Danlos syndrome (Royal College of General Practitioners, 2020).

Even in Lyme disease endemic areas, the potential for misdiagnosis is a concern, both from the perspective of misdiagnosis leading to unnecessary antimicrobial treatment (Kobayashi et al., 2019), and missing other significant diagnoses. Public Health England (2018) stresses it is particularly important to ensure that tumours, multiple sclerosis and motor neurone disease are not misdiagnosed as Lyme disease and has developed a resource to assist with the differential diagnosis of Lyme disease, noting that some of the symptoms of Lyme disease are non-specific and clinicians should consider a range of differential diagnoses. Public Health England notes the differential diagnosis for persistent non-specific systemic symptoms is very wide, depending on the predominant symptoms and their presentation, including:

- Cytomegalovirus (CMV);
- Epstein-Barr virus (EBV);
- Hepatitis B or C;

- HIV
- Syphilis;
- Toxoplasmosis;
- Unusual infections such as *anaplasma*, rickettsia, tick-borne encephalitis, Q fever;
- Autoimmune diseases including rheumatoid arthritis;
- Malignancy;
- Primary psychiatric disorders; and
- Chronic fatigue syndrome, myalgic encephalomyelitis or fibromyalgia.

'Chronic Lyme disease'

Some Australians and healthcare providers believe that a form of 'chronic Lyme disease' exists, globally; however, this is a disputed diagnosis which lacks sufficient supporting evidence (Department of Health, 2018b; Lantos et al., 2010, 2019; Marzec et al., 2017; National Institute for Health and Care Excellence, 2018j; Wormser et al., 2006). Several international guidelines, international authorities, and other authors have reviewed and commented on 'chronic Lyme disease' with respect to its disputed diagnosis and the potential to misdiagnose potentially treatable illnesses as Lyme disease.

NICE does not support the term 'chronic Lyme disease'. In its evidence review for the management of ongoing symptoms related to Lyme disease (National Institute for Health and Care Excellence, 2018h), NICE advised the term 'ongoing symptoms' was preferred for the NICE Lyme disease guideline (National Institute for Health and Care Excellence, 2018j), as it does not attribute cause of symptoms, whereas terms such as 'chronic Lyme disease' imply possible chronic infection and may be misleading.

IDSA/AAN/ACR most recently reviewed the evidence on 'chronic Lyme disease' in their draft Lyme disease guidelines for consultation (Lantos et al., 2019), noting early work in the field sometimes referred to patients, particularly in North America, with Lyme arthritis or European patients with acrodermatitis chronica atrophicans with infection of more than six months duration, as having 'chronic Lyme disease'. The term 'chronic Lyme disease' as currently used lacks an accepted definition for either clinical use or scientific study, has not been widely accepted in the medical and scientific community and in practice has been applied to a highly heterogeneous patient population, including patients with prolonged or unexplained symptoms who lack objective features of Lyme disease, many of whom were proven to have had alternative medical diagnoses (Lantos et al., 2019). IDSA/AAN/ACR cited a systematic study (Reid et al. (1998) in Lantos et al., 2019) which found more than half of patients previously given this diagnosis had other specific disorders including rheumatoid or osteoarthritis, amyotrophic lateral sclerosis, myasthenia gravis or depression. The authors noted while many patients diagnosed with 'chronic Lyme disease' have other diagnosable and potentially treatable disorders, many have **'medically unexplained symptoms'** – poorly understood symptom complexes that lack a unifying medical diagnosis – and suggested studies to better understand this disorder or group of disorders and that the development of effective treatment strategies would be highly beneficial. When evaluating such patients, IDSA/AAN/ACR advises clinicians should proceed to a thorough and individualised history, physical examination, and appropriate laboratory investigation to identify, whenever possible, the best-fitting diagnosis. If an alternative diagnosis is established or suspected,

further evaluation, treatment, and as appropriate, referral should be directed to that diagnosis (Lantos et al., 2019).

The CDC (Marzec et al., 2017) advises in a Morbidity and Mortality Weekly Report (MMWR) that 'chronic Lyme disease' is a non-specific diagnosis without a consistent definition and is a term used by some health care providers as a diagnosis for constitutional, musculoskeletal, or neuropsychiatric symptoms (Feder et al. (2007) and Patrick et al. (2015) in Marzec et al., 2017). Marzec et al. note many of these patients have experienced significant debilitation from their symptoms and have not found relief after consultation with conventional medical practitioners and as a result, some seek treatment from practitioners who might identify themselves as Lyme disease specialists ('Lyme literate' doctors) or from complementary and alternative medicine clinics, where they receive a diagnosis of 'chronic Lyme disease' (Lantos et al. (2015) in Marzec et al., 2017). Citing Feder et al. 2007 and Lantos 2015, the authors went on to explain that a diagnosis of 'chronic Lyme disease' might be based solely on clinical judgment and without laboratory evidence of B. burgdorferi infection, objective signs of infection, or a history of possible tick exposure in an area with endemic Lyme disease. They also noted a belief among persons who support the diagnosis and treatment of 'chronic Lyme disease' (Stanek et al. (2012) in Marzec et al., 2017) that B. burgdorferi can cause disabling symptoms even when standard testing is negative, despite evidence that the recommended two-tiered serologic testing is actually more sensitive the longer *B. burgdorferi* infection has been present, and, additionally, that some practitioners use tests or testing criteria that have not been validated for the diagnosis of Lyme disease (Feder et al. (2007) in Marzec et al., 2017). Marzec et al. highlighted that it is of significant concern to the CDC that after the diagnosis of 'chronic Lyme disease' is made, the actual cause of a patient's symptoms might remain undiagnosed and treated (Lantos et al. (2015), and Nelson et al. (2015) in Marzec et al., 2017). The advice from the CDC by Marzec et al. 2017 in this paper, is further presented in section 5.7, under the discussion about the dangers of long-term antibiotic therapy for the treatment of 'chronic Lyme disease'.

Waddell et al. (2016), in their systematic review of diagnostic test accuracy for Lyme disease in North America, excluded studies on 'chronic Lyme disease' because 'chronic Lyme disease' is not recognised by most infectious disease experts as being caused by *B. burgdorferi*, and occurs in patients exhibiting non-specific illness who do not test positive on FDA approved serological tests (Feder et al. (2007) in Waddell et al., 2016).

In their systematic review of chronic coinfections in patients diagnosed with 'chronic Lyme disease', Lantos and Wormser (2014) found the medical literature does not support the diagnosis of chronic, atypical tick-borne coinfections in patients with chronic non-specific illnesses. The authors noted the controversial and ill-defined diagnosis of 'chronic Lyme disease' is often given to patients with alternative diagnoses or prolonged, medically unexplained physical symptoms, with many of these patients also treated for the chronic coinfections with *Babesia, Anaplasma,* or *Bartonella* in the absence of typical presentations, objective clinical findings, or laboratory confirmation of active infection. In addition, they noted active infection is characterised by objective clinical findings (for example, fever or laboratory abnormalities), but commented that practitioners who frequently offer the diagnosis of 'chronic Lyme disease' often do not rely on more accepted standards of clinical and laboratory testing and in such circumstances, many patients also receive spurious diagnoses of chronic anaplasmosis, babesiosis and bartonellosis (Lantos & Wormser, 2014).

Auwaerter et al. (2011) in their article 'Antiscience and ethical concerns associated with advocacy of Lyme disease', published in Lancet Infectious Diseases, commented a belief

system has emerged for some activists over the last 20 years, although unsupported by scientific evidence, that Lyme disease can cause disabling subjective symptoms even in the absence of objective signs of the disease, that diagnostic tests are often falsely negative, and that treatment with antibiotics for months or years is necessary to suppress the symptoms of the diseases, which often recur despite prolonged antibiotic therapy. As a consequence, some individuals with MUS (Hickie et al. (2006) in Auwaerter et al., 2011) and others with more well defined conditions were diagnosed with, or self-attributed their symptoms to Lyme disease, in the absence of supportive laboratory data. Auwaerter et al. (2011, p. 717) referred to panel 1 in their article which reported 'concepts' about Lyme disease that are unsubstantiated or proven to be inaccurate and that the authors obtained from popular Lyme disease websites, and from public statements and presentations by some 'Lyme literate' medical doctors and 'chronic Lyme disease' activists. In this panel, Auwaerter et al. point out that one 'concept' is that Lyme disease causes autism, Morgellons disease, multiple sclerosis, Parkinson's disease, amyotrophic lateral sclerosis, homicidal behaviour ('Lyme rage'), immune dysfunction, birth defects, and Alzheimer's disease. As such, Auwaerter et al. note many patients, believing they were chronically infected and who sought treatment from 'Lyme literate' medical doctors, and who receive long-term treatment have no convincing evidence of having ever had *B. burgdorferi* infection, by history, (sometimes including never having been exposed to ticks, never having been in an endemic area, and never having had objective clinical findings suggestive of Lyme disease), physical examination, or laboratory test results (Feder et al. (2007) and Hassett et al. (2008) in Auwaerter et al., 2011).

Auwaerter et al. also noted that some 'Lyme literate' medical doctors consider children with autism to have persistent *B. burgdorferi* infection as the cause of the disorder (Bransfield et al. (2008) in Auwaerter et al., 2011). However, as discussed in 3.5.4 Other presentations and considerations in diagnosing Lyme disease, the IDSA/AAN/ACR advised against routinely testing for Lyme disease in children presenting with developmental, behavioural or psychiatric disorders, as their systematic review to inform the draft IDSA/AAN/ACR Lyme disease 2019 draft guideline identified no data to support a causal link between tick-borne infections and childhood developmental delay or behavioural disorders (such as attention deficit hyperactivity disorder, autistic spectrum disorders, Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS), learning disabilities, or psychiatric disorders) (Lantos et al., 2019).

3.5.7. Situation in Australia in considering a differential diagnosis of Lyme disease

In Australia, Lyme disease should be considered in patients presenting with a travel history to Lyme disease endemic areas along with supporting symptoms and/or a known tick bite (Department of Health, 2018b). Follow usual clinical practice to manage symptoms, such as analgesia for headaches or muscle pain, in patients being assessed for Lyme disease (National Institute for Health and Care Excellence, 2018j).

Despite multiple studies which have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australian ticks (Beaman, 2016; Chalada et al., 2016; Collignon et al., 2016; Dehhaghi et al., 2019; Department of Health, 2018b; Gofton, Doggett, et al., 2015; Gofton, Oskam, et al., 2015; Graves & Stenos, 2017; Harvey et al., 2019; Irwin et al., 2017; Loh et al., 2016, 2017; Mackenzie, 2013), nor any other vector that could transmit the disease to humans (Department of Health, 2018a; Graves & Stenos, 2017). It is for this reason that the Australian medical profession does not support the diagnosis of locally acquired Lyme disease in Australia (Department of Health, 2018a). While some Australians and healthcare providers

believe that a form of 'chronic Lyme disease' exists, globally, 'chronic Lyme disease' is a disputed diagnosis which lacks sufficient supporting evidence (Department of Health, 2018a; Lantos et al., 2010, 2019; National Institute for Health and Care Excellence, 2018j; Wormser et al., 2006).

'Chronic Lyme disease' was discussed previously in 3.5.6 Potential to misdiagnose potentially treatable illnesses as Lyme disease, and in 5.8 Management of prolonged or ongoing symptoms following treatment of Lyme disease.

In their 2019 review of human tick-borne diseases in Australia, Dehhaghi et al. (2019, p. 9) noted the presence of Lyme disease (Lyme borreliosis, LB) or Lyme-like disease in Australia is highly controversial; however 'Importantly, there is no convincing evidence for the presence of locally acquired Lyme disease in Australia'. The authors noted the evidence for a potential Lyme Borreliosis pathogen in Australia is limited and there has been no research since 1994. They commented:

> *It is assumed that if the causative species of LB is/are transmitted by ticks within Australia, likely would be (not necessarily) from the Ixodes genus. Research on potential vectors of LB in Australia advises that I. holocyclus and I. tasmani are the two common ticks with the widest geographical distribution in Australia (Dehhaghi et al., 2019, p. 10)*.

In reviewing the evidence, they concluded there is no evidence for transmission of *B. burgdorferi* sensu lato complex with Australian ticks and that while patients in Australia with Lyme-like disease may occasionally have positive Lyme serology, finding the causative agent using PCR or direct culture is regarded as mandatory for confirmation of local acquisition of infection (Dehhaghi et al., 2019). The findings of this latest literature review concur with other reviews (Beaman, 2016; Chalada et al., 2016; Department of Health, 2018b; Graves & Stenos, 2017).

To inform their conclusion, Dehhaghi et al. cited five studies:

- a study (Mackerras and Mackerras (1960) in Dehhaghi et al., 2019), which Dehhaghi et al. noted was the first report of the presence of *Borrelia* species in Australia, when a species of *Borrelia* was isolated from a rat in north-western Queensland in 1956.
- a study (Roberts (1970) in Dehhaghi et al., 2019) which advised that the *I. holocyclus* and *I. tasmani* are the two common ticks with the widest geographical distribution in Australia.
- a study (Wills and Barry (1991) in Dehhaghi et al., 2019) which was reported to have found rigid spirochete-like objects (SLOs) in 41.9 per cent of all Australian ticks collected (167 ticks consisting of *I. holocyclus* and *H. longicornus* from the Hunter Valley and Manning River districts of coastal New South Wales), and that ELISA, immunofluorescence and Western blotting had revealed that at least four bacterial isolates had similar antigenic epitopes with *B. burgdorferi.* Dehagghi et al. noted however that, crucially, the identity of isolates was not confirmed using PCR or further sequencing.
- a study (Russell et al. (1994) in Dehhaghi et al., 2019) of 12,000 ticks (*H. bancrofti, H. longicornis,* and *I. holocyclus)* collected from the New South Wales coast which found that no isolates showed positive binding of monoclonal *B. burgdorferi* antibodies.

- a study (Gofton, Oskam et al. (2015) in Dehhaghi et al., 2019) which found no member of the *B. burgdorferi* sensu lato group in 109 *I. holocyclus* ticks from around New South Wales studied using PCR; however, their results found the presence of a new relapsing fever group *Borrelia.*

Also in 2019, Harvey et al. used a meta-transcriptomics approach to characterise viruses associated with Australian ticks collected from two locations on the central east coast of Australia, including metropolitan Sydney. The transcriptomic data provided no evidence for the presence of *B. burgdorferi* s.l. in any tick sample (Harvey et al., 2019). Harvey et al. commented this study provided further evidence against the presence of Lyme disease in Australia.

In 2017, Irwin et al. in their canine sentinel study, used a combination of serological assays to test dogs living in tick 'hot spots' and exposed to the Australian paralysis tick *I. holocyclus,* for evidence of exposure to *B. burgdorferi* (s.l) antigens and other vector-borne diseases (Irwin et al., 2017). The rational for this approach was that studies conducted in Europe and the US had used dogs as sentinels for tick-associated illness in people because dogs readily contact ticks that may harbour zoonotic pathogens. Irwin et al. found that, of the 555 dogs from four demographic regions recruited into the study, except for one dog presumed to have been exposed to *Anaplasma platys,* infection with *Anaplasma* spp., *B. burgdorferi* (s.l)*, Ehrlichia* spp., and *Dirofilaria immitis,* was not detected in the cohort of dogs. Irwin et al. commented:

> *These results provide further evidence that Lyme borreliosis does not exist in Australia but that cross-reacting antibodies (false positive results) are common and may be caused by the transmission of other tick-associated organisms (Irwin et al., 2017, p. 1)*.

It is noteworthy Irwin et al.'s 2017 paper was not included in the Dehhaghi et al.'s 2019 literature review. However, as there was no methodology for Dehhaghi et al.'s literature review it was not possible to tell the inclusion criteria or date range.

In Graves and Stenos's 2017 review of tick-borne infectious diseases in Australia, the authors concluded Lyme disease bacteria are probably not present in Australian ticks and, given the likely absence of the relevant bacteria in Australian ticks, there is little value in laboratory testing for Lyme disease if the patient has not been to an endemic region of the world (Collignon et al (2016), Gofton, Oskam et al. (2015), and Beaman (2016) in Graves & Stenos, 2017).

Graves and Stenos also cited the study by Loh et al. (2016) which had detected a *Borrelia* species in the Australian echidna tick (*Bothriocroton concolor),* but Graves and Stenos commented this bacteria belongs to a unique clade unrelated to the *Borrelia* species responsible for causing Lyme disease, the tick is not known to bite humans and, as such, the bacterium is unlikely to be human pathogen. Of this study, the authors, Loh et al., reported that, in addition to the finding that the novel *Borrelia* sp. identified in their study does not belong to the *Borrelia burgdorferi* (s.l.) complex, the zoonotic potential and pathogenic consequences of this novel *Borrelia* sp. are unknown at the current time. Loh et al. reported that subsequent analyses confirmed that this novel species of the genus *Borrelia* is more closely related to, yet distinct from, the Reptile-associated (REP) and Relapsing Fever (RF) groups. Additionally, the presence of the *glpQ* gene, which is absent in the Lyme Borreliosis group spirochaetes, further emphasises that the novel species of the genus *Borrelia* characterised in their study does not belong to this group.

Collignon et al. (2016) also found no convincing evidence that classical Lyme disease occurs in Australia, or that there was evidence that the causative agent *B. burgdorferi,* is found in Australian animals or ticks. The authors noted that since the early 1990s, the Australian medical community, especially specialist microbiologists and infectious diseases physicians, have debated whether an indigenous form of classical Lyme diseases occurs in Australia, especially in areas with high rates of tick bites, citing 11 publications, in the following table.

Table 6: Publications cited by Collignon et al. (2016)

Publications
Carley JG, Pope JH. A new species of Borrelia (*B. queenslandica*) from *Rattus villosissimus* in Queensland. Aust J Exp Biol Med 1962; 40: 255-262.
Lawrence RH, Bradbury R, Cullen JS. Lyme disease on the NSW central coast. Med J Aust 1986; 145: 364.
McCrossin I. Lyme disease on the NSW south coast. Med J Aust 1986; 144: 724-725.
Russell RC. Lyme disease in Australia — still to be proven! Emerg Infect Dis 1995; 1: 29-31.
Playford G, Whitby M. Tick-borne diseases in Australia. Aust Fam Physician 1996; 25: 1841-1845.
Hudson BJ, Stewart M, Lennox VA, et al. Culture-positive Lyme borreliosis. Med J Aust 1998; 168: 500-502.
Nash PT. Does Lyme disease exist in Australia? Med J Aust 1998; 168: 479-480.
Russell RC.Vectors vs. humans in Australia — who is on top down under? An update on vector-borne disease and research on vectors in Australia. J VectorEcol 1998; 23: 1-46.
Mayne P, Song S, Shao R, et al. Evidence for *Ixodes holocyclus* (Acarina: Ixodidae) as a vector for human Lyme borreliosis infection in Australia. J Insect Sci 2014; 14: 1-3.
Mayne PJ. Clinical determinants of Lyme borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort. Int J Gen Med 2015; 8: 15-26.
Chalada MJ, Stenos J, Bradbury RS. Is there a Lyme-like disease in Australia? Summary of the findings to date. One Health 2016; 2: 42-54.

Collignon et al. noted that in 1991, *B. burgdorferi* s.l. could not be confirmed in any of 176 tick species examined, (Carley and Pope (1962) and Mayne (2015) in Collignon et al., 2016) and findings of more recent surveys such as Gofton, Oskam et al. (Gofton, Oskam, et al., 2015) and Loh et al. (2016) have also been negative. Collignon et al. did note; however, that many in the 'Lyme disease' community are interested in the novel *Borrelia* species identified in the studies of Australian ticks by Gofton, Doggett et al. (2015), Loh et al. (2016) and Gofton, Oskam et al. (2015), and the possibility that it might cause their illness, but that the novel *Borrelia* species has not yet been shown to be pathogenic (Collignon et al., 2016).

Chalada et al. (2016, p. 43) undertook a review to advise the Australian Government Chief Medical Officer on the current situation of the 'controversial Lyme or Lyme-like illness reported by some to be present in Australia'. The authors identified 10 papers (see the following table) published between 1982 and 2015 in Academic Journals, which reported at least 525 human cases of Lyme-like illness have been mentioned in the scientific literature.

Table 7: Studies reviewed by Chalada et al. (2016) of Lyme-like illness in Australia

Studies reviewed
A. Stewart, J. Glass, A. Patel, G. Watt, A. Cripps, R. Clancy, Lyme arthritis in the Hunter Valley, Med. J. Aust. 1 (1982) 139.
I. McCrossin, Lyme disease on the NSW south coast, Med. J. Aust. 144 (1986) 724.
R. Lawrence, R. Bradbury, J. Cullen, Lyme disease on the NSW central coast, Med. J. Aust. 145 (1986) 364.
N. Stallman, Lyme Borreliosis — A Case Report for Queensland, 21CDI, (1987) 8–9.
B.J. Hudson, M. Stewart, V.A. Lennox, M. Fukunaga, M. Yabuki, H. Macorison, et al., Culture-positive Lyme Borreliosis, Med. J. Aust. 168 (1998) 500–503.
P.J. Mayne, Emerging incidence of Lyme Borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia, Int. J. Gen. Med. 4 (2011) 845.
P.J. Mayne, Investigation of *Borrelia burgdorferi* genotypes in Australia obtained from erythema migrans tissue, Clin. Cosmet. Investig. Dermatol. 5 (2012) 69.
C.Maud, M. Berk, Neuropsychiatric presentation of Lyme disease in Australia, Aust. N. Z. J. Psychiatry 4 (2013) 397–398.
P. Mayne, S. Song, R. Shao, J. Burke, Y. Wang, T. Roberts, Evidence for *Ixodes holocyclus* (Acarina: Ixodidae) as a vector for human Lyme Borreliosis infection in Australia, J. Insect Sci. 14 (2014) 271.
P.J. Mayne, Clinical determinants of Lyme Borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort, Int. J. Gen. Med. 8 (2015) 15.

Chalada et al. (2016) commented that the majority of the reported cases were Lyme-like cases that were suspected, but not confirmed, to represent cases of Lyme Borreliosis, and cautioned that:

> *Unreliability of the published case reports in their diagnostic methods means the evidence for Australian Lyme-like cases remains quite unsubstantial and unconvincing,* and
>
> *Upon investigation, these diagnoses were highly questionable due to significant flaws in the diagnostic process or presentation of results (Chalada et al., 2016, p. 48)*.

Chalada et al. noted that in only one of the studies, (Hudson et al. 1998), a Lyme Borreliosis-causing *Borrelia* species had been cultured from an Australian patient or animal, but this patient had a history of overseas travel to a Lyme disease endemic area of the northern hemisphere, and therefore overseas acquisition could not be ruled out.

Chalada and colleagues reported that four studies, published between 1991 and 2015, have investigated the potential for *B. burgdorferi* s.l. in ticks. The studies, Chalada et al. noted, employed culture with and without PCR and in the most recent studies next generation sequencing. The four studies reviewed and described by Chalada et al. are described below.

Wills and Barry 1991 (Wills and Barry (1991) in Chalada et al., 2016)

Chalada et al. reported that in a letter to the editor of The Medical Journal of Australia in 1991, Wills and Barry published preliminary results of their investigations into the presence of *Borrelia* in Australian ticks. *I. holocyclus and H. longicornus* ticks (177 ticks in all) were collected from the Hunter Valley and Manning River districts of coastal New South Wales and their midguts were cultured in BSK-II media. At least four of the spirochaetes isolated shared antigenic epitopes with *B. burgdorferi* as demonstrated by ELISA, immunofluorescence and Western blotting, suggestive of *Borrelia* species. However, Chalada et al. noted details of the laboratory methods were not published and the organisms recovered were not made available for confirmation by another laboratory, rendering the experiment unable to be replicated. Chalada et al. also commented that false positives in the ELISA, immunofluorescence and Western blotting cannot be ruled out, no PCR or sequencing has been conducted to confirm the identity of the isolates and positive *Borrelia* cultures from Australian ticks have not been reproduced to date. No follow up report to the preliminary findings was published in the scientific literature. Chalada et al. stated 'The use of molecular techniques, especially sequencing, would be ideal for confirmation or dismissal of any SLOs [spirochaete-like objects] as *Borrelia*' (Chalada et al., 2016, p. 45). Note, Dehagghi et al. (2019) had also noted (see above) of this study by Wills and Barry, that crucially the identity of isolates was not confirmed using PCR or further sequencing.

Russell et al. 1994 (Russell et al. (1994) in Chalada et al., 2016)

Chalada et al. commented the reported culture of possible *Borrelia* spirochaetes from 109 ticks by Wills and Barry (1991) (described above) was not reproduced in the study of over 10,000 ticks by Russell et al. (1994). Russell et al.'s 1994 study of approximately 1,200 ticks collected over three years along the New South Wales coast contradicted the findings of Wills and Barry (1991). According to Chalada et al., the Russell study found no definitive evidence for the existence in Australia of *B. burgdorferi,* the causative agent of true Lyme Borreliosis, or for any other tick-borne spirochaete that may be responsible for a local syndrome being reported as Lyme disease. Chalada et al. concluded:

> *The conclusion of Russell et al.'s study – that no spirochaetes were able to be identified through culture or molecular methods in Australian ticks – therefore seems more plausible than the conclusions of Wills and Barry (Chalada et al., 2016, p. 46)*.

Gofton, Oskam et al, 2015a (Gofton, Oskam et al. (2015a) in Chalada et al., 2016)

Chalada et al. reported that Gofton, Oskam et al. found no *B. burdorferi* s. l. in 109 Australian *I. holocyclus* ticks from around New South Wales collected over a 10-year period but did detect a novel relapsing fever group *Borrelia* from a single Australian *I. holocyclus* taken from an echidna. Chalada et al. commented:

> *This work provides further evidence that the cause of the Lyme-like illness in Australia may not be a member of the B. burgdorferi s. l. complex. The finding of a novel relapsing fever Borrelia in an Australian monotreme does provide evidence for the presence of Borrelia in Australia, but it is not known if this organism can infect humans, and should it do so, it is likely that it would present as a relapsing fever illness rather than with Lyme-like symptoms. These factors limit the*

> *likelihood that this novel Borrelia species is the cause of the Lyme-like illnesses seen in Australia (Chalada et al., 2016, p. 46)*.

Chalada et al. (2016) noted a number of limitations of the study including the relatively low number of ticks sampled, the limited geographic range from which they were collected and that no data was presented regarding the distribution of collection sites (urban, rural or wilderness) within that state.

Gofton, Doggett, et al. 2015b (Gofton, Dogget, et al. (2015b) in Chalada et al., 2016)

Chalada et al. (2016) commented that further work using the same protocol as used by Gofton, Oskam et al. (2015a) on a larger cohort of ticks from an Australian-wide catchment and including other tick species (particularly *H. longicornus*) was warranted, given that *H. longicornus* has known competence as a vector as a Lyme-causing *Borrelia* in Japan and would be a superior candidate for potential *B. burdorferi* s.l. transmission in Australia. Chalada et al.; however, noted *H. longicornis* very rarely bites humans. To address this requirement, Gofton, Doggett et al. collected 460 ticks from below the tropic of Capricorn, in Western Australia, and the seaboard Eastern Australia (one from inland Queensland was also included). The ticks were identified as *I. holocyclus (n* = 279), *Amblyomma triguttanum (n*= 167), *H. bancrofti (n*= 7) and *H. longicornis* (*n* = 7). The midguts of all ticks were subjected to 16s ribosomal RNA PCR and next generation sequencing and a *Borrelia* genus specific *flab* nested PCR was also performed on all ticks recovered. Chalada et al. reported that Gofton, Doggett et al. found none of the ticks concerned yielded any *Borrelia* sequences or products (Chalada et al. 2016).

Chalada et al. (2016) also reviewed the evidence on serology, culture and molecular detection from the published papers on Australian Lyme-like cases, and this is discussed in greater detail in 4.7.1 Culture issues of diagnostic testing for Lyme disease in Australia. However, of the evidence and of relevance to this section, was their conclusion:

> *B. burgdorferi s. l. has never been cultured from an Australian patient that could not have acquired the infection overseas and therefore there is currently no proof that B. burgdorferi s. l. or any other kinds of Borrelia species are infecting humans in Australia. If there is a Lyme-like disease that exists in Australia it may well be of a different aetiology (Chalada et al., 2016, p. 52)*.

Similarly, Beaman (2016) reviewed Australian data on Lyme borreliosis and concluded that Lyme disease vectors are not found in Australia and Lyme Borreliosis has not been found in Australian vectors, animals or patients with autochthonous illnesses. Beaman noted that:

- studies commencing in 1998 have not demonstrated the presence of Lyme Borreliosis in non-peripatetic Australians, reservoir hosts or tick vectors (Russell et al. (1994) in Beaman, 2016);
- a more recent study (Gofton, Oskam et al. (2015) in Beaman, 2016) had confirmed the absence of Lyme Borreliosis in Australian ticks (196 *I. holocyclus);* and
- Australia's most common Ixodid tick appears to be incapable of Lyme Borreliosis transmission (Piesman and Stone (1991) in Beaman, 2016).

Beaman commented that the data show countries with demonstrated endemic Lyme disease have no trouble in demonstrating the presence of Lyme borreliosis in vectors, reservoirs or patients and therefore, this would argue strongly against the presence of Lyme disease in Australia. Of relevance to considering Lyme disease in a differential diagnosis in Australian

patients, Beaman noted the recommendation that patients who do not fulfil the case definition of Lyme disease, especially if they have not left Australia, should not be tested for Lyme borreliosis, and additionally alternative causes of rheumatological disease or fatigue syndromes and other infectious agents known to be transmitted by tick bites in Australia (Beaman and Hung (1989) and Graves et al. (1993) in Beaman, 2016) should also be excluded.

Also in 2016 (and updated in 2019), the Royal College of Pathologists of Australia, in its Position Statement, answered the question 'Is there endemic Borreliosis ('Lyme disease' or similar) in Australia?' as follows.

> *There are several important human infectious diseases not thought to be present in Australia, including some transmitted by ticks. With respect to Lyme disease in Australia, there is a spectrum of opinion (both medical and lay) on whether Lyme disease is endemic in Australia or not. The number of cases of Lyme disease in Australian patients remains small and previous research efforts in Australia have failed to demonstrate the presence of Lyme disease-causing Borrelia in Australian ticks. There are Ixodes genus ticks present in Australia, but none of the overseas Ixodes species known to carry Borrelia spp. occur in Australia. The examination of Australian ticks to date (February 2016), has not detected ticks that contain any of the Borrelia spp that are known to cause Lyme disease elsewhere in the world. Further investigations of Australian patients (with symptoms similar to those of Lyme disease) and Australian ticks (especially Ixodes spp) may clarify the issue. Only a genuine case in a non-travelling Australian patient would confirm the disease as being present in Australia (Royal College of Pathologists of Australasia, 2019, p. 1).*

Earlier, in 2013, Mackenzie, in his scoping paper to identify the research needs for an investigation into whether a tick-borne microorganism (*Borrelia*) for Lyme disease exists in Australia noted, similarly to other authors above had noted there has only been one report of *Borrelia* species being found in *I. holocyclus* ticks in Australia (Wills and Barry (1991) in Mackenzie, 2013)(described above), but the cultures were not confirmed and unsustainable; and that experimental vector competence studies had demonstrated that *I. holocyclus* is unable to be infected by a North American isolate of *B. burgdorferi* (Piesman and Stone (1991) in Mackenzie, 2013). Mackenzie also noted while Lyme borreliosis has been reported in Australia (Mayne (2011) and Hudson et al. (1998) in Mackenzie, 2013) he also commented that the vast majority of cases were patients who had travelled to Lyme disease endemic areas.

3.5.8. Diagnosis of Lyme disease in Australia

Lyme disease is not a notifiable disease in Australia.

In a country such as Australia where Lyme disease is not endemic and is not commonly seen in clinical practice, there are additional challenges in diagnosing Lyme disease. The Australian Government Department of Health (Lum et al., 2015) and the Royal College of Pathologists of Australasia (2019) have published guidance on the diagnosis of Lyme disease specific to the Australian context. These guidance documents and the report by Mackenzie (2013) stress that due to the non-specific nature of many clinical signs and symptoms, the diagnosis of Lyme disease in non-endemic Australia cannot reliably be made on clinical signs and

symptoms alone, as many other infectious and non-infectious diseases can have similar features. Laboratory testing is essential. A diagnosis of Lyme disease requires:[2]

- a careful medical history
- a history of overseas travel to areas where Lyme disease is endemic; a patient must have been exposed to ticks however, a history of documented tick bite is not essential as many tick bites go unnoticed
- objective clinical findings, and
- appropriate in vitro diagnostic tests undertaken by NATA/RCPA accredited laboratories.

If Lyme disease is being considered, patients should be referred for Lyme disease serology to the GPs' regular Approved Pathology Practitioner (APP).

The Royal College of Pathologists of Australasia (2019) notes that caution is important in dealing with specimens for Lyme disease testing and in interpreting of positive or indeterminate laboratory results and advises that medical microbiologists should add explanatory comments to all such reports to assist the referring doctor to interpret the laboratory findings correctly.

In addition to the sensitivity and specificity of the diagnostic tests recommended in international guidelines for Lyme disease, the prevalence of the disease or the pre-test probability of a disease strongly influences interpretation of any diagnostic test result. In a region where Lyme disease is uncommon, patients with highly characteristic clinical presentations are rarely found to have Lyme disease and positive test results are seldom associated with clinically probable infection, although the negative predictive value of Lyme disease testing will be very high (Lantos, Branda, et al., 2015). In areas not endemic for Lyme disease [for example Australia], the positive predictive value of the serology test will be low (Chalada et al., 2016; Collignon et al., 2016; Royal College of Pathologists of Australasia, 2019). False positives will occur more frequently in a low prevalence population, such as Australia, with RCPA noting that even with an assay having 98 per cent sensitivity and specificity, in a low prevalence population (for example, 1 per cent), the PPV only approaches 33 per cent (Royal College of Pathologists of Australasia, 2019). Collignon et al. noted tests should only be requested if there is a well-founded suspicion of Lyme disease, and not in situations of low pre-test probability, in order to minimise risk of a false positive result (Moore et al. (2016) in Collignon et al., 2016).

In an area of low Lyme disease incidence in the US, a study of Lyme disease testing showed an 80 per cent false positive rate, which puts patients at risk of incorrect Lyme diagnoses and adverse drug reactions from inappropriate treatment (Lantos, Branda, et al., 2015). Therefore, awareness of epidemiological context and the absence of an alternative diagnosis are necessary for a clinician to decide whether a positive test is explanatory or coincidental. The difficulties in interpreting diagnostic tests for Lyme disease, as described in 4.5.6 Considerations, limitations and important variables in serology testing for Lyme disease, coupled with the difficulties clinicians in Lyme disease endemic countries experience in diagnosing Lyme disease (Brunton et al., 2017) underpin the recommendation that medical professionals seek advice from appropriate experts in infectious diseases or pathology.

[2] A database of NATA accredited facilities can be found at: https://www.nata.com.au/accredited-facility

3.5.9. Confirmed diagnosis of Lyme disease in Australia

A confirmed case of Lyme disease in Australia requires laboratory evidence AND clinical evidence AND epidemiological evidence (Lum et al., 2015).

3.5.10. Cases of overseas-acquired Lyme disease diagnosed in travellers returning to Australia

The literature describes a small number of recent case reports of travellers returning to Australia from Lyme disease endemic areas and being diagnosed with Lyme disease in Australia based on clinical and laboratory evidence (Doolan et al., 2019; Subedi et al., 2015). Additionally, Collignon et al. reported on an analysis of diagnostic testing for Lyme disease in an Australian laboratory (Collignon et al., 2016).

Subedi et al. (2015) reported on the first case of Lyme neuroborreliosis in a returned Australian traveller in 2015. A 58-year-old woman had spent three months in Lithuania where she was bitten by a tick during a trip to a pine forest near Vilnius. Her symptoms had started one month after returning to Australia from Lithuania. She developed two circular non-pruritic rashes, each about 30mm in diameter, which resolved after two weeks without specific intervention. On presentation at a rural hospital in New South Wales in May 2014, the patient had an eight-month history of worsening motor instability, confusion and bilateral occipital headaches associated with photophobia, lethargy and somnolence. She had also experienced continuing headaches, lethargy and a self-limiting episode of diplopia. After tests for herpes simplex viruses 1 and 2, enterovirus, *Mycobacterium tuberculosis,* syphilis treponema and HIV all returned negative laboratory results, serological testing for *Borrelia* in both serum and CSF was requested. With a presumptive diagnosis of Lyme neuroborreliosis, treatment with ceftriaxone (4g daily) was commenced. Serology testing was performed at the Institute of Clinical Pathology and Medical Research at Westmead (Sydney). Serological screening returned positive results for both serum and CSF. Confirmatory Western blotting found the serum of the patient showed IgG responses to two *B. burgdorferi* antigens (molecular weights, 41, 58 kDa) and five *B. alzelii* antigens (22,39,41,58,83kDa). The authors noted only the IgG blotting results for *B. afzelii* antigens met the criteria of the CDC, which stipulates that five or more specific IgG bands are required for a positive serological result (CDC (1995) in Subedi et al., 2015). IgG to the same antigens as well as to a sixth *B. afzelii* antigen (45kDa) were detected in her CSF. The authors also reported the patient also met all three criteria in the European Federation of Neurological Sciences guidelines for a definite diagnosis of Lyme neuroborreliosis:

- Neurological symptoms
- CSF pleocytosis, and
- Detection of intrathecal antibodies or, if symptoms began in the past six weeks, identification of the pathogen in the CSF by PCR or culture (Mygland et al (2010) in Subedi et al., 2015)

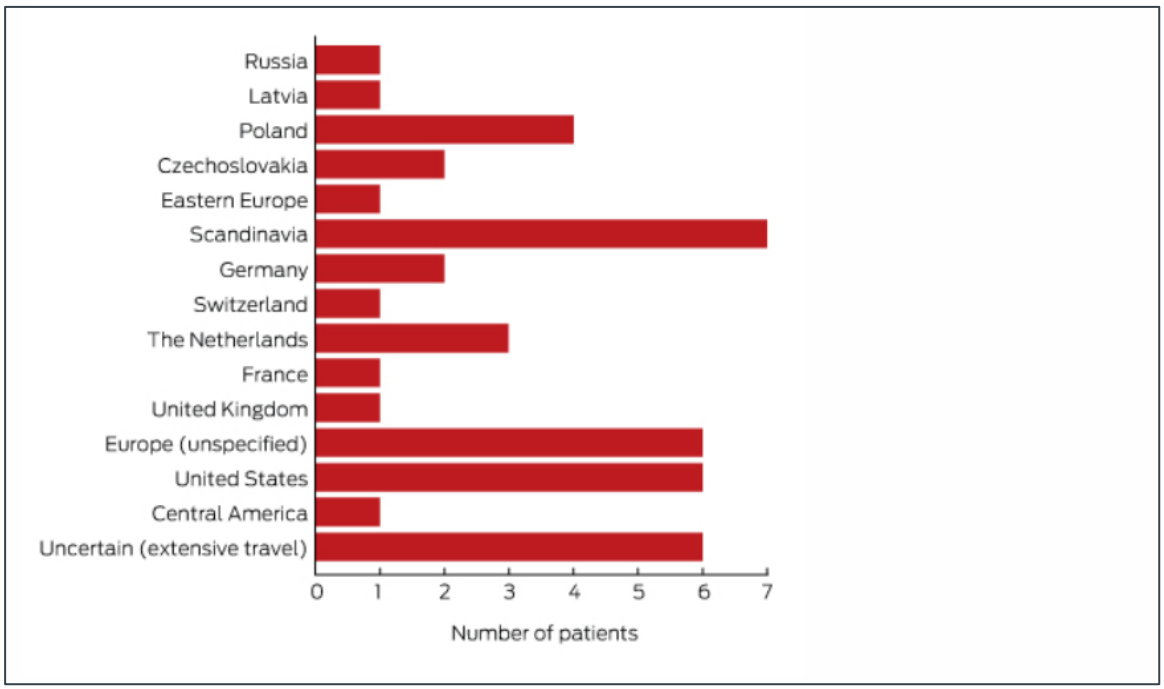
Five months after her treatment of two weeks with intravenous ceftriazone (4g daily) the patient had normal CSF parameters and continued to make a good clinical recovery with her headaches, lethargy and neurological symptoms having all resolved. The authors stressed this case highlighted the importance of obtaining a thorough travel history in a patient presenting with chronic meningoencephalitis and that clinicians should consider neuroborreliosis in patients with a history of travel to an endemic area who present with persistent neurological symptoms. They also noted other differential diagnoses to consider

in patients presenting with chronic meningitis, including cryptococcal meningitis which they noted was endemic in Australia, with *Mycobacterium tuberculosis* infection and tick-borne encephalitis further diagnoses to consider in travellers with meningoencephalitis who have returned from Eastern Europe (Subedi et al., 2015). This case report demonstrates that Australian clinicians and laboratories are able to accurately support diagnosis of Lyme disease using internationally validated serology testing protocols.

More recently, in 2018, Doolan et al. described a case of Lyme disease in a recent migrant, highlighting the infrequent skin disease in the Australian setting (Doolan et al., 2019). This case was in a 43-year-old woman who presented with a four-month history of an erythematous, enlarging, annular lesion consistent with EM rash on her lower calf and a three-month history of localised dysaesthesia and constitutional symptoms including intermittent flu-like illness, lethargy and arthralgia. She had moved from Switzerland to Australia five months earlier. She was initially diagnosed and treated for erythema annulare centrifugum but reported no improvement in her symptoms and developed headaches and sinusitis. The results of full blood examination, inflammatory markers and autoantibodies were all within normal limits. A central lesional biopsy showed a hyperkeratotic epidermis and perivascular polymorphic infiltration of neutrophils and lymphocytes within the dermis, and spirochete staining showed occasional spiral shaped organisms. Serology screening was positive for the Borrelia-specific antibody and subsequent Western blot testing showed five specific IgG bands, confirming *Borrelia* exposure. The patient was treated with oral doxycycline and upon follow-up her rash and constitutional symptoms had resolved but calf dysaesthesia remained (Doolan et al., 2019).

In an analysis of diagnostic testing for Lyme disease conducted by a large private laboratory in Australia, over a 23-month period (September 2014 – July 2016) nearly all (95.5 per cent) of the tests performed in 5395 patients returned negative results. A travel history was available for 37 of the 43 patients with true positive results. All had returned from countries in which Lyme disease is endemic. The analysis found most Lyme disease acquired overseas but diagnosed in Australia was European in origin (30 of 43 cases, or 70 per cent of cases). The following graph is reproduced from Collignon et al. (2016) to demonstrate the countries where travellers had travelled to prior to returning to Australia and being diagnosed with Lyme disease via positive serological testing.

Figure 2: Travel history for patients with positive serological test results for Lyme disease (Box 5. Collignon et al. 2016)



In addition to the finding by Collignon et al. (2016) that the majority of the cases of overseas-acquired Lyme disease was European in origin, the case reports of returning travellers diagnosed in Australia with Lyme disease, described above by Subedi et al. (2015) and Doolan et al. (2018), both had a recent travel history to European countries. These findings are in themselves important, because the geographical location of acquisition of Lyme disease infection has implications for interpretation of serology testing for Lyme disease, and therefore underscores the importance of a comprehensive travel history. The implications of geographical location of acquisition of Lyme disease infection is discussed further in 4.5.6 Considerations, limitations and important variables in serology testing for Lyme disease, and 4.6.1 NATA/RCPA Accreditation and Accredited Laboratories.

3.6. Tick-borne diseases known to be acquired in Australia

In patients who have not travelled overseas to a Lyme disease endemic area AND who have or may have recently been bitten by a tick or in the past or who engage in activities such as bushwalking AND present with acute or chronic symptoms suspect Australian tick-borne diseases (or Australian vector-borne diseases) and seek expert advice.

In patients who have not travelled internationally and present with symptoms suspicious for an Australian tick-borne disease, knowledge of where the patient has travelled in Australia will assist with differential diagnosis. Mosquito-borne diseases may present in the acute phase very similarly and a person who is at risk of tick bites is also likely to be at risk of mosquito bites which can appear very similar if the tick is not actually stuck on the skin, particularly if the wound is inflamed and scratched. It is recommended that medical practitioners keep an open mind when patients speak of symptoms associated with tick bites as while the patient may have other underlying medical issues brought to light at the time of the tick bite, a considered investigation of the whole clinical history is indicated (Graves & Stenos, 2017).

There are 17 human biting ticks known in Australia, only six of these ticks having the ability to act as competent vectors for the transmission of pathogens to humans (Dehhaghi et al., 2019).

Apart from the occasional local bacterial infection at the tick bite site (eschar), the only two systemic bacterial infections that are definitely known to be transmitted by tick bites in Australia are Rickettsial infections from infection with *Rickettsia* spp. (QTT, FISF and ASF) and Q fever (*Coxiella burnetii*) (Dehhaghi et al., 2019; Graves & Stenos, 2017; Mackenzie, 2013). In Australia, Q fever is a notifiable disease, with a Q fever laboratory case definition (Public Health Laboratory Network, 2017).

The species of Australian ticks known to bite humans and transmit bacterial infection are:

- the paralysis tick (*Ixodes holocyclus*), endemic on the east coast of Australia and
  - causes QTT due to *R. australis*
  - causes Q fever due to *C. burnetii*
- the common marsupial tick (*Ixodes tasmani*)
  - causes QTT due to *R. australis*
  - causes ASF due to *R. honei* subsp*. marmionii*
- the southern paralysis tick (*Ixodes cornuatus*)
  - causes QTT due to *R. australis*
- the ornate kangaroo tick (*Amblyomma triguttum*) occurs throughout much of the central, northern and western Australia and
  - causes Q fever due to *C. burnetii*
- the southern reptile tick (*Bothriocroton hydrosauri*) occurs mainly in south-eastern Australia and
  - causes FISF due to *R. honei.*
- the *Haemaphysalis novoaeguinae* (no common name)
  - causes ASF due to *R. honei* subsp*. marmionii* (Graves & Stenos, 2017, p. 321).

Rickettsial diseases

Australia has an almost complete set of Rickettsial infections transmitted to humans by:

- Tick bite (Spotted Fever Group rickettsia)
- Mite bite (scrub typhus)
- Flea bite (murine typhus or cat flea typhus)
- Inhaled infected flea faeces (murine typhus or cat flea typhus) (Graves, n.d., p. 10).

With DSCATT having been attributed to ticks, this literature review only covers Rickettsial diseases transmitted by tick bites. The Rickettsial diseases transmitted by mite bite, flea bite and inhaled infected flea faeces are out of scope.

The symptoms of Rickettsial infections in Australia include eschar, fatigue, fever, headache, myalgia and rash (macular, papular, vesicular) although the severity and duration of Rickettsial diseases vary considerably (Dehhaghi et al., 2019). QTT and ASF have similar core clinical manifestations with a range of other symptoms observed. Early clinical features are often non-specific, making diagnosis challenging (Stewart et al., 2017). Additionally, symptoms may overlap with other infectious diseases including those that are transmitted by non-tick vectors as well as a number of chronic diseases.

While post-infection fatigue, a well-known consequence of several infections including Ross River virus, Q fever and Epstein-Barr virus, is not yet widely recognised as a problem following Rickettsial infection, it has been suggested by a study involving two large cohorts of fatigued and non-fatigued patients and a case report (Graves & Stenos, 2017).

Recommendation: Seek further expert opinion as necessary, depending on the nature of the patient's clinical presentation from appropriate experts in vector-borne diseases including specialist microbiologists with diagnostic experience and ID physicians for diagnosis of vector-borne diseases.

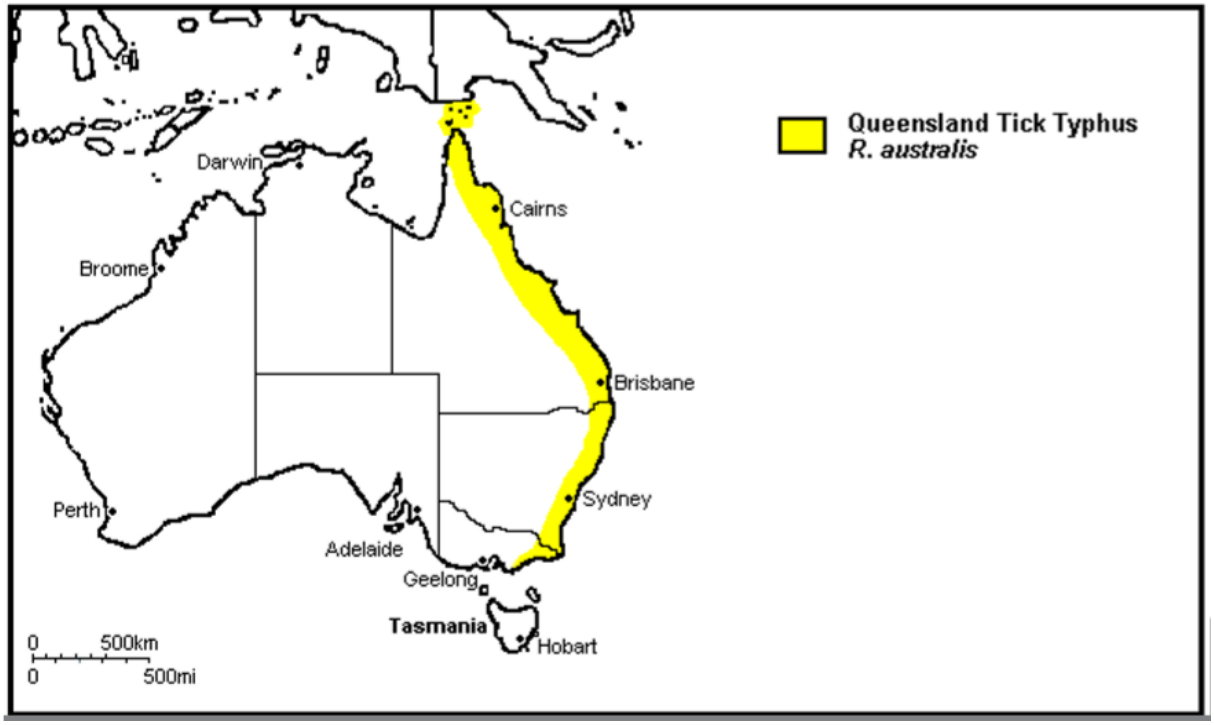
3.6.1. Queensland Tick Typhus

QTT is an emerging public health threat (Dehhaghi et al., 2019; Stewart et al., 2017) and an increasingly recognised important cause of community-acquired acute febrile illness in Eastern Australia (Stewart et al., 2017). Diagnosing *R. australis* infection can be challenging, and in patients presenting with fever and a rash, epidemiologic data and knowledge of high-risk exposure activities can be valuable in considering QTT. A high degree of suspicion is required, as nonspecific symptoms in early QTT can lead to a delay in diagnosis (Stewart et al., 2017). Early recognition and treatment is therefore important.

Transmission and geographic distribution

QTT is regularly seen on the east coast of Australia, from the Torres Strait Islands to the south-eastern corner of Victoria, with the northern suburbs of Sydney a very common location for transmission of this infection (Campbell et al. (1979) and Hudson et al. (1993) in Graves & Stenos, 2017; Stewart et al., 2017). In north-eastern New South Wales, 15.4 per cent of paralysis ticks (*Ixodes holocyclus)* were found to contain *R. australis,* suggesting a one in six risk of being infected with the rickettsia if bitten by this tick in this location (Graves et al., 2016; Graves & Stenos, 2017). The geographical distribution of the known human pathogen that causes Q fever (*R. australis)* is expanding due to changes in climate and human population demographics (Dehhaghi et al., 2019).

Figure 3: Distribution of the Queensland Tick Typhus, as per www.asid.net.au/documents/item/415



Infection by *R. australis* may occur throughout the year in immunocompetent people of all ages and ethnicities, although 80 per cent of documented cases have occurred in winter and spring (June to November) coinciding with increased tick densities in these months (Sexton et al. (1991) and Barker & Walker (2014) in Stewart et al., 2017).

Clinical presentation

In symptomatic infections, QTT is often a mild condition involving fever, headache, malaise, myalgia, a rash, eschar and enlarged lymph nodes (Dehhaghi et al., 2019; Graves & Stenos, 2017; Streeten et al. (1948) in Stewart et al., 2017). However, QTT may be severe, or fatal and may have unusual features (Graves & Stenos, 2017). Less common manifestations of QTT include arthralgia, splenomegaly, abdominal pain, dry cough, sore throat, conjunctivitis and photophobia (McBride et al. (2007) in Stewart et al., 2017). While QTT is not known to directly affect the central nervous system, there have been reports of confusion, seizures and hallucinations as a prominent feature of this disease (Sexton et al. (1991) in Stewart et al., 2017). There are no known identified risk factors for developing severe disease or complications of QTT (Stewart et al., 2017).

Fever: High grade fever of up to 41° (Sexton et al. (1991) in Stewart et al., 2017) is observed in cases; prolonged fever is associated with rickettsaemia, end organ dysfunction and intensive care admissions (Derne et al. (2015) in Stewart et al., 2017). QTT is readily treated with a short course of doxycycline and in acute uncomplicated QTT, fever resolves within 48 hours after initiation of treatment with doxycycline (Graves & Stenos, 2017; Stewart et al., 2017).

Rash: Rash morphology is variable, and can be macular, macropapular (Sexton et al. (1991) in Stewart et al., 2017), vesicular or pustular; the latter two forms can be confused with acute varicella (Hudson et al. (1994) in Stewart et al., 2017). Infrequently the rash is pruritic (Hudson et al. (1993) in Stewart et al., 2017). The rash usually lasts for 10-12 days, can appear as early as 24 hours after a tick bite and typically follows a widespread, global eruption

involving the trunk and limbs. EM) at and around the *Ixodes* attachment site is not uncommon in QTT (Stewart et al., 2017). Of note, EM is observed in other tick-borne illnesses such as *Rickettsia* and *Borrelia* spp. including Lyme disease (Barker & Walker (2014) in Stewart et al., 2017).

In approximately 50-65 per cent of *R. australis* infections, an eschar is seen, with the detection of an eschar being diagnostically valuable; however, it is often difficult to find as it can occur in sites that can be missed on examination such as in the axilla or groin. Tender lymphadenopathy, usually localised to the region draining the tick bite or eschar occurs in approximately 70 per cent of patients (Sexton et al. (1991) in Stewart et al., 2017).

The clinical presentation of a case of QTT in rural Queensland published by Royal Australian College of General Practitioners (RACGP) provides advice to support GPs (Thomas & Wu, 2018).

While post-infection fatigue, a well-known consequence of several infections including Ross River virus, Q fever and Epstein-Barr virus, is not yet widely recognised as a problem following Rickettsial infection, it has been suggested by a study involving two large cohorts of fatigued and non-fatigued patients (Unsworth et al. (2008) in Graves & Stenos, 2017) and a case report (Watts et al. (2008) in Graves & Stenos, 2017).

Diagnostic testing for QTT

For diagnosis of QTT, laboratory investigations of cases include:

- mild-to-moderate thrombocytopaenia commonly early in the disease course transforming into a reactive thrombocytosis during recovery from the disease
- a transient and mild elevation of hepatic transaminases early in the disease
- leukopenia, in mild cases
- neutrophilia and toxic changes on blood film, in patients presenting with severe infection, and
- significantly raised C-reactive protein measurements in systemic Rickettsial infection in contrast to uncomplicated viral infections (Stewart et al., 2017, p. 26).

Serological assays remain the main diagnostic test modality for diagnosing Rickettsial infections. Currently, the indirect microimmunofluoresence assay (IFA) is considered the gold standard assay for diagnosing QTT. Acute and convalescent serum samples are taken 10-14 days apart and a four-fold rise in SFG antibody titre or a single positive titre of 1:256 is used to indicate acute or recent infection (Stewart et al., 2017).

Initial negative serological studies do not rule out Rickettsial infection and should not alter treatment completion in potentially infected patients (Thomas & Wu, 2018).

Additional information is available in Update on Australian Rickettsial Infections (Graves, n.d.)

Factors complicating diagnosis of QTT

A number of factors can complicate the diagnosis, including:

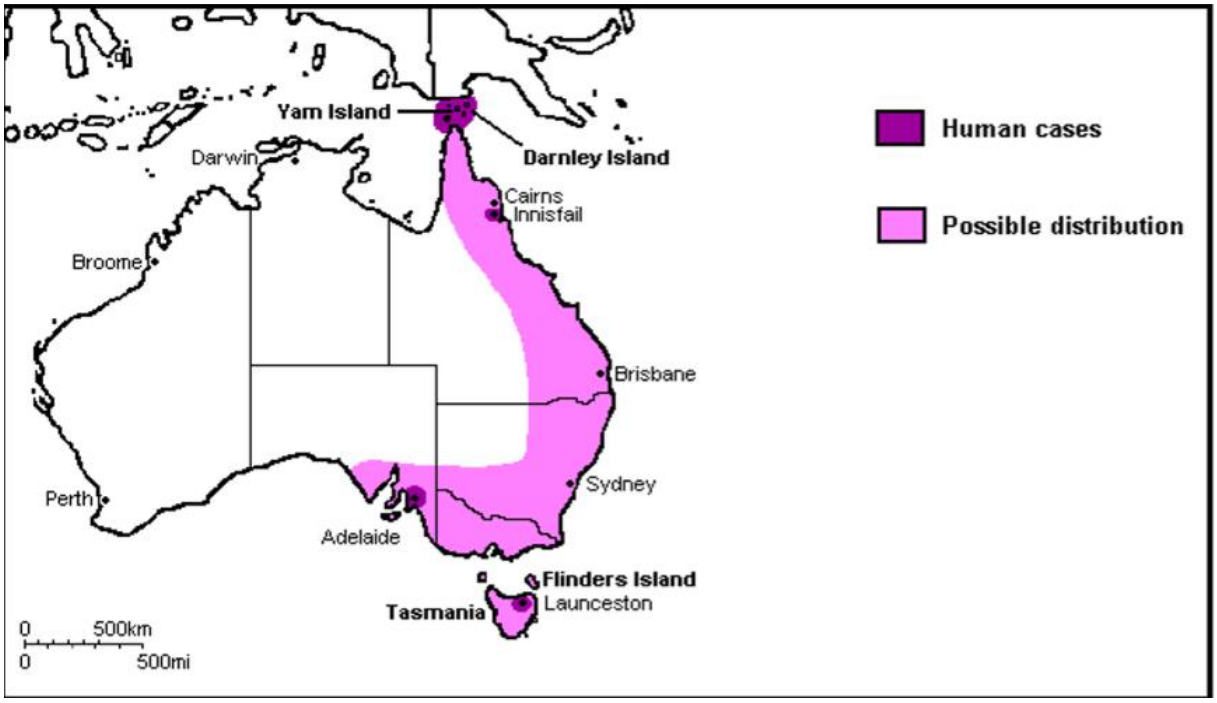
- substantial cross-reactivity of antibodies between some rickettsia and with other species of bacteria such as *Proteus* and *Legionella*
- concomitant illnesses such as rheumatologic- and immune-mediated disorders can yield false positive Rickettsial serological tests
- occasionally, patients infected with *R. australis* do not seroconvert
- serology tests can be difficult to interpret in acute illness; low level titres are associated with previous SFG Rickettsia exposure and not to a patient's current non-Rickettsial infection (Stewart et al., 2017, p. 27).

3.6.2. Australian Spotted Fever

Transmission and geographic distribution

ASF has been reported in the eastern half of Australia and is common in subtropical and tropical areas of Queensland extending down the east coast to East Gippsland in Victoria (Chalada et al., 2016; Dehhaghi et al., 2019; Graves & Stenos, 2017).

Figure 4: Distribution of Australian Spotted Fever, as per www.asid.net.au/documents/item/415



Clinical presentation

Symptoms of ASF include fever, headache and muscle aches with a stiff neck, vomiting and mental confusion also being possible (Banks & Hughes, 2012).

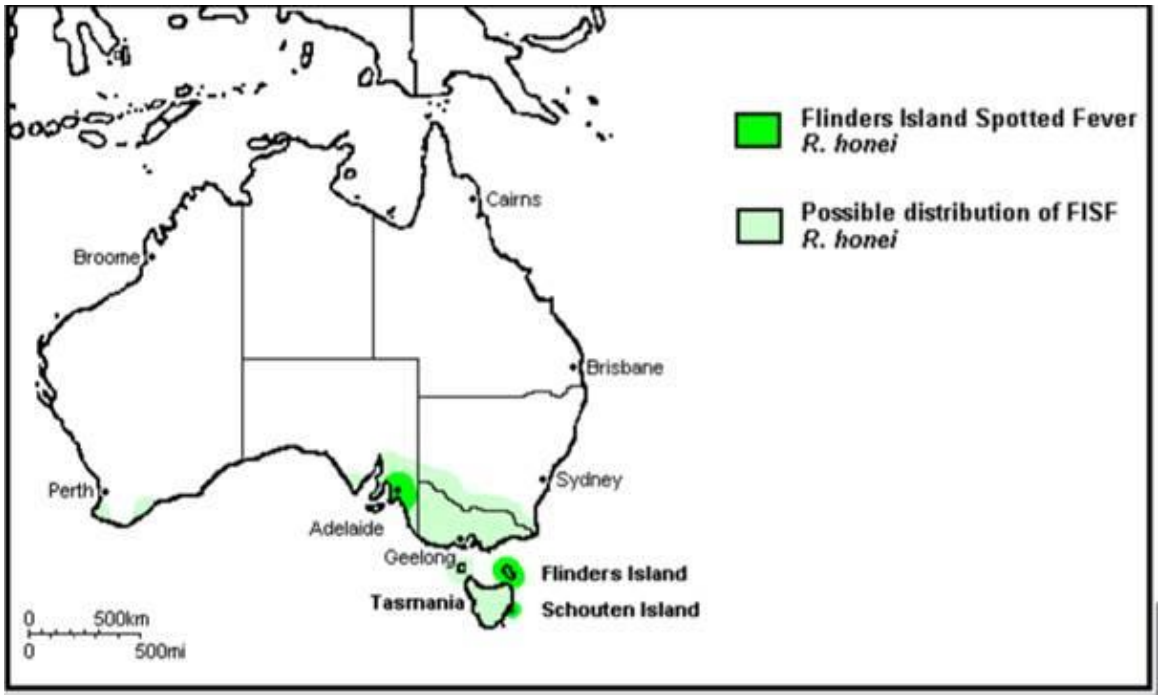
Additional information is available in Update on Australian Rickettsial Infections (Graves, n.d.)

3.6.3. Flinders Island Spotted Fever

Transmission and geographic distribution

FISF is transmitted by the tick *Bothriocroton hydrosauri* and has been reported in Flinders Island, mainland Tasmania, Southern-eastern Australia, south-western coastal areas of Western Australia in Salisbury Island and Walpole, and south-eastern coastal regions of South Australia near Adelaide (Dehhaghi et al., 2019; Graves & Stenos, 2017; Willis et al., 2019)

Figure 5: Distribution of Flinders Island Spotted Fever, as per www.asid.net.au/documents/item/415



The Department of Health, Tasmania, reported in 2019 that confirmed cases of FISF have been acquired in Tasmania, including in the midlands of Tasmania (Willis et al., 2019).

Clinical presentation

Symptoms of FISF include cough, fever, headache, maculopapular rash, myalgia and arthralgia (Dehhaghi et al., 2019).

Additional information is available in Update on Australian Rickettsial Infections (Graves, n.d.)

3.6.4. Q fever

In Australia, Q fever is a notifiable disease, with a Q fever laboratory case definition (Public Health Laboratory Network, 2017) and is included in the CDNA National Guidelines for Public Health Units (Communicable Diseases Network Australia, 2018). In Australia, Q fever is the most commonly reported zoonotic disease (Eastwood et al., 2018). As Q fever can be mistaken for other conditions, including other zoonotic diseases (for example, leptospirosis, brucellosis), the work up should be determined by a detailed history, examination and initial screening investigation, with a useful algorithm having been developed for general practitioners (GPs) (Eastwood et al., 2018).

Transmission and geographical distribution

Q fever is acquired via various modes of transmission, a minority of which is tick-borne.

While *Coxiella burnetii* are present in both the paralysis tick and ornate kangaroo tick, most cases of Q fever infection by this bacterium occur by inhalation of infectious aerosols from carrier (reservoir) vertebrate animals such as goats, sheep cattle, kangaroos and domestic pets or dust particles contaminated by birth fluids, faeces or urine from infected animals (Communicable Diseases Network Australia, 2018; Dehhaghi et al., 2019; Graves & Stenos, 2017; Mackenzie, 2013). Rats may also harbour the tick *Amblyomma triguttum trigutum* which is a natural host for the *Coxiella burnetii* bacterium that causes Q fever in humans.

The incubation period is typically 2-3 weeks; person to person spread rarely occurs (Communicable Diseases Network Australia, 2018). Persons at increased risk of Q fever are:

- at risk occupational groups with contact of high risk animal products, including (but not limited to): abattoir and meat workers; agriculture, livestock and dairy farmers/workers; laundry workers handling clothes of at-risk workplaces; veterinary professionals and staff; animal shooters/hunters; dog/cat breeders and anyone regularly exposed to parturient animals
- other people through non-occupational, environmental exposures including (but not limited to): family members of occupationally exposed groups; people living in close proximity to a high risk industry (neighbouring livestock farms and stockyards); visitors to at risk environments; people involved in mowing which aerosolises dust potentially contaminated by animal excreta, and
- persons at increased risk for chronic Q fever after experiencing an acute infection include: immunosuppressed persons; pregnant women; persons with valvular heat disease/valvular prosthesis; persons with aneurysms/vascular grafts (Communicable Diseases Network Australia, 2018, p. 7).

In 2016, there were 551 cases (2.3 per 100,000 population) in the annual Q fever notification. The majority of Australian Q fever notifications were reported from Queensland and New South Wales during 2011-2015, with the notification rate remaining highest in southwest/central-west Queensland and northwest New South Wales, and generally reflecting the intensity of local cattle, sheep, and goat husbandry, and associated processing industries (Communicable Diseases Network Australia, 2018), although it is emerging in other regions, including the Northern Territory and southwest Western Australia (Dehhaghi et al., 2019).

Clinical presentation

Q fever may present as an acute or chronic illness; the majority (60 per cent) of cases will be asymptomatic/subclinical presentations (Chalada et al., 2016; Communicable Diseases Network Australia, 2018). People who become sick often have a severe flu-like illness.

Acute Q fever

The most common manifestation is an influenza-like illness which might occur in conjunction with hepatitis and/or pneumonia, which can appear similar to other aetiologies of atypical pneumonia such as those associated with *Legionella* or *Mycoplasma,* requiring consideration of differential diagnoses (Communicable Diseases Network Australia, 2018). Commonly reported signs and symptoms include: fever, chills, sweats, severe headache, (especially behind the eyes), photophobia, weakness, anorexia, nausea, myalgia, cough and weight loss

(Communicable Diseases Network Australia, 2018; Eastwood et al., 2018). The most prevalent acute symptoms are fever (95 per cent), headaches (53 per cent) and myalgia (38 per cent) (Chalada et al., 2016).

In a minority of infected cases (≤1 per cent), patients may develop pericarditis, myocarditis or neurologic complications (e.g. meningoencephalitis, encephalomyelitis) (Communicable Diseases Network Australia, 2018). Unlike Rickettsial infections (see above), Q fever is unlikely to be associated with a rash (Dehhaghi et al., 2019).

Chronic Q fever

Chronic Q fever is the most serious form of the disease and can occur from one month to several years after acute illness as a result of persistence of *C. burnetii* infection in the host after primary infection (Communicable Diseases Network Australia, 2018; Eastwood et al., 2018). Sometimes there is no history of acute illness. Chronic Q fever may present as one of three forms according to the focus of infection: endocarditis; osteoarticular infections; and vascular infections with the abdominal or thoracic aorta the most frequent site for vascular infections (Communicable Diseases Network Australia, 2018). Chronic Q fever may also manifest as chronic hepatitis, pericarditis, and very rarely as adenopathies, lung or splenic pseudotumours, or chronic neuropathy (Chalada et al., 2016). As such, Q fever may sometimes present as an infection similar to Lyme carditis or Lyme neuroborreliosis (Chalada et al., 2016).

Q fever fatigue syndrome

Q fever fatigue syndrome refers to systemic symptoms that fail to recover more than 12 months after the acute illness (Communicable Diseases Network Australia, 2018) and is the most common sequela following acute infection in Australia, occurring in approximately 10-15 per cent of patients (Marmion (2009) in Communicable Diseases Network Australia, 2018). The initial infection may be mild or severe, and patients present with a 'chronic fatigue-like' picture (Eastwood et al., 2018). Typical features include: profound fatigue, arthralgia, myalgia, concentration and memory problems, sleeping problems, sweats and headaches (Morroy et al. (2016) in Communicable Diseases Network Australia, 2018). Alcohol intolerance is a commonly reported feature (Eastwood et al., 2018). The severity of the initial acute infection is the only known risk factor for the development of the post-Q-fever fatigue (Eastwood et al., 2018).

Diagnostic testing for Q fever

The Q fever *National Guidelines for Public Health Units* developed by the Communicable Diseases Network Australia (CDNA) specify that, if acute Q fever infection is suspected, a series of blood specimens should be requested and should include:

- unclotted blood or serum for Q fever PCR (and possible culture) AND
- paired (acute and convalescent) serum/clotted blood specimens taken 2-3 weeks apart for serology.

The collection of convalescent sera from all cases is critical, even if the patient has since recovered. See Q fever CDNA *National Guidelines for Public Health Unit*s section 8 Laboratory testing for specific detail (Communicable Diseases Network Australia, 2018, p. 12).

Diagnosis of Q fever

Diagnosis of Q fever can be made by a medical professional based on symptoms, clinical examination, and laboratory tests on blood samples. Two or more blood samples on separate occasions are often required to confirm a Q fever diagnosis (Communicable Diseases Network Australia, 2018).

CDNA provide details on laboratory tests (PCR and serology testing) and interpreting results for Q fever (Communicable Diseases Network Australia, 2018).

Advice by RACGP to assist GPs in diagnosing Q fever, including in aspects of diagnostic tests is also available (Eastwood et al., 2018).

While achieving a timely, definitive diagnosis of Q fever is challenging, early treatment is beneficial and empirical antibiotic therapy should be considered if the presentation and clinical history suggest a zoonotic disease (Eastwood et al., 2018). Moreover, the Q fever CDNA guidelines for Public Health Units specify that if Q fever is suspected clinically (in people with appropriate symptoms AND who are at high risk of contracting Q fever), empirical treatment should be commenced without waiting for laboratory tests (Communicable Diseases Network Australia, 2018)

3.7. Tick-borne infections reported to be found in Australian patients but not known to be acquired in Australia

In 3.2 Diseases or disorders patients experiencing DSCATT symptoms have been diagnosed with, we reported Dr Schloeffel told the DSCATT Forum (TMS Consulting Pty Ltd, 2018a) that nine infective organisms had been found in Australian patients with vector-borne disease patients. These diseases included: *Borrelia* including relapsing fever, Rickettsias, *Bartonella,* Ehrlichiosis, Anaplasmosis, *Babesia*, *Coxiella burnetti, Mycoplasma*, and viruses. Additionally, The House of Representatives Standing Committee on Health noted Dr Schloeffel had listed 10 groups of coinfections associated with tick-borne or Lyme-like illness including relapsing fever, Rickettsias and chronic viral infections including HIV in his submission to the Inquiry. In contrast, Professor Graves had found no evidence of babesiosis or rickettsiosis in a small group of patients with Lyme-like illness in the Austin Health ID Program, after extensive investigation based on laboratory evidence or failure to respond to medical therapy that is usually effective against these two diseases (TMS Consulting Pty Ltd, 2018a).

Additionally, in 3.2 Diseases or disorders patients experiencing DSCATT symptoms have been diagnosed with, many patients who identified as having Lyme disease or Lyme-like illness, told the Senate Inquiry that they had been also diagnosed with coinfections including *Bartonella* and Babesiosis when their samples were referred for testing to non-accredited laboratories in Australia or laboratories in the US or Germany. Additionally, the LDAA had told The House of Representatives Standing Committee on Health that 55 per cent of patients with tick-borne or Lyme-like illness reported being diagnosed with at least one coinfection with the rate in Australia being 'much higher' than that reported in the US (House of Representatives Standing Committee on Health, 2016).

The Senate Inquiry (2016a) reported that ticks are hosts and vectors of a number of parasites, bacteria and viruses, with the main organisms that may be transmitted by ticks and associated with disease known in Australia being:

- *Anaplasma* – which causes disease in cattle (bovine anaplasmosis, or 'bovine tick fever') and dogs (canine anaplasmosis);
- *Babesia* – a significant cause of disease in cattle (Bovine babesiosis) and dogs (Canine babesiosis);
- *Bartonella* – which causes disease in domestic and wild animals including cats and kangaroos – uncertain whether it can cause human disease;
- *Ehrlichia* – which causes disease in dogs worldwide but has not been recognised in Australia;
- *Francisella* – which is relatively rare and there is no evidence to suggest it is pathogenic for humans (Senate Community Affairs References Committee, 2016a, pp. 29–30).

Within our search for literature relevant to Australian ticks, several peer-reviewed papers that reported on findings of organisms in Australian ticks were identified. While the scope for the DSCATT Clinical Pathway is focussed on known tick-borne infections in Australia, infections or organisms identified in Australian ticks where human infection has not been established is out of scope. However, we have provided the references relevant to each of these tick-borne diseases below, for completeness.

Anaplasmosis and Ehlichiosis: (Dehhaghi et al., 2019; Gofton, Doggett, et al., 2015; Graves et al., 2016; Graves & Stenos, 2017; Mackenzie, 2013)

Babesiosis: (Chalada et al., 2016; Dawood et al., 2013; Dehhaghi et al., 2019; Graves & Stenos, 2017; Lantos & Wormser, 2014; Mackenzie, 2013; Senanayake et al., 2012)

Bartonellosis: (Chalada et al., 2016; Dehhaghi et al., 2019; Gofton, Doggett, et al., 2015; Mackenzie, 2013)

Borrelia: (Chalada et al., 2016; Gofton, Doggett, et al., 2015, 2015; Gofton et al., 2018; Graves et al., 2016; Graves & Stenos, 2017; Loh et al., 2016)

Candidatus Neoehrlichia mikuensis: (Chalada et al., 2016; Gofton, Doggett, et al., 2015; Gofton, Oskam, et al., 2015; Graves & Stenos, 2017; Mackenzie, 2013)

Francisella: (Dehhaghi et al., 2019; Graves & Stenos, 2017; Mackenzie, 2013)

Viral tick-borne infections: (Dehhaghi et al., 2019; Graves & Stenos, 2017; Harvey et al., 2019; Mackenzie, 2013)

Other Australian research related to ticks: (Banks & Hughes, 2012; Greay et al., 2016; Kwak, 2018)

Tick-borne coinfections*:* (Collignon et al., 2016; Lantos & Wormser, 2014; Mackenzie, 2013)

3.8. Other likely differential diagnoses in patients presenting with persistent debilitating symptoms

3.8.1. If tick-borne disease is not indicated, consider alternative diagnoses

Of the non-infectious diseases, Chalada et al. (2016) noted fibromyalgia, chronic fatigue syndrome, delusional parasitosis and multiple sclerosis as some examples of conditions that may be misdiagnosed as DSCATT, particularly in Australia where the infectious aetiology for Lyme-like illness has not been elucidated.

Additionally, Australian literature notes post fatigue syndrome is a well-known consequence of several infections including Ross River virus, Q fever and Epstein-Barr virus; however, the antecedent infection may not be clearly identified in the patient (Graves & Stenos, 2017).

The following causes should be considered when developing a differential diagnosis:

- infectious – including blood-borne or sexually transmitted infections, vector-borne diseases, travel related, food and water-borne
- autoimmune – including inflammatory arthritis, motor neurone disease, multiple sclerosis
- neoplastic
- psychological – including depression, anxiety and reactions to traumatic events
- inflammatory
- vascular
- neurological
- cardio-respiratory, and
- lifestyle related – including diet, exercise, sleep and stress.

This section is also relevant to 3.5.6 Potential to misdiagnose potentially treatable illnesses as Lyme disease, where we presented findings from the literature reviewed, including international authority advice about the potential to misdiagnose potentially treatable conditions. Further detail about infectious and non-infectious diseases and conditions to consider in a differential diagnosis is contained in that section.

3.8.2. Medically unexplained symptoms

MUS are defined as physical symptoms persisting for more than several weeks and for which adequate medical examination has not revealed a condition that adequately explains the symptoms (Olde Hartman et al., 2017). Patients with MUS may be very unwell and require complex care. MUS exist along a continuum ranging from self-limiting symptoms to recurrent and persistent symptoms through to symptom disorders. People experiencing debilitating symptoms attributed to ticks, without any definitive diagnosis could be considered to fall within the definition of MUS.

Brown (2018), in his analysis of 698 publicly available submissions made to the Senate Inquiry by people who identified as have Lyme disease or DSCATT, noted the most commonly reported symptoms by patients (fatigue, disordered thinking, or 'brain fog', sensory disturbance, arthralgia and myalgia, and headache), coupled with submissions showing a 'striking female preponderance' (80.3 per cent when reported), were prominent components

of both fibromyalgia and chronic fatigue syndrome (CFS), the two most prominent MUPS disorders (Brown, 2018, p. 424). Brown further commented that the non-specific symptoms, female preponderance, and lack of confirmatory laboratory testing suggested patients were more likely to be experiencing a MUPS disorder (such as CFS) than an active or latent infection, as had been found by investigators of 'chronic Lyme disease' in the US, which reached the same conclusion by actively comparing healthy, CFS and 'alternatively diagnosed Lyme' groups (Patrick et al. (2015) in Brown, 2018, p. 425).

Fibromyalgia and CFS have also been raised by other authors as considerations in the differential diagnosis for patients presenting with symptoms attributed to Lyme-like illness or DSCATT (Chalada et al., 2016; Collignon et al., 2016). Collignon et al., noted the chronic debilitating symptoms reported by patients to the Senate Inquiry overlap to a considerable degree with those of CFS and related disorders and for most patients diagnosed with CFS, the exact cause is unknown. However, Epstein-Barr virus, cytomegalovirus and other viruses have all been implicated in syndromes characterised by fatigue-like symptoms that can persist for more than six months (Collignon et al., 2016). Chalada and colleagues discussed fibromyalgia and CFS in relation to DSCATT with key points presented in the following table.

Table 8: Fibromyalgia and chronic fatigue syndrome in relation to DSCATT, as per Chalada et al. (2016)

Non-infectious disease	Symptoms
Fibromyalgia	• Widespread musculoskeletal pain, hyperalgesia, fatigue, insomnia, memory loss and poor concentration, headache and irritable bowel syndrome. • Diagnosed based on widespread musculoskeletal pain, sensitivity in a number of 'tender spots', and the presence of other associated symptoms such as headaches, sleep disturbances and memory loss. • Fibromyalgia may present as sequelae of infections with *C. burnetti, Chlamydophilia pneumoniae,* Epstein-Barr virus and Parvo-virus B19.
Chronic fatigue syndrome (CFS)	• Very similar to fibromyalgia in that it is a syndrome of unknown aetiology characterised by persistent fatigue, musculoskeletal pain, insomnia and cognitive impairment and headaches. • CFS and fibromyalgia commonly co-occur with evidence suggesting that the two syndromes are merely symptom amplification of the same somatic syndrome. • Both syndromes are more common in women than men. • CFS diagnosis is based on onset of unexplained persistent or relapsing chronic fatigue that is not substantially alleviated by rest, accompanied by symptoms including short term memory or poor concentration, sore throat or lymph nodes, muscle or joint pain and headaches. • CFS may present as sequelae of infections with *C. burnetti, Chlamydophilia pneumoniae,* Epstein-Barr virus and Parvo-virus B19.

3.8.3. Diagnostic testing in patients presenting with unresolved debilitating symptoms and no diagnosis

Investigations should be underpinned by clinical evidence. International evidence indicates patients with MUS are at risk of potentially harmful additional testing (Olde Hartman et al., 2017) and are often subjected to repeated diagnostic investigations, and unnecessary and costly referrals and interventions (Royal College of Psychiatrists, 2017). Unnecessary investigations that do not show anything, are often not reassuring. They can make someone worry even more that there is something still to be found and more tests are needed.

For fatigue, diagnostic testing is determined by the differential diagnosis as per normal clinical practice (Murtagh, 2003). Fatigue was the most prevalent self-reported symptom among patients whose submissions to the Senate Inquiry were analysed (Brown, 2018). An Australian diagnostic model for the diagnosis of fatigue in general practice by Murtagh and published by RACGP concluded that before diagnosing tiredness as psychological, pathological as well as physical causes must be excluded. Additionally, GPs must be perceptive in their approach without resorting to over investigation. Key points to GPs included:

- when patients complain of fatigue, believe them
- always consider underlying psychological distress, especially depressive order
- take a searching history particularly regarding lifestyle and drug intake
- keep in mind haemochromatosis - the key test is transferrin saturation (Murtagh, 2003, p. 876).

3.8.4. Diagnosis of medically unexplained symptoms

People with MUS may obtain a diagnosis over time as symptoms develop and guide to the origin of the illness. Others may find that symptoms resolve over time without ever identifying a cause.

The diagnosis of MUS, including the identification of the symptom complex associated with DSCATT is a diagnosis of exclusion and requires ongoing review as new symptoms arise or treatments are trialled. A full history and examination are critical.

4. RESEARCH QUESTION 3: WHAT ARE THE CURRENT ISSUES ASSOCIATED WITH DIAGNOSTIC TESTING FOR LYME DISEASE IN AUSTRALIA AND BY OVERSEAS LABORATORIES?

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4.1. Overview and key findings

This section provides the findings of the literature reviewed to answer research question 3:

What are the current issues associated with diagnostic testing for Lyme disease both in Australia and by overseas laboratories?

Lyme disease is a recognised and documented infectious disease, defined as being caused by the bacteria *Borrelia burgdorferi* s. l**.** endemic in the US, Europe and Asia. Despite multiple studies which have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australian ticks.

While this section focusses specifically on issues associated with diagnostic testing for Lyme disease in Australia and in overseas laboratories, it also addresses the concerns raised to the Senate Inquiry about diagnostic testing used to diagnose patients who identified as having Lyme disease or Lyme-like illness. It also highlights the issues internationally with inappropriate diagnostic testing and the use of unvalidated diagnostic tests to diagnose patients with Lyme disease or 'chronic Lyme disease'. Acknowledging the limitations with current diagnostic tests, international developments in diagnostic testing being undertaken via the CDC and National Institutes of Health (NIH) are also briefly covered.

4.1.1. Key findings

Diagnostic tests are used to help identify those cases in which Lyme disease is the cause.

The symptoms of Lyme disease, other than EM, such as facial palsy, joint pains or nerve pains can be seen in many other conditions. Diagnostic tests are used to help identify those cases in which Lyme disease is the cause, so that appropriate treatment can be given and ensure that other important diseases are not misdiagnosed as Lyme disease. It is important that the tests used have both the ability to identify infection with the Lyme disease bacteria and to discriminate this from other causes of infection or disease. Following the discovery of *B. burgdorferi* as the causative agent of Lyme disease, numerous tests were developed by clinical and private laboratories. As direct detection of *B. burgdorferi* by PCR or culture has been challenging, most diagnostic test development has focussed on indirect detection of infection by assessing the antibody response of the patient. As such, indirect tests through serological assays for antibodies to *B. burgdorferi* s.l. are the mainstay of laboratory diagnosis, the most common diagnostic methodologies employed, the prerequisite laboratories facilities are widely available and specimens are easy to obtain.

There is strong international consensus on the two-tier serology test protocol for diagnosing Lyme disease.

There is strong international consensus on the use of the two-tier serology testing protocol within the literature and by international authorities and guidelines. A 2019 review of European and US guidelines (16 guidelines from seven countries) indicated that the diagnosis of Lyme disease is currently based on a two-tier serology at all stages of infection except for the early localised dermatological presentation, EM.

The CDC and other international guidelines recommend a two-tier serology approach to improve specificity, the two steps consisting of a sensitive EIA or ELISA, followed by immunoblotting (Western blot) of samples that are positive or indeterminate in the first step with strict interpretative criteria. The rationale for this approach is that the overall sensitivity

and specificity are maximised when these tests are performed in sequence. The final result of serological testing is considered positive only when the ELISA is reactive (positive or equivocal) and the Western blot is also positive. Thus, the two-tiered system maximises the sensitivity and specificity of the assays and increases the likelihood of observing a seroconversion (from IgM to IgG) that is evident in most *bona fide B. burgdorferi* infections. Not following the two-tiered algorithm (for example, performing a Western blot alone or after the ELISA is negative) can increase the frequency of false positive results, with false positive results potentially leading to possible misdiagnosis and unnecessary treatment.

Very recent (2019) international guidance from IDSA/AAN/ACR in the US advises that serologic (serum antibody) testing is highly sensitive in patients with non-cutaneous manifestations of Lyme disease, as these manifestations typically develop after weeks to months of infection; serologic testing is also highly specific when performed and interpreted according to current guidelines; predictive value is increased when results are correlated with clinical features, patient history and risk factors; and currently, the only FDA cleared or approved diagnostic tests for Lyme disease are antibody tests.

There are recognised limitations of serology tests for Lyme disease and the usefulness of serological tests for Lyme disease depend on the pre-test probability and subsequent predicative values in the setting where the tests are being used.

Currently available tests for Lyme disease carry limitations. All diagnostic tests produce both false positive and false negative results. The frequency depends on the specificity and sensitivity of the test and the prevalence of the disease in the population. Four systematic reviews, some with meta-analysis, all found accuracy of serology tests increased with progression of the disease, with test sensitivity increasing with progression of *B. burgdorferi* infection from early to late. However, all reviews found marked variation and heterogeneity in study findings of sensitivity and specificity for each test technology, whether it be ELISA, Western blot, or two-tiered test methodology. The three reviews that assessed study quality all found the studies to be at high risk of bias.

While both NICE and IDSA/AAN/ACR, in their guidelines, identify the currently available protocol as reliable when used appropriately, both note limitations of the testing protocol. Limitations highlighted include: that false positive and false negative results can occur; and possible reduction of accuracy of the test can occur if testing is carried out too early (before antibodies have developed) or the person has reduced immunity, for example in people on immunosuppressant treatments. Additionally, in a seropositive patient it can be difficult to determine whether antibody reactivity is due to past infection, active/current infection, or both.

The interpretation of serological assays in Lyme disease requires an understanding of the clinical indications and limitations of the tests, and the usefulness of serological tests for Lyme disease depends on the pre-test probability and subsequent predicative values in the setting where the tests are being used. The most common cause of poor performance in serological testing (as in other infectious diseases diagnosed by antibody testing) is their use in unselected patient populations with a low pre-test probability of Lyme disease. The most crucial factor governing pre-test probability for Lyme disease is exposure history.

Despite multiple studies which have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australia. In areas not endemic for Lyme disease [for example, Australia] the positive predictive value of the serology test will be low. Some people believe that they have acquired Lyme disease in Australia because the results of screening antibody tests to *B. burgdorferi* are positive.

However, where a patient has not travelled overseas, these positives are all likely to be false positive test results. Even a highly specific test will produce some false positives, so that people who have never been exposed to *B. burgdorferi* can have reactive antibody results. Tests for Lyme disease should only be requested if there is well-founded suspicion of Lyme disease and not in situations of low pre-test probability, to minimise the risk of a false positive result. In an area of low Lyme disease incidence in the US, a study of Lyme disease testing showed an 80 per cent false positive rate, which puts patients at risk of incorrect Lyme disease diagnosis and adverse drug reactions from inappropriate treatment.

Noting the limitations of serology testing, there is significant international work being undertaken to improve laboratory diagnosis of Lyme disease, particularly by the NIH in the US.

Diagnostic testing for Lyme disease in Australia follows international best practice.

In a country such as Australia where Lyme disease is not endemic and is not commonly seen in clinical practice, there are additional challenges in diagnosing Lyme disease. The current standard laboratory protocol for diagnosing Lyme disease in Australian diagnostic laboratories follows international best practice and uses a two-tier serology system. There is established Australian guidance for diagnostic testing for Lyme disease.

In Australia, laboratory diagnostic testing for Lyme disease is required for two reasons:

1. Unless the clinician is familiar with the path pathognomonic EM rash, it is clinically safer to obtain supportive evidence of infection through diagnostic testing (culture or PCR of the tissue or more usually antibody testing on a convalescent sample).
2. Diagnostic laboratory support is preferred for patients presenting with non-specific signs and symptoms of a disease syndrome, notwithstanding the limitations of the tests.

Diagnostic testing for Lyme disease should only be initiated following advice from appropriate experts such as a consultant ID physician or a specialist microbiologist and should only be undertaken in Australia in a pathology laboratory accredited by NATA and Royal College of Pathologists of Australasia (RCPA) to conduct such testing.

NATA accreditation is highly regarded both nationally and internationally as a reliable indicator of technical competence. All pathology laboratories in Australia receiving funding via Medicare must be accredited by the NATA/RCPA Laboratory Accreditation Program. In Australia, the NRL review of serological assays to diagnose Lyme disease determined the tests used by accredited laboratories to diagnose Lyme disease had equivalent reliability to tests used in overseas laboratories. This therefore means Australian NATA/RCPA accredited laboratories are able to confidently diagnose classical Lyme disease acquired in patients who have travelled to endemic areas and have contracted the infection more than four weeks prior to testing, noting that most patients seroconvert within four to eight weeks of infection. A follow up paper to the NRL report noted that in the known negative population, specificities of the immunoassays ranged between 87.7 per cent and 99.7 per cent, and in Australia's low prevalence population this would translate to a positive predictive value of <4 per cent.

There are commercially available laboratory testing methods to be avoided.

Unvalidated commercially available laboratory testing methods not recommended, based on evidence, by international authorities and guidelines such as the CDC and IDSA/AAN/ACR include: non-standard serology interpretation, urine antigen or DNA testing, lymphocyte

transformation tests, quantitative CD57 lymphocyte assays, culture, immunofluorescence staining, or cell wall-deficient or cystic forms of *B. burgdorferi*, 'Reverse Western Blots', in-house criteria for interpretation of immunoblots, measurements of antibodies in joint fluid (synovial fluid) and IgM or IgG tests without previous ELISA/EIA/IFA.

The quality of the evidence on the current issues associated with diagnostic testing for Lyme disease in Australia and in international laboratories is robust and meets the scientific quality to underpin an evidence-based clinical pathway.

4.1.2. Literature reviewed

Australian Government reports	(Senate Community Affairs References Committee, 2016a, 2016b)
Australian Department of Health reports, reports to, and guidance	(Department of Health, 2018c; Lum et al., 2015; Mackenzie, 2013; National Serology Reference Laboratory Australia, 2017)
(Inter)national authority and intergovernmental reports, evidence reviews, guidelines and guidance	(Brunton et al., 2017; Centers for Disease Control and Prevention, n.d., 2018, 2019c; Mead et al., 2019; Moore et al., 2016; National Institute for Health and Care Excellence, 2018b, 2018j; National Institute of Allergy and Infectious Diseases, 2019)
Guidelines and guidance (International and Australian) by clinical and professional bodies	(Dessau et al., 2018; Lantos et al., 2019; Royal College of Pathologists of Australasia, n.d., 2019)
Systematic Reviews (with/without meta-analysis)	(Cook & Puri, 2016; Leeflang et al., 2016; Waddell et al., 2016)
Narrative literature reviews and reviews	(Aguero-Rosenfeld & Wormser, 2015; Auwaerter et al., 2011; Borchers et al., 2015; Chalada et al., 2016; Collignon et al., 2016; Eldin et al., 2019; Halperin, 2015; Lindsay et al., 2014; McManus & Cincotta, 2015)
Randomised controlled trials	
Prospective cohort studies	
Case control studies	
Observational studies	(Brown, 2018; Kobayashi et al., 2019)
Research articles	(Best et al., 2019)
Other resources and websites	(Australian Rickettsial Reference Laboratory, n.d.; National Association of Testing Authority, Australia, n.d.)

4.1.3. Quality of the evidence

The evidence base on the current issues associated with diagnostic testing for Lyme disease in Australia and in international laboratories is robust. We drew heavily on recommendations, guidelines and guidance from international authorities and Australian and international clinical professional associations. Literature reviewed also included four systematic or evidence-based reviews with or without meta-analyses, and several

international reviews by prominent authors in the field of diagnostic testing for Lyme disease. Australian evidence included reports to the Department of Health, and guidance from Australian authorities.

4.2. Issues raised about diagnostic testing for Lyme disease in Australia

The Senate Committee reported that diagnostic testing of samples, usually blood, taken from patients suspected of having Lyme disease was perhaps the most controversial issue to emerge from the inquiry. Observations reported by the committee included:

- much, if not most, of the evidence presented was contradictory with most of it confidently articulated by qualified, experienced and respected professionals
- a number of prominent and experienced doctors have questioned the reliability of laboratory tests used to diagnose or rule out Lyme-like illness, classical and 'chronic' Lyme disease or other Lyme-like illnesses
- the issue of test quality was a key part of the matter (i.e. understanding which testing protocol is optimal and how the tests are to be interpreted),
- that the question can be seen from two perspectives:
    - classical Lyme disease, caused by *Borrelia* bacteria, cannot be contracted in Australia - a position held by the Australian medical authorities, and many experts in relevant fields and supported by the fact that accredited Australian laboratories return negative results when testing for Lyme disease
    - an illness with considerable similarities to Lyme disease can and has been contracted in Australia and pathogens that cause Lyme disease do exist here - a position held by some doctors and scientists and supported by the fact that patients who have not travelled overseas have had positive laboratory test results when tested for Lyme disease by some Australian and overseas laboratories (Senate Community Affairs References Committee, 2016b).

Other matters identified or debated with respect to diagnostic testing for Lyme disease in Australia included:

- the reliability (including concerns around sensitivity, specificity, false positives and false negatives) of the two-tier testing serological diagnostic protocol
- the issue of discordant results between accredited laboratories in Australia and non-accredited Australian and overseas laboratories
- the use of and reliability of PCR testing by one unaccredited Australian laboratory
- the preference by some Australian medical practitioners to send test samples to laboratories in Germany:
    - in the belief they are better placed to test for *Borrelia,*
    - including that they will do the Western blot if requested, whereas Australian laboratories will only do so if the ELISA test is positive (Senate Community Affairs References Committee, 2016a, 2016b).

Discordant laboratory results between accredited laboratories in Australia and non-accredited Australian and overseas laboratories were seen to cause confusion and frustration for patients (Senate Community Affairs References Committee, 2016a).

The Senate Committee acknowledged evidence provided by Australian Medical authorities indicating that accredited laboratories, following established best-practice testing processes, have not found classical Lyme disease in Australian patients, with the exception of those who most likely contracted the disease overseas. The Senate Inquiry also noted the Australian

Department of Health had contracted the NRL to conduct a review of serological assays used in Australia and overseas to diagnose Lyme disease. (Senate Community Affairs References Committee, 2016b). This review is discussed in 4.6.1 NATA/RCPA Accreditation and Accredited Laboratories.

Patients told the Senate Inquiry about diagnostic testing to support their diagnoses. In his analysis of submissions by patients to the Senate Inquiry, Brown reported on diagnosis, including diagnostic laboratory testing, and other methods of diagnosis. (Brown, 2018). Brown's findings are described below.

Regarding the diagnostic testing that had supported submitters' diagnoses, Brown reported that nearly three-quarters (508 patients; 72.8 per cent of the 689 submitters reported data on 'any' diagnostic laboratory testing. Of the 137 submissions that disclosed a NATA/RCPA accredited diagnostic pathology test, only 14 patients; (10.2 per cent) reported positive serology (representing 2.8 per cent of all who reported pathology and 2.0 per cent of all submissions). Ten patients had travelled overseas and the four other patients who had either not travelled overseas or did not mention travel did not report the result of confirmatory (Western blot) serological testing. Additionally, two patients reported they had contracted Lyme disease overseas (US and France) and another two patients who reported travel also reported explicitly that only first-tier testing was positive. Of the small minority of patients who reported a positive Lyme disease serology test from a NATA/RCPA accredited laboratory, Brown commented that a proportion of these may be positives from overseas exposure unrelated to their current illness, and some may represent only positive first-tier testing and not confirmatory testing as required by the RCPA position statement on laboratory testing for Lyme disease (Royal College of Pathologists of Australasia (2016) in Brown, 2018).

Additionally, nearly one in ten patient submissions (68, 9.8 per cent) reported having self-diagnosed with Lyme disease after media reports, with a similar proportion (67, 9.8 per cent) reported having self-diagnosed with Lyme disease by research or on the internet. Two submissions (0.3 per cent) reported Lyme disease was acquired congenitally (Brown, 2018).

Table 9: Diagnostic information reported in submissions

Diagnostic method	Number and per cent of all patients	Number and per cent of patients who reported data
Diagnostic laboratory testing		
Any	508 (72.8 per cent)	508 (100 per cent)
Pos NATA/RCPA	14 (2.0 per cent)	14 (2.8 per cent)
Neg NATA/RCPA	123 (17.6 per cent)	123 (24.2 per cent)
Pos non-NATA/RCPA	454 (65.0 per cent)	454 (89.4 per cent)
Neg non-NATA/RCPA	27 (3.9 per cent)	27 (5.3 per cent)
Neg NATA/RCPA, Pos non-NATA/RCPA	83 (11.9 per cent)	83 (16.4 per cent)

Source: Brown, 2018

4.3. The need for diagnostic testing in Lyme disease

The symptoms of Lyme disease, other than EM, such as facial palsy, joint pains or nerve pains can be seen in many other conditions (Borchers et al., 2015; National Institute for Health and Care Excellence, 2018j). Diagnostic tests are used to help identify those cases in which Lyme disease is the cause, so that appropriate treatment can be given and ensure that other important diseases are not misdiagnosed as Lyme disease. It is important that the tests used have both the ability to identify infection with the Lyme disease bacteria and to discriminate this from other causes of infection or disease (National Institute for Health and Care Excellence, 2018j). As all manifestations of Lyme disease except EM are not specific and require laboratory confirmation, it is vital to obtain a detailed history in order to establish probable exposure to *Ixodes* ticks at an appropriate time of year and to obtain appropriate and definitive laboratory confirmation (Borchers et al., 2015; Eldin et al., 2019; Royal College of Pathologists of Australasia, 2019).

As discussed in 3.5.7 Situation in Australia in considering a differential diagnosis of Lyme disease, despite multiple studies that have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australia. This means that Australia is a non-endemic country for Lyme disease, and it is not possible to reliably diagnose Lyme disease on clinical symptoms and signs alone. Laboratory testing is essential, as many other infectious and non-infectious diseases can have similar features to Lyme disease (Lum et al., 2015; Royal College of Pathologists of Australasia, 2019) and all stages of Lyme disease have features that mimic other medical conditions.

4.4. Overview of the challenges in laboratory techniques to diagnose Lyme disease

There are a wide variety of diagnostic tests available to assist in the diagnosis of Lyme disease, and there is considerable debate about the accuracy and reliability of some of the tests. At this time, there is no single laboratory test that is 100 per cent sensitive and specific (that is, there is no perfect laboratory test) for the confirmation of Lyme disease. A further complication is that not all individuals who are infected with *B. burgdorferi* present in the same way (Lindsay et al., 2014).

The normal hierarchy of laboratory tests used for diagnosis of an infectious disease is:

1. Culture of the causative microbe from a patient sample in the laboratory.
2. Detection of the DNA/RNA of the causative microbe in a patient sample by molecular detection methods (for example, PCR followed by a gene or genome sequencing).
3. Detection of antibodies in the patient's serum, directed against antigens of the known causative microbe, through serology (Royal College of Pathologists of Australasia, 2019).

The culture of *Borrelia* bacteria is difficult: culture is expensive and requires special media and laboratory expertise, the number of spirochaetes in clinical specimens is low and culture is used/attempted usually only in reference laboratories (Borchers et al., 2015; Collignon et al., 2016; Halperin, 2015; Lum et al., 2015; Moore et al., 2016; National Institute for Health and Care Excellence, 2018b; Royal College of Pathologists of Australasia, 2019). While other tests such as PCR can identify fragments of bacteria DNA, they are not useful for the majority of people with Lyme disease (National Institute for Health and Care Excellence, 2018b). These techniques are mostly used in research laboratories as opposed to diagnostic laboratories and are discussed in more detail in 4.7 Less commonly used laboratory methods for direct detection of *B. burgdorferi* in clinical tissue specimens.

As a result, laboratory support for the diagnosis of Lyme disease relies on testing the host response to the infecting organism with blood tests looking for antibodies to the Lyme disease bacteria *B. burgdorferi* s.l. (serological tests) being the most common tests performed when Lyme disease is suspected (Aguero-Rosenfeld & Wormser, 2015; Borchers et al., 2015; Centers for Disease Control and Prevention, 2019c; Dessau et al., 2018; Eldin et al., 2019; Halperin, 2015; Lantos et al., 2019; National Institute for Health and Care Excellence, 2018j; Royal College of Pathologists of Australasia, 2019). While currently the mainstay of laboratory diagnosis for Lyme disease internationally and in Australia, serology testing has acknowledged limitations, with variables and considerations that can affect the diagnostic accuracy of the tests. These limitations are discussed in 4.5.4 Latest international guidance and advice to health professionals and patients about two-tier testing for Lyme disease and 4.5.6 Considerations, limitations and important variables in serology testing for Lyme disease.

Noting the limitations of serology testing, there is significant international work being undertaken to improve laboratory diagnosis of Lyme disease, particularly by the NIH in the US. These international developments are covered in 4.9 International developments and recommendations in testing for Lyme disease.Importantly, within the body of literature reviewed, including international guidelines and international authority advice on diagnostic testing for Lyme disease, there are unvalidated tests for Lyme disease that are not

recommended. These tests are covered in 4.8 Commercially available laboratory testing methods to be avoided.

4.5. Serology testing

Most diagnostic test development has focussed on indirect detection of infection by assaying the antibody response of the patient and as such, the most widely used laboratory method to confirm infection and diagnose Lyme disease is through testing for antibodies to *B. burgdorferi* sp. through serology testing (Aguero-Rosenfeld & Wormser, 2015; Borchers et al., 2015; McManus & Cincotta, 2015; Moore et al., 2016).

Tests for detection of antibodies are not perfect, as they depend on factors such as the duration it takes for detectable levels to be produced in an infected individual (which may be several weeks after infection), and the quality of the method used to detect them, the latter involving using antigens that have desirable sensitivity and specificity to confirm the presence of antibodies that react with *B. burgdorferi* (Aguero-Rosenfeld & Wormser, 2015).

4.5.1. The serological immune response to *B. burgdorferi* sp. infection

As with most spirochaetes, such as *Treponema pallidum,* the agent of syphilis, infection with *B. burgdorferi,* leads to the production of antibodies (Aguero-Rosenfeld & Wormser, 2015). Disease development depends on the immune response, and most patients will already have detectable antibodies at the time of clinical presentation, except in patients with clinical disease of short duration (Dessau et al., 2018).

Infection with *B. burgdorferi* s.l. leads initially to an IgM antibody response, followed two weeks later by an IgG antibody response (Mackenzie, 2013). Antibodies against *Borrelia* spp are slow to develop, with IgM generally not being detectable for the first one to two weeks after infection and IgG often not appearing for four to six weeks (Borchers et al., 2015). As noted by Dessau et al. in their position paper of ESGBOR, the ESCMID study group for Lyme borreliosis, antibodies are expected to develop in almost all patients (> 99 per cent) within six to eight weeks (Stanek et al. (2011), Wilske et al. (1983), Hansen et al. (1998), Hansen and Asbrink (1989), Hansen and Lebech (1991), Wormser et al. (2006), and Hansen (1994) in Dessau et al., 2018). Aguero-Rosenfeld and Wormser concurred, and advised by six weeks following infection, virtually all should be seropositive (Wormser et al. (2006) in Aguero-Rosenfeld & Wormser, 2015).

The IgM response tends to be relatively short-lived in most patients, but the IgG remains for decades following infection (Mackenzie, 2013). In some individuals an IgM antibody response can persist for months or even years, or for life after treatment or past infection, although Dessau et al. noted this is not associated with a (persistent) infection with *B. burgdorferi* (Kalish et al. (2001), Glatz et al. (2006), Seriburi et al. (2012), and Goosens et al. (1999) in Dessau et al., 2018).

However, while most patients infected with *B. burgdorferi* would manifest a classical immune response as described above, approximately 50 per cent of patients with EM remain seronegative (Dessau et al., 2018). Borchers et al. similarly noted that some patients with EM as their only manifestation may never seroconvert (Aguero-Rosenfeld et al. (1993) and Engstrom et al. (1995) in Borchers et al., 2015), particularly in Europe (Strle et al. (1996), Oski et al. (2001), and Glatz et al. (2006) in Borchers et al., 2015).

This natural history has important implications, both for the timing of successful serological testing, and for the ability (or not) to differentiate between ongoing infection and residual antibody responses to previous infection (Aguero-Rosenfeld & Wormser, 2015).

4.5.2. Serology testing for Lyme disease

Following the discovery of *B. burgdorferi* as the causative agent of Lyme disease, numerous tests were developed by clinical and private laboratories. As mentioned above, as direct detection of *B. burgdorferi* by PCR or culture has been challenging because spirochaetes only transiently enter the bloodstream, most diagnostic test development has focussed on indirect detection of infection by assessing the antibody response of the patient (Moore et al., 2016). As such, indirect tests through serological assays for antibodies to *B. burgdorferi* s.l. are the mainstay of laboratory diagnosis, the most common diagnostic methodologies employed, the prerequisite laboratories facilities are widely available, and specimens are easy to obtain (Mackenzie, 2013).

Serologic testing has developed over time, with most efforts aiming to improve specificity. Initial work used ELISAs using sonicated whole organisms as the target antigens, with a number of interpretive criteria chosen to try to balance sensitivity and specificity (Halperin, 2015). Halperin noted extensive studies in the early 1990s with large populations of patients with and without Lyme disease led to the currently recommended two-tier approach (Dressler et al. (1993), and CDC (1995) in Halperin, 2015) using a highly sensitive ELISA as a screening test, and then a Western blot to provide specificity (Halperin, 2015).

Two-tiered serologic testing

Evaluation of evidence in 1994 by the Second National Conference on the Serologic Diagnosis of Lyme disease found that no single test was sufficient on its own (Moore et al., 2016). Borchers et al. also noted this finding, reporting that single serological tests yield false positive results in people with other spirochaetal infections, such as spirochaetal infection of gums, a very common infection (Borchers et al., 2015). In 1995 there was an attempt to standardise and improve specificity of Lyme disease antibody testing. The CDC and other agencies and guidelines, including in Europe, recommended the use of a two-step algorithm (Aguero-Rosenfeld & Wormser, 2015; Borchers et al., 2015; Collignon et al., 2016; Moore et al., 2016).

The first tier uses a highly sensitive EIA (ELISA) or rarely indirect immunofluorescence assay (IFA) that detects IgG and IgM antibodies, and that, if the result is positive or equivocal, is followed by a highly specific Western blot run on the same sample with strict interpretative criteria (Aguero-Rosenfeld & Wormser, 2015; Borchers et al., 2015; Centers for Disease Control and Prevention, 2019c; Collignon et al., 2016; Lindsay et al., 2014; Mackenzie, 2013; Moore et al., 2016; National Institute for Health and Care Excellence, 2018j; Royal College of Pathologists of Australasia, 2019).

Serology involving screening with EIA followed, if positive, by an immunoblot assay is the current standard protocol in Australian Reference Laboratories (Lum et al., 2015; Royal College of Pathologists of Australasia, 2019).

The rationale for this approach is that the overall sensitivity and specificity are maximised when these tests are performed in sequence (Lindsay et al., 2014). The final result of serological testing is considered positive only when the ELISA is reactive (positive or equivocal) and the Western blot is also positive. Thus the two-tiered system maximises the sensitivity and specificity of the assays and increases the likelihood of observing a seroconversion (from IgM to IgG) that is evident in most *bona fide B. burgdorferi* infections (Lindsay et al., 2014).

Second level or confirmatory tests usually involve immunoblot tests, notably Western blots. These involve separating out antibodies that detect different antigens by electrophoresis, then revealing antibodies picking up these antigens as stained bands on gel plates. Collignon et al. commented that second assays are similarly used to maximise specificity when diagnosing HIV and syphilis infections (Collignon et al., 2016). The CDC notes that serological test results must be interpreted according to strict criteria, including clinical presentation, a thorough history, and whether Lyme disease is endemic to a particular area (Moore et al., 2016).

Not following the two-tiered algorithm (for example, performing a Western blot alone or after the ELISA is negative) can increase the frequency of false positive results, with false positive results potentially leading to misdiagnosis and unnecessary treatment (Lindsay et al., 2014).

When performed and interpreted in accordance with current guidelines, Moore et al. noted two-tiered serologic testing is a valuable and highly specific clinical tool for diagnosis of disseminated Lyme disease, citing evidence for a sensitivity of ~70 per cent - 100 per cent and a specificity >95% for disseminated Lyme disease (Moore et al., 2016). Aguero-Rosenfeld and Wormser concurred, commenting that despite recognised limitations (discussed further below), antibody detection following the CDC guidelines performs well for patients who have objective manifestations consistent with Lyme disease other than EM (Aguero-Rosenfeld & Wormser, 2015). As such, two-tier serologic testing is the standard of care in diagnosing disseminated Lyme disease, but Moore et al. cautioned the analysis requires appropriate clinical judgement when ordering the test and interpreting the results (Moore et al., 2016).

First tier

The first test tier involves measuring the overall antibody response (typically IgM and IgG) of a patient to *B. burgdorferi* antigens. It is used as a screening test to detect IgM and/or IgG antibodies in serum that are directed against the bacterium that causes Lyme disease (Lindsay et al., 2014).

First level assays currently in use include Enzyme-linked Assays (ELISA), usually revealed with either immunofluorescence or peroxidase. The antigen capture layers vary – consisting either of bacterial fragments, or genetically engineered recombinant antigens from specific organisms. Some combine multiple strains. Some detect both IgM and IgG specific antibodies, others just IgG (National Institute for Health and Care Excellence, 2018b).

While both the ELISA and IFA have been approved (by the FDA) as first tier tests, ELISA is more easily automated (and therefore more commonly performed) with the additional benefit of ELISA being that it provides a quantitative value of the relative concentrations of antibodies in the serum compared with that of a control, enabling use of objective cut-off values (Moore et al., 2016). In addition to the beneficial quantifiable nature of the ELISA, Mackenzie (2013) noted the ELISA was more sensitive than IFA. However, while most immunoassays are highly sensitive, they may lack specificity (that is, false positives can occur as a result of other medical conditions) (Lindsay et al., 2014).

Moore et al. reported that (at the time of publication) most laboratories in the US use a whole cell sonicate preparation of *B. burgdorferi* antigen for the ELISA, with this test approach having high sensitivity because of multiple antigens in the whole-cell preparation; however, because some of these antigens are cross-reactive with antigens from the host or other pathogens, specificity of the ELISA alone is not optimal (Aguero-Rosenfeld et al. (2005) in Moore et al., 2016).

Second tier

The Western blot is used as a corroborative test and has greater specificity than the enzyme immunoassay. It detects antibodies in serum that are directed against electrophoretically separated antigen extracts and recombinant antigens native to *B. burgdorferi* (Lindsay et al., 2014). Antibody reactivity to these antigens (indicated by bands on the Western immunoblot) is considered present if bands are visualised with intensity equal to or greater than the control (Moore et al., 2016). Western immunoblot was included in response to a multicentre evaluation of laboratories performing Lyme disease testing which had found that using the Western immunoblot in addition to EIA increased specificity to >98 per cent, reducing false positives produced by the first tier-EIA (Craven et al (1996) in Moore et al., 2016).

The use of an immunoblot as a first line or stand-alone test is not recommended (Aguero-Rosenfeld & Wormser, 2015; Dessau et al., 2018). Aguero-Rosenfeld and Wormser cautioned against the use of immunoblots as first stage tests due to their lack of sensitivity, but also due to most, if not all, *B. burgdorferi* antigens being cross-reactive; meaning that immunoblot interpretation is dependent on experience, and the number, position on the gel, and type of immunoreactive bands that are found (Aguero-Rosenfeld & Wormser, 2015).

Halperin (2015) explained Western blot criteria were not selected based on the uniqueness of any *Borrelia* epitopes, but rather on statistical analyses of findings to identify those combinations with the greatest positive and negative predictive values. As a result of these studies, a set of three IgM and ten IgG bands were selected. Patients with early disease typically have at least two of the three IgM bands, while patients with long standing disease typically have at least five of the ten IgG bands. Halperin noted two important facts need to be kept in mind.

1. The Western blot criteria were developed in individuals with positive or borderline ELISAs; as such, interpretation in patients with negative ELISAs is quite problematic and should only be attempted with great caution.
2. The IgM tests are quite cross-reactive, so false positives are commonplace. Patients with disease of more than one-month or two-month duration should be IgG seropositive, so only IgG plots provide reliable information. Any IgM findings in this setting should be considered at best, uninterpretable, and more correctly as spurious.

The second tier should not be performed if the first-step enzyme assay is negative. Aguero-Rosenfeld and Wormser (2015) explained the reason for this is that most first-step assays generate an objective value (as noted above) that corresponds with the intensity of the antibody reaction, as opposed to second-step immunoblots that, for the most part, are read and interpreted visually. This subjective reading and interpretation can lead to erroneous positive results if weak bands are scored as positive in samples with negative enzyme immunoassay.

This addresses one of the points raised to the Senate Inquiry by some practitioners who send their patient's samples to some Germany laboratories for testing as it was reported that these laboratories will undertake the immunoblot when the ELISA is negative (Senate Community Affairs References Committee, 2016a, 2016b).

Surveillance versus Clinical Diagnostic testing - Misconception about the 2-tiered serologic analysis

Moore et al. (2016), in their continuing medical education (CME) article, noted the inaccuracy of the misconception that the two-tiered serologic analysis is intended only for surveillance rather than patient diagnosis. They explained the inaccuracy is an apparent conflation of clinical serologic testing recommendations for Lyme disease and the surveillance case definition of the Council of State and Territorial Epidemiologists (citing http://wwwn.cdc.gov/nndss/conditions/lyme-disease/). They noted that recommendations for two-tiered testing are meant to aid diagnosis of individual patients in the clinical setting and that while serologic test results might be used by public health officials to determine whether a given illness meets the surveillance case definition, the methods themselves were not developed for this purpose. Furthermore, they noted that for practical reasons, serological results might be used slightly differently in surveillance than is recommended in the clinical setting. An example being that a positive IgG result by Western immunoblot alone is accepted as laboratory evidence of infection for surveillance purposes whereas it is not recommended to perform Western immunoblot without a first tier ELISA for laboratory diagnosis. Moore et al. explained that this operational definition enables simplification of reporting practices because it can be difficult to track down records of the first-tier test. However, they state 'it does not represent best clinical practice' (Moore et al., 2016, p. 1175).

Since the publication of Moore's CME article (cited on the CDC website) the CDC updated its Case definition for Lyme disease in 2017 (Centers for Disease Control and Prevention, n.d.).

The Laboratory Criteria for Diagnosis in the CDC's 2017 Case definition for Lyme disease (Centers for Disease Control and Prevention, n.d.) is detailed below.

For the purposes of surveillance, laboratory evidence includes:

- A positive culture for *B. burgdorferi*, **OR**
- A positive two-tier test. (This is defined as a positive or equivocal enzyme immunoassay (EIA) or immunofluorescent assay (IFA) followed by a positive Immunoglobulin M[1] (IgM) or Immunoglobulin G [2] (IgG) Western immunoblot (WB) for Lyme disease) **OR**
- A positive single-tier IgG[2] WB test for Lyme disease[3].

[1] IgM WB is considered positive when at least two of the following three bands are present: 24 kilodalton (kDa) outer surface protein C (OspC)*, 39 kDa basic membrane protein A (BmpA), and 41 kDa (Fla). Disregard IgM results for specimens collected >30 days after symptom onset.

[2] IgG WB is considered positive when at least five of the following 10 bands are present: 18 kDa, 24 kDa (OspC)*, 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa flagellin (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa.

[3] While a single IgG WB is adequate for surveillance purposes, a two-tier test is still recommended for patient diagnosis.

*Depending upon the assay, OspC could be indicated by a band of 21, 22, 23, 24 or 25 kDA.

4.5.3. International consensus on the use of two-tier serology testing for diagnosis of Lyme disease

There is strong international consensus on the use of the two-tier serology testing protocol within the literature and by international authorities and guidelines (Centers for Disease Control and Prevention, 2019c; Dessau et al., 2018; Eldin et al., 2019; Lantos et al., 2019; National Institute for Health and Care Excellence, 2018j).

The CDC recommendations for diagnosis and testing for Lyme disease are:

> CDC currently recommends a two-step testing process for Lyme disease. Both steps are required and can be done using the same blood sample. If this first step is negative, no further testing is recommended. If the first step is positive or indeterminate (sometimes called 'equivocal'), the second step should be performed. The overall result is positive only when the first test is positive (or equivocal) and the second test is positive (or for some tests equivocal) (Centers for Disease Control and Prevention, 2019c).

NICE (2018j) recommends the following laboratory investigations to support diagnosis of Lyme disease.

> Diagnose and treat Lyme disease without laboratory testing in people with EM. Laboratory testing is unnecessary for people presenting with EM because the rash is very specific to Lyme disease and prompt treatment will prevent further symptoms developing. However, as most other symptoms associated with Lyme disease have other more diagnosis common causes, testing may be helpful to ensure accurate diagnosis and appropriate treatment.
>
> - Use a combination of clinical presentation and laboratory testing to guide diagnosis and treatment in people without EM. Based on the evidence of test accuracy test results need careful interpretation alongside clinical assessment to guide diagnosis. Due to the limitation of tests, Lyme disease should not be ruled out by negative tests if it is strongly suggested by the clinical assessment.
> - If there is a clinical suspicion of Lyme disease in people without EM, offer an ELISA test for Lyme disease. Treatment could be started at the same time as testing if clinical assessment strongly suggested Lyme disease because prompt treatment is important.
> - A strategy of two-tier testing (an initial and confirmatory test) is recommended with the evidence indicating this was potentially cost saving. Test for both IgM and IgG antibodies using ELISAs based on purified or recombinant antigens derived from the VlsE protein or its IR6 domain peptide (such as C6 ELISA). Initial testing with a combination IgM and IgG ELISA for Lyme disease should be offered because the evidence generally showed better accuracy (both sensitivity and specificity) for combined tests compared to IgM-only or IgG-only tests. The evidence was best for tests based on purified or recombinant antigens derived from the VlsE protein or its IR6 domain peptide (such as C6).
> - If the ELISA for Lyme disease is negative and the person still has symptoms, review their history and symptoms and think about the possibility of an alternative diagnosis. The clinical review is to ensure that alternative diagnoses are not missed.

- If Lyme disease is still suspected in people with a negative ELISA who were tested within four weeks from symptom onset, repeat the ELISA four to six weeks after the first ELISA test. As antibodies take some time to develop, repeat testing would be warranted for people who may have had the initial test too early, before an immune response has developed.
- If Lyme disease is still suspected in people with a negative ELISA who have had symptoms for 12 weeks or more, perform an immunoblot test. An immunoblot would help rule out or confirm diagnosis where uncertainty still remains.
- Diagnose Lyme disease in people with symptoms of Lyme disease and a positive immunoblot test.
- If the immunoblot test for Lyme disease is negative (regardless of the ELISA result) but symptoms persist, consider a discussion with or referral to a specialist to:
  - Review whether further tests may be needed for suspected Lyme disease (for example, synovial fluid aspirate or biopsy, or lumbar puncture for cerebrospinal fluid analysis) or
  - Consider alterative diagnoses (both infectious, including other tick-borne diseases, and non-infectious diseases)
  - Choose a specialist appropriate for the person's history or symptoms (for example, an adult or paediatric infection specialist, rheumatologist or neurologist)
- If the immunoblot test for Lyme disease is negative and symptoms have resolved, explain to the person no treatment is required.
- Carry out tests for Lyme disease only at laboratories that:
  - are accredited by the UK accreditation service (UKAS) and
  - use validated tests (validation should include published evidence on the test methodology, its relation to Lyme disease and independent reports of performance) and
  - participate in a formal external quality assurance programme
- Do not routinely diagnose Lyme disease based only on tests done outside the NHS, unless the laboratory used is accredited, participates in formal external quality assurance programmes and uses validated tests. If there is any doubt about tests:
  - Review the person's clinical presentation and
  - Carry out testing again using a UKAS-accredited laboratory and/or seek advice from a national reference laboratory (National Institute for Health and Care Excellence, 2018j).

More recently, in 2019, the draft clinical guideline for diagnosis and treatment of Lyme disease from IDSA/AAN/ACR advised several general principles regarding diagnostic testing for Lyme disease (Lantos et al., 2019). These principles included:

- Based on performance of characteristics and practical considerations, antibody tests are first-line for the laboratory diagnosis of Lyme disease.
- Serologic (serum antibody) testing is highly sensitive in patients with non-cutaneous manifestations of Lyme disease, as these manifestations typically develop

after weeks to months of infection (Molins et al. (2016), and Steere et al. (2008) in Lantos et al., 2019).

- IgG seronegativity in a patient with prolonged symptoms (months to years) essentially rules out the diagnosis of Lyme disease, barring laboratory error or a rare host immune deficiency affecting humoral immunity.
- Serologic testing is also highly specific when performed and interpreted according to current guidelines (Steere et al. (2008), and Molins et al. (2014) in Lantos et al., 2019).
- Serum antibody tests should be performed using a two-tiered testing protocol employing clinically validated assays (Moore et al. (2016) and CDC (1995) in Lantos et al., 2019).
- Predictive value is increased when results are correlated with clinical features, patient history and risk factors.
- Currently, the only FDA-cleared or approved diagnostic assays for Lyme disease are antibody tests (Lantos et al., 2019).

Lantos et al. (2019) noted that as an indirect method, antibody testing has some important limitations including: false negative results in the first few days to weeks following initial exposure; and, in a seropositive patient it can be difficult to determine whether antibody reactivity is due to past infection, active/current infection, or both.

In addition to the recommendations by the CDC, NICE and IDSA/AAN/ACR for two-tier serologic testing for diagnosis of Lyme disease, two recent reviews have examined the recommendations of international guidelines for diagnostic testing for Lyme disease (Dessau et al., 2018; Eldin et al., 2019) and found the two-tier principle is also part of many current guidelines in laboratory testing for Lyme borreliosis in Europe.

A 2019 review by Eldin et al. of European and American guidelines (16 guidelines from seven countries) for the diagnosis of Lyme disease found all guidelines indicated that the diagnosis of Lyme disease is currently based on a two-tier serology at all stages of infection, except for the early localised dermatological presentation known as EM (Eldin et al., 2019). The recommendation from 15 of the 16 international guidelines was no serology testing in the case of EM suspicion due to early serology not being sensitive enough (40 per cent to 60 per cent) to confirm Lyme diagnosis at the EM stage. The one discordant recommendation was from the German Borreliosis Society that recommended (relative indication) a one-tier serology in case of early infection suspicion with or without EM and a lymphocyte transformation test (Eldin et al., 2019). Eldin et al. noted many guidelines state that less than 24-48 hours for the rash onset, disappearing within a few days without extension, should rule out the diagnosis of EM.

Eldin et al. noted that in recent years the issue of the diagnosis of Lyme disease has been highly publicised on the Internet and other media in Europe and America, with numerous pieces of information about Lyme disease having emerged, mostly as patient testimonials. As such, many patients, associations of patients and some physicians share the perception that the laboratory diagnosis of Lyme disease in France and other European countries is not relevant and they should be tested abroad (mainly in Germany) to benefit from reliable tests. Eldin et al. commented this phenomena can result in mistrust of patients towards the French medical community (Eldin et al., 2019). Eldin et al. therefore aimed to provide an overview

of existing guidelines on the diagnosis of Lyme disease in countries where the disease is prevalent (Eldin et al., 2019).

The review analysed 16 guidelines including:

- one each from France (SPILF 2006), the US (the IDSA 2006 guideline), Canada (Canadian Public Health Laboratory Network, 2006), Switzerland (Swiss Infectious Diseases Society, 2006), Belgium (Belgian Society of Infectious Diseases and Clinical Microbiology, 2016) and Poland (Polish Society of epidemiology and infectious diseases, 2015)
- two from the UK (NICE guidelines draft 2017; British Infections Association, 2011)
- two from Europe (EFNS, 2010; ESGBOR, 2017), and
- six from Germany (Committee for infectious diseases and vaccinations of the German academy for pediatrics and adolescent health, 2012; German Borreliosis Society, 2010; German Rheumatology Society and German Association of Children and Adolescent Health, 2013; German Neurological Society, 2012; German Society of Hygiene and Microbiology 2017; German Dermatology Society, 2016).

Eldin et al. (2019) noted the German guidelines were analysed with a special interest because patients in France are often convinced that German physicians have a different approach to disease. Five of the six German guidelines were issued by academic societies and available on the website of the Association of Scientific Medical Societies in Germany, whereas the other guideline from the German Borreliosis Society was reported by Eldin et al. to be defined as a 'transdisciplinary medical association' of physicians and researchers working on Lyme and tick-borne diseases. Eldin et al. noted this society is not officially recognised by the German authorities as an academic society.

In addition to reviewing and comparing the evidence-based guidelines from North America and Europe, Eldin et al. analysed the guidelines for quality, using the following criteria: references; method for searching evidence; systematic search of evidence; explicit link between recommendation and evidence; gradation; and single or multiple organism(s). The highest quality score was six, which was obtained by the European Federation of Neurological Societies (EFNS) 2010 guidelines and the NICE 2017 draft guidelines (which were finalised as the 2018 NICE guidelines, referred to extensively in this section and in other sections on Lyme disease in this literature review). The German Borreliosis Society showed the lowest quality score. Eldin et al. also noted the guideline from the German Borreliosis Society had discordant recommendations when compared to other guidelines, 'possibly explained by its low quality score'.

Of the review Eldin et al. were able to conclude that

> *Contrary to the intense debate taking place on the Internet and in the European and American media, our analysis shows that the great majority of the medical scientific guidelines with a high quality score, agree on the clinical diagnostic methods of Lyme disease (Eldin et al., 2019, p. 121).*

Dessau et al. in their 2018 position paper of ESGBOR, the ESCHMID study group for Lyme borreliosis 'To test or not to test', noted that while the two-tier testing for Lyme borreliosis is under debate in the US, the principle is also part of many current guidelines in laboratory testing for Lyme disease in Europe, citing the following papers (Wilske et al. (2007), Dessau

et al. (2013), Hundfeld and Kraiczy (2009), Brouqui et al. (2004), Wilske et al. (2000), Dessau et al. (2011), Wormser et al. (2014), and Robertson et al. (2000) in Dessau et al., 2018).

4.5.4. Latest international guidance and advice to health professionals and patients about two-tier testing for Lyme disease

The interpretation of serological assays in Lyme disease requires an understanding of the clinical indications and limitations of the tests, and the usefulness of serological tests for Lyme disease depends on the pre-test probability and subsequent predicative values in the setting where the tests are being used (Leeflang et al., 2016).

While the IDSA/AAN/ACR, NICE and CDC identify that the currently available protocol is reliable when used appropriately, all three international authorities also note the limitations of the testing protocol (Centers for Disease Control and Prevention, 2019c; Lantos et al., 2019; National Institute for Health and Care Excellence, 2018j). Additionally, the European Society of Clinical Microbiology and Infectious Diseases (ESGBOR-ESCMID) provided recommendations to support both the clinical diagnosis and initiatives for a more rational use of laboratory testing in patients with clinically suspected Lyme borreliosis (Dessau et al., 2018), described below.

NICE (2018j) recommends clinicians provide the following information to patients being tested for Lyme disease:

- tests for Lyme disease have limitations and that false positive and false negative results can occur and what this means
- most tests for Lyme disease assess for the presence of antibodies and the possible reduction of accuracy of the test if:
    - testing is carried out too early (before antibodies have developed), and
    - the person has reduced immunity, for example in people on immunosuppressant treatments, which might affect the development of antibodies
- the symptoms and signs associated with Lyme disease overlap with those of other conditions
- they will be assessed for alternative diagnoses if their tests are negative and their symptoms have not resolved, and
- symptoms such as tiredness, headache and muscle pain are common and a specific medical cause is often not found (National Institute for Health and Care Excellence, 2018j).

In 2019 IDSA/AAN/ACR advised in their draft Lyme disease guidelines:

- serologic (serum antibody) testing is highly sensitive in patients with non-cutaneous manifestations of Lyme disease, as these manifestations typically develop after weeks to months of infection
- serologic testing is also highly specific when performed and interpreted according to current guidelines
- predictive value is increased when results are correlated with clinical features, patient history and risk factors, and
- currently, the only FDA-cleared or approved diagnostic assays for Lyme disease are antibody tests (Lantos et al., 2019).

The CDC advises the key points to remember about diagnosis and testing for Lyme disease are:

- most Lyme disease tests are designed to detect antibodies made by the body in response to infection.
- antibodies can take several weeks to develop, so patients may test negative if infected only recently.
- antibodies normally persist in the blood for months or even years after the infection is gone; therefore, the test cannot be used to determine cure.
- infection with other diseases, including some tick-borne diseases, or some viral, bacterial, or autoimmune diseases, can result in false positive test results.
- some tests give results for two types of antibody, IgM and IgG. Positive IgM results should be disregarded if the patient has been ill for more than 30 days (Centers for Disease Control and Prevention, 2019c).

The position statement of ESGBOR-ESCMID noted, currently, a large volume of diagnostic testing for Lyme borreliosis is reported, whereas the incidence of clinically relevant disease manifestations is low, indicating the overuse of diagnostic testing for Lyme borreliosis with implications for patient care and cost-effective management (Dessau et al., 2018). Key points and recommendations of ESGBOR-ESCMID were:

- diagnosis of Lyme borreliosis is based on a complete diagnostic workup, including medical history, with compatible clinical symptoms, objective signs, possible exposure to tick bites, and exclusion of other diseases, but not laboratory testing alone.
- patients with a typical EM should be diagnosed clinically and treated promptly without serological testing, which is insensitive at this stage of the disease.
- pathology is elicited by the host immune response. Detection of antibodies to *B. burgdorferi* is necessary to support the clinical diagnosis of Lyme borreliosis manifestations other than EM.
- in patients with suspected Lyme neuroborreliosis examination of cerebrospinal fluid is strongly recommended.
- the use of *Borrelia* serology in patients with non-specific subjective symptoms is discouraged.
- in patients with disease duration of >6 weeks a specific IgG response is a prerequisite, but an isolated IgM response is of no diagnostic relevance.
- detection of antibodies to *B. burgdorferi* cannot discriminate between active, latent or past infection.
- *Serology* Detection of specific IgG and IgM antibodies is recommended for routine laboratory testing for Lyme borreliosis. This is because direct detection of the pathogen in clinical samples has a lower sensitivity (Dessau et al., 2018).

4.5.5. Recent evidence-reviews, systematic reviews and meta-analyses on accuracy of serology tests for Lyme disease

The accuracy and reproducibility of commercially produced Lyme disease kits is a widely recognised issue (Aguero-Rosenfeld & Wormser, 2015; Cook & Puri, 2016; Mackenzie, 2013), underpinning the importance that commercial laboratories utilise validated kits (Lantos et al., 2019; Mackenzie, 2013; National Institute for Health and Care Excellence, 2018j). The wide variation in sensitivity and specificity of currently available serological tests for antibodies to *Borrelia*, is seen to have consequent implications for their correct interpretation (Aguero-Rosenfeld & Wormser, 2015).

Mackenzie, in 2013, noted there has been very limited interassay standardisation – particularly in the European market; different test methodologies can result in differences with respect to test quality; and in Germany alone, a study had reported at least 55 different companies provide a variety of diagnostic tests which can lead to a high number of false negative and false positive results (Müller et al. (2012) in Mackenzie, 2013).

Within the last five years, four comprehensive evidence and systematic reviews (with or without meta-analysis) have been undertaken on the accuracy of diagnostic tests including serology tests and test kits. These were:

- an evidence-based review (no meta-analysis) of diagnostic tests [part c: diagnostic tests] by NICE that examined initial tests, confirmatory tests and combination tests for Lyme disease, to inform the NICE Lyme disease guideline and recommendations (National Institute for Health and Care Excellence, 2018b)
- a systematic review and meta-analysis to assess the diagnostic accuracy of serological tests for the diagnosis of the different manifestations of Lyme borreliosis in Europe (Leeflang et al., 2016)
- a systematic review and meta-analysis on North American evidence published since 1995 on the accuracy of diagnostic tests and test regimes used to diagnose Lyme disease in patients presenting with clinical symptoms in North America at various stages of the disease and to address whether there is evidence of superior, equivalent or poor performance by the commercial (approved by the FDA and/or Health Canada) and in house laboratory tests (Waddell et al., 2016). Waddell et al. noted this systematic review was complementary to the systematic review by Leeflang et al. (2016)
- a meta-analysis of studies concerning the accuracy of test kits that were commercially available, where samples were proven to be positive using serology testing, evidence of an EM rash and/or culture, test specificity was ≥85 per cent, and studies were published from 1995 onwards (Cook & Puri, 2016).

While all of the systematic and evidence-based reviews looked at accuracy, there are some fundamental differences in the reviews. Leeflang et al. (2016) only reviewed studies on serological tests from Europe, Waddell et al. (2016) reviewed studies on serology and other tests from North American research only, whereas NICE (2018b) and Cook and Puri (2016) did not limit their inclusion criteria by continent. NICE (2018b) was the only evidence-based review to have PICO tables. Additionally, NICE, Leeflang et al. and Waddell et al. all undertook quality assessments, including risk of bias, using the QUADAS-2 tool for the studies included in their respective reviews; Cook and Puri did not assess quality of the studies, but made some comments about quality. NICE intended to undertake meta-analyses but did not (with the reasons provided below), whereas Cook and Puri only undertook a meta-analysis.

Despite the differences in the parameters of the four reviews, all found accuracy of serology tests increased with progression of the disease, with test sensitivity increasing with progression of *B. burgdorferi* infection from early to late. However, all reviews found marked variation and heterogeneity in study findings of sensitivity and specificity for each test technology, whether it be ELISA, Western blot, or two-tiered test methodology. The three reviews that assessed study quality all found the studies to be at high risk of bias.

Several themes emerged from these reviews including the findings on accuracy, issues with the studies and study types, including risk of bias, and the implications for clinical practice and decision making. These themes are discussed below.

NICE (2018)

The 2018 NICE evidence review on diagnostic tests included three review questions that aimed to determine the most accurate initial test, confirmatory test and test combination. The review questions were:

- In people with suspected (or under investigation for) Lyme disease, what is the most accurate initial test to identify whether Lyme disease is present?
- In people with a positive test for Lyme disease, what is the most accurate test to confirm or rule out Lyme disease?
- In people with suspected (or under investigation for) Lyme disease, what is the most accurate combination of tests to identify whether Lyme disease is present? (National Institute for Health and Care Excellence, 2018b).

All review questions included a PICO table. Only studies published in English were included. No date limits were set for the search. Cross-sectional studies in which the index test(s) and the reference standard test are applied to the same people and two-gate/case-control study designs that compare the results of the index test in people with an established diagnosis with its results in healthy controls were included. Case reports and case series were excluded. Included and excluded studies were presented in the evidence review. Three different reference standards were identified for the review: *Borrelia burgdorferi* s.l. culture, PCR and clinical diagnosis. Culture was noted as difficult, slow and not compatible with providing a rapid diagnostic result and therefore rarely used as a reference standard in clinical studies. In cases where *B. burgdorferi* s.l. culture or PCR were used as an index test in any of the included studies, clinical diagnosis would function as the reference standard. For this review sensitivity and specificity were prioritised over positive predictive value and negative predictive value because the authors noted they are intrinsic to the test and do not depend on the prevalence of Lyme disease (National Institute for Health and Care Excellence, 2018b).

For all studies included in the review (across all three research questions) the NICE committee stated

> *The included studies varied significantly by test, study population and clinical presentation, which made it impossible to meta-analyse the large number of results. Given the general lack of evidence from cross-sectional studies, which are the most robust study design for diagnostic accuracy studies, case-control studies were also included in this review. The committee considered the entirety of the evidence when making recommendations (National Institute for Health and Care Excellence, 2018b).*

Findings on accuracy of tests

Initial tests for Lyme disease

NICE reviewed 123 studies: 114 involved adults, 102 of which were case-control studies and nine were cross-sectional studies; and nine studies involved children (five case-control and four cross-sectional studies). Overall, NICE found the evidence generally showed better sensitivity and specificity results for combined IgM and IgG tests for different clinical presentations of Lyme disease compared to IgM-only and IgG-only and there was no clear advantage of ELISA tests or immunoblots or vice versa for any clinical presentation.

The following table on the clinical evidence statements for initial tests for Lyme disease is reproduced from NICE Evidence Review for diagnostic tests - Initial tests for Lyme disease (National Institute for Health and Care Excellence, 2018b).

Table 10: Clinical evidence statements: Initial tests for Lyme disease (National Institute for Health and Care Excellence, 2018b)

Clinical Evidence Statements
Overall, the evidence was of Very Low quality due to the case-control study design, risk of bias and imprecision. The included studies varied significantly by test, study population and clinical presentation. It was not possible to meta-analyse the large number of results because studies with comparable tests differed in how clinical presentations were reported, how tests were conducted and analysed and how the test results were interpreted.
Generally, combined IgM/IgG tests showed better sensitivity and specificity results for different clinical presentations of Lyme disease than IgM-only and IgG-only tests. There was no clear advantage of ELISAs over immunoblots or Western blots or vice versa for any clinical presentation. Borrelia burgdorferi s.l. culture and polymerase chain reaction (PCR), which also functioned as reference standards in this review, showed poor results when compared to clinical diagnosis. There was only limited evidence for other tests, which required caution when interpreting the results.
The analyses by time point did not show any clear advantage of one test over the other. IgM tests tended to have a higher sensitivity in the early stages of Lyme disease, such as the erythema migrans, and a lower sensitivity in later stages of Lyme disease. By contrast, the sensitivity of IgG test increased with disease progression.
There was only limited evidence in children. The sensitivity of tests was generally lower in children than in adults. There was no noticeable difference in specificity between adults and children for different clinical presentations of Lyme disease.

Confirmatory tests for Lyme disease

This review included five studies: four case-control studies and one cross-sectional study. The review did not identify any studies in children.

The following table on the clinical evidence statements for initial tests for Lyme disease is reproduced from NICE Evidence Review for diagnostic tests - Initial tests for Lyme disease (National Institute for Health and Care Excellence, 2018b).

Table 11: Clinical evidence statements: Confirmatory tests for Lyme disease (National Institute for Health and Care Excellence, 2018b)

Clinical Evidence Statements
Evidence on the accuracy of confirmatory tests in confirming Lyme disease was very limited. Very Low quality evidence from 3 case-control studies in adults showed a higher sensitivity of IgG-specific tests compared to a test detecting IgM antibodies for confirming Lyme disease in people with an EM. Specificity across the included studies was generally very high although there is a risk of overestimation due to the case-control study design. Very Low quality evidence from 1 cross-sectional study showed a very high sensitivity, but low specificity of an IgG-specific immunoblot for confirming Lyme disease in adults. The very limited evidence on combined IgM/IgG immunoblots was inconclusive. No evidence in children could be identified.

Combination of diagnostic tests for Lyme disease

This review included fifteen studies; 14 were in adults and one study was in children (cross-sectional). Overall, NICE found the evidence suggested that the combination of initial combined IgM and IgG/ELISA and confirmatory IgM and IgG immunoblot testing had a high sensitivity and specificity, particularly for Lyme arthritis, Lyme carditis and ACA. Only one of the studies on Lyme arthritis was conducted in a European setting and all studies in Lyme arthritis and ACA were conducted in the US.

The following table on the clinical evidence statements for initial tests for Lyme disease is reproduced from NICE Evidence Review for diagnostic tests - Initial tests for Lyme disease (National Institute for Health and Care Excellence, 2018b).

Table 12: Clinical evidence statements: Combination of diagnostic tests for Lyme disease (National Institute for Health and Care Excellence, 2018b)

Clinical Evidence Statements
Overall, the evidence was of Very Low quality due to the case-control study design, risk of bias and imprecision. The included studies varied significantly by test, study population and clinical presentation. It was not possible to meta-analyse the large number of results because studies with comparable tests differed in how clinical presentations were reported, how tests were conducted and analysed and how the test results were interpreted. Very Low quality evidence from 14 case-control studies in adults showed that a combination of ELISAs and immunoblots where both tests detect both IgM and IgG antibodies had the highest coupled sensitivity and specificity for detecting and confirming Lyme disease. Overall sensitivity of test combinations increased with disease progression. Although tests that are less frequently used in clinical practice, such as C6 or WCS ELISAs, also showed a relatively high sensitivity, there was considerably higher variance around the point estimates and the point estimates of these less frequently used tests were mostly lower than for combined IgM/IgG ELISAs. Low quality evidence from 1 cross-sectional study in children showed similarly high sensitivity and specificity point estimates for C6 and WCS ELISAs in combination with IgM/IgG immunoblots for detecting and confirming Lyme disease. No evidence in widely used combined IgM/IgG ELISAs in children was, however, identified. Nearly all of the identified evidence showed a specificity of 99 per cent to 100 per cent.

Implications for clinical practice

Within this evidence-review the committee also undertook an exploratory analysis to estimate the additional cost of two-tier testing (ELISA including C6 IgM and IgG followed by confirmatory immunoblot if ELISA is positive) over initial testing only (ELISA including C6 IgM and IgG) in people with suspected Lyme disease to evaluate what the cost of a misdiagnosis (either false positive or false negative) would need to be for two-tier testing to be cost neutral. Overall, the committee considered that a misdiagnosis was very likely to cost at least £381, (as it was noted these people would have a number of healthcare interactions whether the misdiagnosis was a false positive or false negative), and agreed that from this analysis two-tier testing is very likely to be cost neutral compared to initial testing only and may even be cost saving. However, the committee also noted that if health benefits had been incorporated into the analysis, two-tier testing would likely be cost effective compared to initial testing only.

Based on this analysis and the clinical evidence the committee recommended two-tier testing is done in current practice (National Institute for Health and Care Excellence, 2018b).

The NICE recommendations for Laboratory investigations in the NICE guideline for Lyme disease published in 2018 are summarised below (National Institute for Health and Care Excellence, 2018j).

Table 13: Summary of NICE recommendations for laboratory investigations

The committee agreed that laboratory testing is unnecessary for people presenting with EMs, because the rash is very specific to Lyme disease and prompt treatment will prevent further symptoms developing. However, most other symptoms associated with Lyme disease have other more common causes, so testing may be helpful to ensure accurate diagnosis and appropriate treatment.
Based on the evidence on test accuracy, the committee agreed that test results need careful interpretation alongside clinical assessment to guide diagnosis. Because of the limitations of tests, Lyme disease should not be ruled out by negative tests if it is strongly suggested by the clinical assessment. The committee decided that treatment could be started at the same time as testing if clinical assessment strongly suggests Lyme disease, because prompt treatment is important.
The committee agreed a strategy of two-tier testing (an initial and confirmatory test), which the evidence indicated was potentially cost saving. Initial testing with a combination IgM and IgG ELISA for Lyme disease should be offered because the evidence generally showed better accuracy (both sensitivity and specificity) for combined tests compared to IgM-only and IgG-only tests. The evidence was best for tests based on purified or recombinant antigens derived from the VlsE protein or its IR6 domain peptide (such as a C6).
For people with a negative ELISA result who continue to have symptoms, the committee agreed that clinical review would ensure that alternative diagnoses are not missed. In addition, because antibodies take some time to develop, repeat testing would be warranted for people who may have had the initial test too early, before an immune response has developed. If symptoms have been present for 12 weeks, the committee agreed that an immunoblot would help rule out or confirm diagnosis where uncertainty remains.
The committee agreed that testing should be done in UKAS-accredited laboratories and that any tests used for diagnosis should be validated before they are used to diagnose Lyme disease to avoid unreliable and misleading results, which may lead to misdiagnosis.
Based on their knowledge and experience, the committee agreed that *Borrelia burgdorferi sensu lato (sl)* infection does not behave differently in children than adults but acknowledged that a young child's immune responses might not be as rapid and effective. The limited evidence in children did not show a noticeable difference in test accuracy compared with adults. Therefore, the committee decided that separate recommendations for testing in children were unnecessary.
The committee considered it important that people being tested for Lyme disease understand how the tests work, their limitations and the importance of basing decisions on tests that are valid.

With regard to how the recommendation to carry out an immunoblot test despite a negative initial ELISA when there is clinical suspicion of Lyme disease would affect clinical practice, NICE advised the following:

> *A 2-tiered testing system is used in current practice, in which a positive result on an initial ELISA leads to a confirmatory immunoblot test. A negative result on an initial ELISA would not usually lead to a confirmatory immunoblot test. Therefore, the recommendation to carry out an immunoblot test, despite an initial negative ELISA when there is clinical suspicion of Lyme disease would be a change to practice and increase the number of people receiving this test. However, this would only apply to a small population, so this recommendation is not likely to have a significant resource impact (National Institute for Health and Care Excellence, 2018j). p29*

The above recommendations were informed by a diagnostic evidence review Lyme disease: diagnosis and management [C] Evidence reviews for diagnostic tests (National Institute for Health and Care Excellence, 2018b).

Limitations with studies

In addition to issues with the quality of the evidence identified above for all three review questions, NICE made a number of statements and observations about the quality of the evidence overall. Of the case-control studies included in the review, NICE identified several limitations (National Institute for Health and Care Excellence, 2018b).

- The majority of evidence was from case control studies and was of very low quality because of risk of bias, study design and imprecision, with particular concerns about the selection of people, the lack of blinding, the limited information on the index tests, and the inadequate reference standard.
- Many studies were of US populations or were old studies using discontinued tests; no studies were on UK populations.
- There is a strong potential of the results being an overestimate of the true sensitivity and specificity values due to the way the case-control studies were conducted.

NICE explained that populations in case-control studies tend to differ from 'true populations' found in clinical practice as cases tend to be more severely ill than the average patient population in clinical practice in order to fill inclusion criteria of studies; whereas controls are usually drawn from a healthy population or include known specific cross-reactivity controls.

Additionally, the evidence from cross-sectional studies was assessed as low to very low quality, mainly due to issues with the index tests and reference standards. Additional concerns about the included cross-sectional studies included:

- the majority of cross-sectional studies, as was the case for case-control studies, did not provide sufficient information on the tests used
- concerns regarding lack of blinding
- many of the studies were small, with samples of fewer than 100 participants, and
- the evidence on tests other than ELISA or immunoblot was often based on single studies.

NICE also commented on the 'modern' ELISAs (tests based on the C6 or validated sets of purified antigens), noting that evidence from two cross-sectional studies suggested these tests have a relatively high degree of sensitivity for detecting Lyme disease in people with neuroborreliosis. NICE noted that evidence for modern types of ELISAs could not necessarily be extrapolated to other types of ELISAs as other types of ELISAs do not include highly immunogenic antigens such as C6 (which cause an early antibody response useful for diagnostic testing) (National Institute for Health and Care Excellence, 2018b).

Waddell et al. (2016)

Waddell et al. (2016), in their systematic review and meta-analysis, included 48 studies on diagnostic tests (serology, culture, PCR) used in North America published since 1995. They found:

- there is a dramatic increase in test sensitivity with progression of *B. burgdorferi* infection from early to late Lyme disease
- recent studies examining the sensitivity and specificity of various test protocols noted that the Immunetics ® C6 B. burgdorferi ELISA ™ and the two-tier approach have superior specificity compared to proposed replacements, and the CDC recommended Western blot algorithm has equivalent or superior specificity over other proposed test algorithms
- direct detection methods, culture and PCR of tissue or blood samples were not as sensitive or timely compared to serological testing
- QUADAS-2 tool assessment found the majority (84 per cent) of studies had an unclear risk of bias, meaning the study received an unclear or high risk of bias score on one or more domains (Waddell et al., 2016).

Another finding of note was a large number of both commercial and in-house developed tests used by private laboratories which had not been evaluated in the primary literature (Waddell et al., 2016). We discuss these 'in house' and unvalidated tests further in 4.8 Commercially available laboratory testing methods to be avoided.

Findings on accuracy of tests

For two-tiered serological test versus clinical diagnosis, from 13 studies, meta-analytic summaries demonstrated low sensitivity for early stage 1 Lyme disease patients (46.3 per cent; 95%CI: 39.1-53.7) and increasing sensitivity for stage 2 (89.7 per cent; 95%CI: 78.3-95.4) and stage 3 Lyme disease (99.4 per cent; 95%CI: 95.7-99.9). There was relatively high specificity (98.3 per cent to 99.9 per cent) across control groups; most false positives in the controls groups were patients with diseases known to produce antibodies that cross-react in serological tests for *B. burgdorferi* (Waddell et al., 2016).

For ELISA versus clinical diagnosis, from 23 studies with a mix of FDA-licenced tests and in-house tests, similar to the two-tiered tests, test performance for patients with stage 1 Lyme disease was highly variable and had poor sensitivity, but in later stages the sensitivity improved. The meta-analytic summary for sensitivity for early (stage 1) Lyme disease from 16 studies was 54.0 per cent (95%CI: 42.9-64.8); the meta-analytic summary for specificity for the same stage of disease was 96.8 per cent (95%CI: 95.0-98.0). The overall specificity varied by test and between studies more than was reported for the two-tier tests. For assays used in cases of late Lyme disease, the sensitivity and specificity was higher and more consistent compared with early Lyme disease (Waddell et al., 2016).

Waddell et al. reported the findings of their review were in agreement with others, who had found sensitivity was highest for ELISAs targeting C6 and these showed less variability in test sensitivity compared to other test protocols (Fallon et al. (2014) in Waddell et al., 2016). Waddell et al. also noted that Fallon et al. had found that the C6 ELISA alone and the two-tier approach has superior specificity compared to proposed replacements and the CDC Western blot algorithm has equivalent or superior sensitivity over other proposed algorithms (Waddell et al., 2016).

Implications for clinical practice

Waddell et al. (2016) noted that Hinckley et al. (2014) had recently estimated that less than 12 per cent of Lyme disease tests in the US were for true infections, and that the overuse of these assays to diagnose Lyme disease has been an ongoing discussion and challenge for topic-specific specialist and physicians (Hinkley et al. (2014) and Maraspin et al. (2011) in Waddell et al., 2016). Lyme disease results for patients who do not meet the clinical criteria can be used to rule out Lyme disease but a positive test in such patients is likely to be a false positive (Waddell et al., 2016). At the early stage of Lyme disease the two-tier testing method was found by this review to be good for ruling in Lyme disease if the patient tested positive, but had poor predictive value for ruling out Lyme disease which is why it is recommended to retest after 30 days. For convalescent patients treated at stage 1 Lyme disease, sensitivity remained low even after 30 days (Waddell et al., 2016).

Regarding diagnostic test performance in early Lyme disease, Waddell et al. (2016) noted the challenge in testing for Lyme disease in patients exhibiting signs and symptoms of Lyme disease for less than 30 days, as the performance of test protocols is not optimal for making clinical decisions and that this was largely due to the time required for the infected individuals' immune system to mount a reaction. As such, they noted researchers have explored the use of a variety of targets including VlsE and C6 expressed after infection, Osp C and Fla B expressed by the feeding tick to detect infection sooner; however, cross reactivity and genetic variability within the targets has limited the diagnostic performance of any target (Branda et al. (2013) and Sillanpaa et al. (2007) in Waddell et al., 2016).

Limitations with studies

Similar to the findings of NICE (2018b) and Leeflang et al. (2016), Waddell et al. (2016) identified several issues from their risk of bias assessment using the QUADAS-2 tool. Issues included unclear risk of bias in 84 per cent of studies, inappropriate blinding was not addressed in many papers, and unexplained exclusion of observations from the analysis was another common problem reporting issue. Additionally, 28.6 per cent of studies had authors employed or funded by commercial companies that supplied one or more of the tests evaluated and in four studies the risk of funding bias was identified to be very high (Waddell et al., 2016).

Leeflang et al. (2016)

In their systematic review and meta-analysis of 78 studies of serological tests for Lyme borreliosis in Europe, Leeflang et al. (2016) found that overall:

- the diagnostic accuracy of ELISAs and immunoblots for Lyme borreliosis in Europe varies widely, with an average sensitivity of approximately 80 per cent and a specificity of approximately 95 per cent
- there was no evidence that ELISAs have a higher or lower accuracy than immunoblots or that two-tiered approaches have a better performance than single tests
- sensitivity was found to be highly heterogeneous
- sensitivity was lowest in the early stage of infection (EM) with summary estimates of 50 per cent (95% CI: 40-61). Summary estimates for sensitivity for detecting neuroborreliosis was higher at 77 per cent (95% CI: 67-85), and highest for ACA at 97 per cent (95% CI: 94-99)
- in studies with healthy controls, specificity was around 95 per cent but in cross-sectional studies specificity was around 80 per cent
- none of the studies had low risk of bias for all QUADAS-2 domains (Leeflang et al., 2016).

Of their findings, Leeflang et al. (2016) stressed caution in interpreting the results of the review and meta-analyses due to 'much' variation in the results and that the included studies were all assessed at high risk of bias.

The included studies had evaluated an ELISA or an immunoblot assay against a reference standard (the test or testing algorithm used to define whether someone has Lyme borreliosis or not). Most studies included in the review used clinical criteria sometimes in combination with serology. Studies on ELISAs, immunoblots, two-tiered testing algorithms of an ELISA followed by an immunoblot and specific antibody index measurement were included. Studies performed in Europe and published in English, French, German, Norwegian, Spanish and Dutch were included. No date limit was reported in the methodology. The 57 included case-control studies ranged in date from 1987 to 2011; the 18 cross-sectional studies included ranged from 1992 to 2008 (Leeflang et al., 2016).

Implications for clinical practice

Leeflang et al. (2016, p. 10) noted that interpretation of serological assays in Lyme borreliosis requires an understanding of the clinical indications and limitations of the currently available tests and the usefulness of serological tests for Lyme disease depends on the pre-test probability and subsequent predictive values in the setting where the tests are being used. Although the authors were not able to meta-analyse predictive value, they commented 'the sensitivity and specificity estimates from their review may be used to provide an idea of the consequences of testing when the test is being used in practice'. Additionally, the authors highlighted the immunoblot was not analysed in a way that is representative for practice as most immunoblots were analysed on the same samples as the ELISAs while in practice immunoblots will only be used on ELISA-positive samples (Leeflang et al., 2016).

As such, and as noted above, the authors stressed caution in interpreting the results of the review and meta-analyses due to 'much' variation in the results and that the included studies

were all assessed at high risk of bias. They highlighted that the observed heterogeneity and risk of bias complicate the extrapolation of their results to clinical practice and concluded:

> *We found no evidence that ELISAs have a higher or lower accuracy than immunoblots; neither did we find evidence that two-tiered approaches have a better performance than single tests. However, the data in this review do not provide sufficient evidence to make inferences about the value of the tests for clinical practice. Valid estimates of sensitivity and specificity for the tests as used in practice require well-designed cross-sectional studies, done in the relevant clinical patient populations. Furthermore, information is needed about the prevalence of Lyme borreliosis among those tested for it and the clinical consequences of a negative or positive test result. The latter depend on the place of the test in the clinical pathway and the clinical decisions that are driven by the test results or not. Future research should primarily focus on more targeted clinical validations of these tests and research into appropriate use of these tests (Leeflang et al., 2016, p. 11)*

As such, Leeflang et al. (2016) commented that the actual added value of testing for Lyme disease requires information about subsequent actions and consequences of testing noting that imperfect laboratory tests may still be valuable for clinical decision making if subsequent actions improve the patient's outcome, with the challenge for clinicians being to deal with the uncertainties of imperfect laboratory tests.

Limitations of the studies

Leeflang et al. (2016) identified limitations including the representativeness of the results, the poor reporting of study characteristics, the lack of a true gold standard, and that most included studies in their review were case-control studies which the authors commented may be easier to perform in a laboratory setting than cross-sectional designs but their results are less representative for clinical practice.

Leeflang et al. (2016) noted in their methodology that the ideal study type to answer their question on the accuracy of serological tests for Lyme borreliosis in Europe would be a cross-sectional study, including a series of representative, equally suspected patients who undergo both the index test and the reference standard, as such studies provide valid estimates of sensitivity and specificity and would also directly provide estimates of prevalence and predictive values. However, the cross-sectional studies were anticipated by Leeflang et al. to be sparse and therefore case control studies were included - the same decision taken by NICE in its evidence-based review above (National Institute for Health and Care Excellence, 2018b). Leeflang et al. explained case-control studies estimate the sensitivity of a test in a group of cases (patients for whom it is relatively sure that they do have Lyme disease) and estimate the specificity in a group of controls (patients for whom it is relatively sure that they do not have Lyme disease). Controls are healthy volunteers, or patients with other diseases other than Lyme disease. Leeflang et al. commented that future studies should be prospective cross-sectional studies including a consecutive sample of presenting patients stratified by the situation in which a patient presents (for example, tertiary Lyme referral centre versus general practice), but cautioned also that better designed diagnostic accuracy studies will not improve the accuracy of test themselves, rather they will only provide more valid estimates of the tests' accuracy including predictive value.

Cook and Puri (2016)

Cook and Puri (2016) also compared sensitivities for serological testing regimes in a meta-analysis of test accuracy, including 18 studies published in the previous 20 years where the tests were commercially available, samples were proven to be positive using serology testing, there was evidence of an erythema rash and/or culture and the test sensitivity was ≥ 85 per cent. Consistent with the findings of NICE (2018b), Leeflang et al. (2016) and Waddell et al. (2016) above, Cook and Puri noted the high risk of bias in all studies.

Findings on accuracy of tests

From their meta-analysis, Cook and Puri (2016) found that sensitivity varied widely (from 30.6 per cent to 86.2 per cent between tests), with weighted mean sensitivity of all test kits with all samples reported as 59.5 per cent, with one individual test reported as low as 7.4 per cent (noted as a Western blot used to identify IgG antibodies in samples defined by the authors as acute stage). The weighted mean specificity for all studies and for all tests was 96.1 per cent.

While the most sensitive test methodology was found to be Western blot with a weighted mean of studies of 62.4 per cent (range 53.5 - 76.6), the sensitivity of this methodology was not statistically significantly different from the ELISA which had a weighted mean of all tests of 62.3 per cent (range 45.0 - 82.2).

For the six studies using C6 peptide ELISA tests the mean sensitivity was 53.9 per cent (range 42.1 - 53.9) and for studies of two-tier tests the mean was 53.7 per cent (38.9 - 67.5). Noting the lack of definitions and standardisation of disease stage along with high risk of bias in all studies, Cook and Puri reported the tests were most sensitive at 89.7 per cent when neurological and/or arthritic symptoms were present, while the sensitivity for samples at the acute stage was lowest at 35.4 per cent, but higher in the convalescent stage (64.5 per cent). However, due to these limitations the authors commented the sensitivity of the tests presented for disease stages should be considered as indicative and not definitive, although they noted the studies did demonstrate that sensitivity increases with severity of symptoms and dissemination to joints, the heart, and the central nervous systems. Additionally, Cook and Puri (2016) found no evidence that commercial test sensitivity had improved significantly over time, with linear regression analysis of data from included studies demonstrating an increase in test sensitivity of four percentage points over the 20-year period.

Implications for clinical practice

Consistent with Leeflang et al. (2016), Cook and Puri (2016) noted the sensitivities achieved by the studies included in their meta-analyses do not represent test performance in clinical settings, due to methods for selection of samples used in the studies, such as eliminating samples from patients demonstrating a weak antibody response, and also that clinical samples will include those taken soon after infection before antibodies have developed, those from patients already treated with antibiotics and steroids which suppress antibody production and depress test sensitivity, and those from patients with weakened immune systems. Cook and Puri stressed an important clinical implication for their conclusion that current Lyme disease testing lacks sensitivity was that many genuine cases of Lyme disease may be underdiagnosed and that based on their meta-analysis they recommended that clinicians not assume negative laboratory investigation results exclude a diagnosis of Lyme disease. NICE addressed the issues of negative Lyme serology in their recommendations above (National Institute for Health and Care Excellence, 2018b).

4.5.6. Considerations, limitations and important variables in serology testing for Lyme disease

While serology is currently the mainstay of laboratory diagnostics for Lyme disease, there are a number of considerations, limitations and important variables that international guidelines have highlighted (see 4.5.4 Latest international guidance and advice to health professionals and patients about two-tier testing for Lyme disease) and were also discussed in the body of literature reviewed above for this research question. These include the prevalence of Lyme disease in the population, antigenic variation between different *Borrelia* spp., stage of the disease, and that the test cannot be used to determine cure. In this section, we discuss these considerations in greater detail. We also cover the evidence on improper use of serological tests or interpretation, an issue that has relevance to 4.8 Commercially available laboratory testing methods to be avoided.

Exposure pre-test probability and unnecessary testing

As previously discussed, in 3.4, epidemiological context is important including travel history and possible exposure to ticks that transmit Lyme disease. The IDSA/AAN/ACR (Lantos et al., 2019) advises predictive value is increased when results are correlated with clinical features, patient history and risk factors, while the CDC (Centers for Disease Control and Prevention, 2019c) advises healthcare providers should consider the signs and symptoms of Lyme disease, the likelihood that the patient has been exposed to black-legged ticks, the possibility that other illnesses may cause similar symptoms and the results of laboratory tests, when indicated.

This section explores in greater depth the evidence on pre-test probability and the influence this has on the accuracy of serology tests for Lyme disease. This section has specific relevance to Australia, a country that is not endemic for Lyme disease.

In addition to the sensitivity and specificity of the diagnostic tests recommended in international guidelines for Lyme disease, the exposure to the organism that causes Lyme disease along with the prevalence of the disease or the pre-test probability of a disease in the population strongly influences interpretation of any diagnostic test result. The interpretation of serological assays in Lyme disease requires an understanding of the clinical indications and limitations of the tests, and the usefulness of serological tests for Lyme disease depends on the pre-test probability and subsequent predicative values in the setting where the tests are being used (Leeflang et al., 2016).

Before using diagnostic tests in clinical practice, theoretical considerations on positive and negative predictive values (PPV and NPV) need to be taken into account (Dessau et al., 2018). The most common cause of poor performance of serologic testing (as in other infectious diseases diagnosed by antibody testing) is their use in unselected patient populations with a low pre-test probability of Lyme disease (Aguero-Rosenfeld & Wormser, 2015). The most crucial factor governing pre-test probability for Lyme disease is exposure history (Moore et al., 2016). Aguero-Rosenfeld and Wormser explained poor performance in low pre-test probability populations is a consequence of none of the serologic assays being 100 per cent specific, giving the example that even if a serologic test were 99 per cent specific and 99 per cent sensitive, if the pre-test probability was one per cent in a given population, the post-test probability that a positive test is a true is only 50 per cent. In populations where the pre-test probability were 0.1 per cent, the post-test probability that a positive test is a true positive would be less than 10 per cent (Aguero-Rosenfeld & Wormser, 2015).

Dessau et al. (2018), in their ESGBOR-ESCMID position paper regarding positive predictive value (PPV - the ability of a diagnostic test to rule in the diagnosis of a possible disease), advised the 'rule of thumb' that the PPV is <5 per cent when the pre-test probability remains <5 percent. In such cases, a false positive result is highly probable. Dessau et al. advised the pre-test probability (disease prevalence) should approach 10 per cent before ordering a diagnostic assay, in order to reach a reasonable PPV of 50-80 per cent (Dessau (2013), and Bunikis and Barbour (2002) in Dessau et al., 2018). Dessau et al. stressed that as a consequence of these considerations around PPV, clinicians are advised to avoid serological testing whenever the clinical symptoms are not indicative of Lyme disease according to the case definitions, as this will avoid positive test results that have no clinical meaning in patients with non-specific symptoms and a low pre-test probability for Lyme borreliosis (Dessau et al., 2018).

In areas not endemic for Lyme disease [for example Australia], the positive predictive value of the serology test will be low (Chalada et al., 2016; Collignon et al., 2016; Royal College of Pathologists of Australasia, 2019). RCPA advised that false positives will occur more frequently in a low prevalence population, such as Australia, as even with an assay having 98 per cent sensitivity and specificity, in a low prevalence population (for example, 1 per cent), the PPV only approaches 33 per cent (Royal College of Pathologists of Australasia, 2019). Concordant with the advice of Dessau et al. (2018) in the previous paragraph, Collignon et al. (2016) noted that even a highly specific test will produce some false positives, so that people who have never been exposed to *B. burgdorferi* can have reactive antibody results and that tests should only be requested if there is a well-founded suspicion of Lyme disease and not in situations of low pre-test probability, in order to minimise risk of a false positive result (Moore et al. (2016) in Collignon et al., 2016). This underscores the need for a comprehensive travel history with certainty that a patient has indeed travelled to an area endemic for Lyme disease.

In a region where Lyme disease is uncommon, patients with highly characteristic clinical presentations are rarely found to have Lyme disease and positive test results are seldom associated with clinically probable infection, although the negative predictive value of Lyme disease testing will be very high (Lantos, Branda, et al., 2015). In an area of low Lyme disease incidence in the US, a study of Lyme disease testing showed an 80 per cent false positive rate which puts patients at risk of incorrect Lyme diagnoses and adverse drug reactions from inappropriate treatment (Lantos, Branda, et al., 2015). Moore et al. (2016) also cited this study by Lantos et al. and noted the findings that only 0.7 per cent of patients without recent travel history who had potential signs of disseminated infection (arthritis, cranial neuropathies, or meningitis) were ultimately given a diagnosis of Lyme disease, which indicated that even clinical signs considered consistent with Lyme disease have poor predictive value in low incidence regions (Lantos et al. (2015) in Moore et al., 2016).

Additionally, Moore et al. (2016) advised that even EM-like lesions, once considered pathognomonic for Lyme disease, can be caused by other conditions, such as Southern tick-associated rash illness, a tick-borne illness found primarily in the south-eastern US, for which an infectious aetiology has not been identified, (Masters et al. (2008) in Moore et al., 2016). For these reasons, positive results for Lyme serologic analysis provide little diagnostic value for patients in areas to which Lyme disease is not endemic and with no history of recent travel to disease endemic areas.

In a non-endemic country such as Australia, with low pre-test probability, antibody testing in a large Australian diagnostic laboratory over a 23-month period between September 2014

and July 2016 found that nearly all (5,372, 95.5 per cent) tests from 5,395 patients returned negative results for Lyme disease (Collignon et al., 2016). Concordant with the advice of Dessau et al. (2018) above, Collignon et al. noted tests should only be requested if there is a well-founded suspicion of Lyme disease and not in situations of low pre-test probability, in order to minimise risk of a false positive result (Moore et al (2016) in Collignon et al., 2016). In this study, test referrals came from all Australian states, with most from New South Wales (45 per cent) and Queensland (27 per cent) with women aged 30-50 years being the largest group tested. Seventy-nine samples (one per cent of all samples) returned positive results for both the screen immunoassay and initial immunoblot. Of these 79 patients, 29 who had a low pre-test probability of infection such as no symptoms or epidemiological risk factors were negative on a second immunoblot. The total number of true positive tests was therefore 50 (0.9 per cent of all tests) from a total of 43 patients. Additionally, the total number of false positives was 206 of 256 positive screening tests (80.5 per cent). The authors noted that a travel history was available for 37 of the 43 patients with true positive results and all had returned from countries in which Lyme disease is endemic (Collignon et al., 2016).

Even in Lyme disease endemic areas, the potential for misdiagnosis is a concern, both from the perspective of misdiagnosis leading to unnecessary antimicrobial treatment (Dessau et al., 2018; Kobayashi et al., 2019) and missing other significant diagnoses. Despite case definitions for Lyme disease in Europe, laboratory testing for *Borrelia*-specific antibodies continues to be frequently used in many clinical situations where testing is not recommended by current guidelines and while the consequences of over-testing for Lyme disease have not been documented directly in clinical studies. The main concerns are delay of other relevant diagnoses, adverse effects and the development of antimicrobial resistance (Dessau et al., 2018). Kobayashi et al. (2019) noted that while Lyme disease is the most common vector-borne infection in the US, diagnostic accuracy in community settings is not well characterised. In their retrospective cohort study of patients referred to an academic centre with a presumed diagnosis or concern for Lyme disease between 2000 and 2013, of 1261 patients, most (911 patients, 72.2 per cent) did not have Lyme disease. Of these patients without Lyme disease, the majority (764 patients, 83.9 per cent) had received antimicrobials to treat Lyme disease or their coinfections. The authors noted the percentage of patients established to have Lyme disease was lower than in earlier studies of referred populations and concluded incorrect diagnosis and unnecessary antibiotic treatment were common both for Lyme disease and co-infections (Kobayashi et al., 2019).

Collignon et al. (2016) also highlighted this, commenting that even a highly specific test will produce some false positives, so that people who have never been exposed to *B. burgdorferi* can have reactive antibody results. This underscores the need for a comprehensive travel history with certainty that a patient has indeed travelled to an area endemic for Lyme disease.

Therefore, awareness of epidemiological context and the absence of an alternative diagnosis are necessary for a clinician to decide whether a positive test is explanatory or coincidental. The difficulties in interpreting diagnostic tests for Lyme disease, as described above, coupled with the difficulties clinicians in Lyme disease endemic countries experience in diagnosing Lyme disease (Brunton et al., 2017) underpin the recommendation that medical professionals in Australia seek advice from appropriate experts in infectious diseases or specialist microbiologists.

Geographic location of acquisition of *B. burgdorferi* infection

The geographic location where a person is bitten by a tick carrying a spirochaete species of the *B. burgdorferi* s.l. complex has important implications in the two-tier diagnostic testing for Lyme disease, hence underlining the necessity of a comprehensive travel history. As noted in 3.5.1 Transmission and geographical distribution of Lyme disease, more than 18 spirochaete species comprise the *B. burgdorferi* s.l. complex. Four species are found only in North America, eleven species occur in and are restricted to Eurasia and three species occur in North America and Europe (Mackenzie, 2013). The antigenic variation by different species of *B. burgdorferi* s.l. species and applicability of the immunoblot interpretation using a method developed in one geographic area to other geographic areas was raised in several articles (Borchers et al., 2015; Chalada et al., 2016; Collignon et al., 2016; Royal College of Pathologists of Australasia, 2019).

Collignon et al. (2016) highlighted that diagnosis is complicated by antigenic variations in the organisms used for developing the assays, noting that not only are there antigenic differences between the *B. burgdorferi* s.l. species, but many of their genes are differentially expressed in tick and mammalian environments. However, the use of newer recombinant antigens rather than whole cell lysates have improved the reliability of serological tests (Borchers et al., 2015; Collignon et al., 2016; Mackenzie, 2013; Moore et al., 2016; Royal College of Pathologists of Australasia, 2019).

Chalada et al. (2016) noted the greater number of strains of *B. burgdorferi* in Europe and Asia that cause Lyme disease than in the US and that different strains of *B. burgdorferi* may express only some of the antigens detected in immunoblot, may constitutionally lack certain genes for certain proteins, or comprise immunodominant antigens of molecular weights that differ from those used in the immunoblot. For these reasons, the immunoblot interpretation using a method developed in one geographical area may not be applicable to other geographic areas, and therefore standardisation of immunoblotting methods for Lyme disease diagnosis in Europe and Asia is unfeasible (Rizzoli et al. (2011), and Robertson et al. (2000) in Chalada et al., 2016).

Borchers et al. (2015) noted the CDC had published recommendations on the number and types of IgM and IgG bands that have to be present in order to consider immunoblot results positive, but that these rules should not be applied to patients who were infected in Europe since the existence of three pathogenic requires species-specific interpretation rules (Borchers et al., 2015). Borchers noted factors such as *Borrelia*-specific antibody repertoire appearing more restricted, fewer patients with EM alone ever developing seropositivity (≤70 per cent compared with 80-86 per cent) and subclinical infection appearing to be considerably more common in European compared to US patients were likely to lower the sensitivity and specificity of serological tests in the diagnosis of Lyme disease acquired in Europe. Borchers et al. stressed how critical it is that known positive and negative samples must be included in all assays. As such, conventional US two-tiered testing has very poor sensitivity in infections acquired in Europe (Borchers et al., 2015).

Similarly, Best et al. (2019), in their Australian study that reported on the performance of serological assays used for Lyme disease testing in Australia and informed the 2017 NRL report (discussed in 4.6.1 NATA/RCPA Accreditation and Accredited Laboratories), noted that in the CDC's most recent case definition an IgG immunoblot is not considered definitively positive for surveillance and diagnosis unless reactivity is observed to five *B. burgdorferi* proteins, an approach recommended by the CDC in 1995 and still followed today (Centers for Disease Control (1995) in Best et al., 2019). However, Best et al. considered whether

interpreting immunoblots using CDC criteria would be appropriate for their study as they noted differences in immunological responses to different strains of *B. burgdorferi* s.l. had been reported with infected US individuals showing reactivity to a greater number of *B. burgdorferi* proteins than their European counterparts (Hauser et al. (1997), and Dressler et al. (1994) in Best et al., 2019). Noting the 1997 study by Hauser et al. had shown using CDC criteria in European patients had resulted in reduced detection in well-characterised infected sera, Best et al. decided not to use CDC criteria to interpret immunoblot results in their study as only 60 of the 639 clinical specimens used in their study originated from North America (Best et al., 2019).

With respect to the Australian situation, the above findings and considerations regarding the influence of geographical location of acquisition of Lyme disease infection and interpretation on immunoblots underpin the importance of taking a comprehensive travel history and including this information on the laboratory request form or in discussions with appropriate experts in infectious diseases. Indeed, as discussed above in the section on pre-test probability, Collignon et al. (2016) reported that in an analysis of serology tests for Lyme disease conducted in a large private diagnostic laboratory in Australia most Lyme disease acquired overseas but diagnosed in Australia was European in origin (30 or 43, or 70 per cent of cases). The travel history of the 43 patients with positive serological tests for Lyme disease included Russia, Latvia, Poland, Czechoslovakia, Eastern Europe, Scandinavia, Germany, Switzerland, The Netherlands, France, the UK, Europe (unspecified), the US, and Central America. A small number of patients (6) were reported as uncertain (extensive travel).

Background seropositivity

Background seropositivity is a major consideration when testing for Lyme disease (Borchers et al., 2015; Dessau et al., 2018; Moore et al., 2016). Dessau et al. noted that concerning clinical specificity, the natural background or cross-reactivity of *Borrelia*-specific antibodies in otherwise healthy individuals remains relatively low, but not negligible in the majority of the European population with a modern urbanised lifestyle. Background seropositivity can vary depending on lifestyle. Dessau et al. noted a German nationwide survey found 9.4 per cent of the generalised population had IgG antibodies to *B. burgdorferi*, but that background immunity above 20 per cent has been described in selected populations with outdoor activities in geographical hotspots. Based on this evidence, Dessau et al. advised physicians should be aware of the local seroprevalence relevant to the diagnostic tests in a given geographical region (Dessau et al., 2018).

Seropositivity can result from previous exposure as IgG and IgM against *B. burgdorferi* can remain for many years after initial infection (Hilton et al. (1999), and Kalish et al. (2001) in Moore et al., 2016). Of the study by Hilton et al. (1999) Moore et al. reported this seroepidemiologic study conducted in New York had found five per cent of study participants had antibodies against *B. burgdorferi*; however, 59 per cent of seropositive patients denied a prior diagnosis of Lyme disease.

Borchers et al. (2015) also cited the 1999 study by Hilton et al. along with two other studies to support the finding that background positivity rate in the general population may be high in endemic areas, ranging from 5 to 8.4 per cent in US studies with two-tier testing (Hilton et al. (1999), Smith et al. (1998), and Krause et al. (1996) in Borchers et al., 2015). In various European and some Chinese provinces, Borchers et al. reported studies had found background seropositivity rates from 3 to 15 per cent (Tiernberg et al. (2007), Dehnert et al. (2012), Skogman et al. (2010), and Hao et al. (2013) in Borchers et al., 2015).

Of seropositivity, Moore et al. (2016) commented that in such persons, seropositivity might indicate a false positive result or be due to a prior undiagnosed infection that either resolved spontaneously or was treated incidentally with antimicrobial drugs prescribed for another indication.

Testing in the early phase of infection

International guidance and guidelines discussed previously highlighted that, as antibodies take several weeks to develop, serology testing in the early phase of infection with *B. burgdorferi* may affect the accuracy of the result and patients may test negative if infected only recently. Similarly, the systematic reviews and meta-analyses reviewed in section 4.5.5 all found accuracy of serology tests increased with progression of the disease, with test sensitivity increasing with progression of *B. burgdorferi* infection from early to late (Aguero-Rosenfeld & Wormser, 2015; Cook & Puri, 2016; Leeflang et al., 2016; National Institute for Health and Care Excellence, 2018b; Waddell et al., 2016).

This section examines the findings of the literature in more depth with respect to the limitations of serology testing for Lyme disease in the early phase of infection.

Serology provides a snapshot of the immune status of the patient at the time of specimen collection. It is expected that antibodies might not be present early in the course of infection, as is the case in most infectious diseases diagnosed by serology (Aguero-Rosenfeld & Wormser, 2015; Halperin, 2015). This period is known as the window period; it is common to all serologic testing, and, as such, clinicians must consider the timing of the patient's illness when ordering and interpreting Lyme disease tests (Moore et al., 2016).

Antibodies against *Borrelia* spp. are slow to develop, with IgM generally not being detectable for the first 1 to 2 weeks after infection and IgG often not appearing for 4 to 6 weeks (Borchers et al., 2015). Very early in the course of Lyme disease, such as during the acute EM rash, as many as 50 per cent of patients will be seronegative, whereas in individuals with symptoms of more than one-to two months duration, essentially every patient is seropositive (Wormser et al. (2006) in Aguero-Rosenfeld & Wormser, 2015; Wormser et al. (2006) in Halperin, 2015).

Serologic testing has low sensitivity during the first weeks of infection while the antibody response is still developing (Aguero-Rosenfeld & Wormser, 2015; Cook & Puri, 2016; Eldin et al., 2019; Lantos et al., 2019; Leeflang et al., 2016; Moore et al., 2016; National Institute for Health and Care Excellence, 2018b; Waddell et al., 2016), and this can result in false negatives when patients are tested less than two weeks after development of the skin lesion (Steere et al. (2008), Aguero-Rosenfeld et al. (1993), and Aguero-Rosenfeld et al. (1996) in Lantos et al., 2019).

While IgM tests tended to have a higher sensitivity in the early stages of Lyme disease, such as the EM rash, and a lower sensitivity in later stages of Lyme disease, by contrast the sensitivity of IgG test increased with disease progression, which is in keeping with the general understanding of how an immunological response to infection develops (National Institute for Health and Care Excellence, 2018b).

The limited sensitivity of antibody assays during early infection is not considered a problem in most cases as the diagnosis of Lyme disease at this point [in endemic areas] is confirmed clinically by the recognition of the presence of the characteristic skin lesion EM (Aguero-Rosenfeld & Wormser, 2015). Indeed, Cook and Puri noted the lack of antibody response in early stage disease is well recognised by the main guidelines [in Lyme disease endemic

countries] (Wormser et al. (2006), British Infection Association, and Stanek et al. (2011) in Cook & Puri, 2016), all of which define the need to diagnose and treat Lyme disease if an EM rash is present, usually giving instructions that serology tests are not necessary (Cook & Puri, 2016). As noted above, Eldin et al.'s review of European and North American guidelines for the diagnosis of Lyme disease (16 guidelines from seven countries, and including the 2018 NICE guideline discussed below) also found the recommendation from 15 of the 16 international guidelines was for no serology testing in the case of EM suspicion due to early serology not being sensitive enough (40-60 per cent) to confirm Lyme diagnosis at the EM stage (Eldin et al., 2019). Additionally, Dessau et al. (2018), in their position paper of ESGBOR-ESCMID, noted that the main recommendations according to current European case definitions for Lyme borreliosis are that typical EM should be diagnosed clinically and does not require laboratory testing, whereas the remaining disease manifestations require testing for serum antibodies to *B. burgdorferi* and diagnosis of Lyme neuroborreliosis requires laboratory investigation of the spinal fluid including intrathecal antibody production.

The most recent international guideline providing recommendations on diagnostic testing for Lyme disease, by the IDSA/AAN/ACR (Lantos et al., 2019), included recommendations for diagnostic testing for patients presenting with EM. The recommendation was concordant to the findings of Eldin et al. (2019) discussed above and with the 2018 NICE guideline that was included in Eldin et al.'s review, and with the findings of Dessau et al. (2018). While IDSA/AAN/ACR and NICE both recommended clinical diagnosis over laboratory testing in patients with EM, neither guideline excluded diagnostic testing to support diagnosis at this stage of the disease. IDSA/AAN/ACR recommended the following diagnostic testing strategy for EM.

- In patients with skin lesions compatible with EM, clinical diagnosis is recommended over laboratory testing **(Strong recommendation, moderate quality evidence)** (The clinical diagnosis assumes that a patient has had plausible exposure to infectious ticks in a region endemic for Lyme disease).
- In patients with one or more skin lesion suggestive of but atypical for EM, antibody testing on an acute-phase sample (followed by a convalescent phase sample if the initial sample is negative) is recommended rather than currently available direct detection methods such as PCR or culture performed on blood or skin samples **(Weak recommendation, low-quality evidence)** (Lantos et al., 2019)**.**

While NICE advised testing is unnecessary for people presenting with EM, because the rash is very specific to Lyme disease and prompt treatment will prevent further symptoms developing, the NICE committee also recommended that as most other symptoms associated with Lyme disease have other more common causes, testing [for people presenting with EM] may be helpful to ensure accurate diagnosis and appropriate treatment (National Institute for Health and Care Excellence, 2018j).

Patients with illnesses suspicious of early Lyme disease but lacking typical EM can present a diagnostic dilemma as serologic test results might be negative at this time (Moore et al., 2016). In most infections serodiagnosis relies on assessment of acute and convalescent specimens, reflecting that in any infection, there is little or no measurable antibody, but as infection persists, the host response reflected in the antibody concentration will substantially increase (Halperin, 2015). Where Lyme disease is suspected on the basis of symptoms but early serological testing is negative, follow up testing on a convalescent sample is recommended (Borchers et al., 2015; Lantos et al., 2019; Lindsay et al., 2014). IDSA/AAN/ACR noted several studies had shown when paired (acute and convalescent-

phase) sera are analysed in patients with EM, seroconversion can be documented in approximately 60-70 per cent of treated cases (Steere et al. (2008), Aguero-Rosenfeld et al. (1996), Steere et al. (1983), Shrestha et al. (1985), and Branda et al. (2011) in Lantos et al., 2019).

Moore et al. (2016) noted the specific Western blot test and its subsequent interpretation are dependent on the time course of the illness, citing evidence to support that IgM response appears first and is generally directed at the most immunogenic antigens; therefore IgM Western immunoblot should be performed along with IgG Western immunoblot on a reflex basis for patients with signs and symptoms lasting ≤30 days. Moore et al. added that the IgG response generally follows that of IgM and involves a larger number of antigens and because most patients have a detectable IgG response beyond 30 days, IgG Western immunoblot as the second-tier test is typically sufficient for diagnosis. Moore et al. advised, at this stage, IgM Western immunoblot is unnecessary and increases the risk for false positives. Aguero-Rosenfeld and Wormser (2015) concurred and emphasised that IgM seropositivity is only of diagnostic use during the first month of early disease and should not be used to support the diagnosis in patients with a prolonged disease who are IgG negative. This advice is echoed by the CDC, and as reported in 4.5.4 Latest international guidance and advice to health professionals and patients about two-tier testing for Lyme disease, the CDC advises that positive IgM results should be disregarded if the patient has been ill for more than 30 days (Centers for Disease Control and Prevention, 2019c).

As noted previously Waddell et al. (2016), in their systematic review and meta-analysis of diagnostic tests used in Northern America, noted the challenge in testing for Lyme disease in patients exhibiting signs and symptoms of Lyme disease for less than 30 days with poor and highly variable sensitivity of serological tests in the initial stages of the disease when an individual is mounting an immune response to *B. burgdorferi.* As such, researchers have explored the use of a variety of targets including VlsE and C6 expressed after infection, Osp C and Fla B expressed by the feeding tick to detect infection sooner. However, Waddell et al. noted cross-reactivity and genetic variability within the targets has limited the diagnostic performance of any target (Branda et al. (2013), Sillanpaa et al. (2007) in Waddell et al., 2016).

Persistence of antibodies, differentiating past and newly acquired infections and testing for cure

The persistence of both IgM and IgG *Borrelia*-specific antibodies for years in some patients make it difficult or impossible to distinguish between past and newly acquired infection, active/current infection, or both based on seropositivity alone (Aguero-Rosenfeld & Wormser, 2015; Borchers et al., 2015; Centers for Disease Control and Prevention, 2019c; Dessau et al., 2018; Lantos et al., 2019; Moore et al., 2016), with this being a recognised limitation for two-tier serologic testing for Lyme disease (Aguero-Rosenfeld & Wormser, 2015; Lantos et al., 2019).

As reported earlier in 4.5.4 Latest international guidance and advice to health professionals and patients about two-tier testing for Lyme disease, the CDC advises that as antibodies normally persist in the blood for months or even years after the infection is gone, the test cannot be used to determine cure. Moore et al. (2016) highlighted that serologic diagnosis of patients with possible reinfection poses a major dilemma for clinicians (Nadelmanand Wormser (2007) in Moore et al., 2016) and also why serologic testing is not useful as a test

of cure. Retesting to assess whether the patient is cured is not justified and illogical and often leads to unnecessary repeat courses of antibiotics (Aguero-Rosenfeld & Wormser, 2015).

In cases of suspected reinfection, a detailed history and physical examination including a thorough examination are essential because most patients will have EM (Moore et al., 2016). For patients without EM, serologic analysis is still recommended but results should be interpreted with caution and in these cases, it might be helpful to conduct an acute-phase and convalescent-phase serologic analysis to detect an increase in EIA titer or an increase in number of antibody bands that might indicate active infection (Aguero-Rosenfeld and Wormser (2005), and Nadelman and Wormser (2007) in Moore et al., 2016).

The effect of antibiotic treatment and the development of seropositivity in patients infected with *B. burgdorferi* was also discussed in the literature. Borchers et al. (2015) noted the rate of seropositivity correlates with the duration of symptoms before diagnosis and treatment not only in samples taken at presentation, but also in samples obtained during follow up, thereby indicating that early antibiotic treatment may abrogate the development of seropositivity (Glatz et al. (2006), Stanek et al (1999) in Borchers et al., 2015). After antibiotic therapy, the production of IgG and IgM antibodies may vary individually and the individual immune response cannot be correlated with the clinical course of the disease or the success of the antibiotic treatment (Peltomaa et al (2003), and Fleming et al. (2004) in Dessau et al., 2018).

On the other hand, studies performed in the 1980s had suggested that early but incomplete treatment with antibiotics might permanently abrogate the antibody response (Dattwyler et al. (1988) in Halperin, 2015). However, Halperin noted these studies relied in large part on diagnosing patients based on measures of T-cell response to *B. burgdorferi* and that subsequent work had shown the T-cell assay to be quite non-specific (Wormser et al. (2006), Dressler et al. (1991) in Halperin, 2015). Halperin noted these later studies rendered the conclusion about the abrogation of antibiotic treatment on serology development incorrect and commented that only if very early treatment eradicates the infection, eliminating any ongoing immune stimulation would treatment blunt the antibody response. Halperin further commented that some have interpreted these early studies to indicate that simply ingesting antibiotics would render a patient seronegative while the antibiotics were present in the patient's system but there has never been any evidence to support this conclusion nor is there any biologically plausible basis for making such an assertion.

With this recognised limitation in two-tier serologic testing, Aguero-Rosenfeld and Wormser (2015) commented it would be desirable to have another type of assay to judge whether new onset symptoms are actually due to Lyme disease in patients who are known to be seropositive and also in patients who are seropositive because of prior asymptomatic infection that had resolved.

Improper use of serologic tests or interpretative criteria and unvalidated tests

As discussed previously, in 4.5.2 Serology testing for Lyme disease and 4.5.3 International consensus on the use of two-tier serology testing for diagnosis of Lyme disease, the CDC has strict interpretive criteria alongside clinical assessment to guide diagnosis. Serologic testing is highly specific when performed and interpreted according to current guidelines. However, improper use of serologic tests or the use of diagnostic tests or interpretative criteria that have not been fully validated may lead to misdiagnosis and unnecessary antibiotic treatment (Lindsay et al., 2014).

This section has relevance to, and should be read in conjunction with section 4.8 Commercially available laboratory testing methods to be avoided.

The interpretation of the Western immunoblot depends on the number of bands present. In the US, where *B. burgdorferi* sensu stricto is the only causative agent of Lyme disease, a positive IgM Western immunoblot result is indicated by the scored presence of ≥two of three bands (21-24, 39, and 41 kDa) and a positive IgG result is indicated by the scored presence of ≥five of 10 bands (18,21-24, 28,30, 39, 41,45,58,66, and 93 kDa) (Chalada et al., 2016; Halperin, 2015; Moore et al., 2016). Halperin noted two important facts need to be kept in mind regarding Western blots and interpretation of the three IgM and ten IgG bands.

1. The Western blot criteria were developed in individuals with positive or borderline ELISAs; as such interpretation in patients with negative ELISAs is quite problematic and should only be attempted with great caution.
2. The IgM tests are quite cross-reactive so false positives are commonplace; patients with disease of more than one- or two-month duration should be IgG seropositive, so only IgG plots provide reliable information. Any IgM findings in this setting should be considered at best, uninterpretable, and more correctly as spurious (Halperin, 2015).

Moore et al. (2016) stressed it was imperative to avoid interpreting fewer bands as a positive result or evidence of infection, because antibodies to several antigens are cross-reactive with non-Borrelial antigens. They noted that, for example, the 41kDa bands indicates reactive antibody against a *B. burgdorferi* flagellin protein; however, this antibody cross-reacts with other flagellar proteins and in a study by Bacon et al., Moore et al. reported this was found in 43 per cent of healthy controls including many persons with little or no exposure risk for Lyme disease (Bacon et al. (2003) in Moore et al., 2016). Therefore the presence of one IgM band or ≤ four IgG bands does not indicate a positive result with evidence indicating overinterpreting a small number of antibody bands leads to reduced specificity and potential misdiagnosis (citing the CDC and a 2015 study by Nelson et al. on neoplasms diagnosed as 'chronic Lyme disease') (Centers for Disease Control and Prevention (2005), and Nelson et al. (2015) in Moore et al., 2016).

Aguero-Rosenfeld and Wormser (2015) concurred, and noted that subjective reading and interpretation can lead to erroneous positive results if weak bands are scored as positive in samples with negative enzyme immunoassay. Additionally, they noted that some healthcare providers in the US believed that Western immunoblots can be used independently of the first step (a practice that Aguero-Rosenfeld and Wormser stressed should be discouraged as it might lead to erroneous results). They noted another knowledge gap was some providers interpreting the presence of any band as positive. They noted that most if not all *B. burgdorferi* antigens are cross-reactive therefore immunoblot interpretation is dependent on the number and type immunoreactive bands that are found (Aguero-Rosenfeld & Wormser, 2015).

Borchers et al. (2015) highlighted that not only are immunoblots subject to intra and interlaboratory variation but also to subjective interpretation and concluded 'over reporting comes from an overzealous diagnosis based on misinterpretation of the serologic findings and inaccuracies of internet information' (Borchers et al., 2015, p. 104).

Moore et al. (2016), in their continuing medical education paper, noted alternative laboratories might use standard Western immunoblot techniques but apply nonstandard interpretation criteria or fail to perform the recommended first tier EIA commenting that 'unfortunately, many of these alternative laboratories have appealed to patients because they

often claim to specialise in testing for tick-borne diseases and assert that their tests have better sensitivity than standard two-tiered serologic analysis' (Moore et al., 2016, p. 1175). They highlighted the recommendation that clinicians use only Lyme disease tests that have been clinically validated and cleared by the FDA, and if there is ever any question regarding testing protocols or interpretation, clinicians should discuss with an infectious disease specialist (Moore et al., 2016). Moore et al.'s additional evaluation of the evidence on unvalidated tests and interpretation criteria is in 4.8 Commercially available laboratory testing methods to be avoided.

The CDC also identified in-house criteria for interpretation of immunoblots as unvalidated laboratory tests that are not recommended for diagnosis of Lyme disease (Centers for Disease Control and Prevention, 2019c).

False positive results in the ELISA from cross-reactive antibodies from other infections or from autoimmune or inflammatory conditions

Earlier we noted that the CDC advised that infection with other diseases, including tick-borne diseases, or some viral, bacterial, or autoimmune diseases can result in false positive test results (Centers for Disease Control and Prevention, 2019c). Additionally, in section 3.4, we noted Lindsay et al. (2014) advised of the importance of taking a clinical history as infection with other related pathogens (e.g. syphilis) and autoimmune disorders may cause false positive results.

There are many species of spirochaetes (including *Borrelia*) present in the normal human gastrointestinal tract (including the oral cavity) and some of these may potentially cause cross-reacting antibodies to be produced by the patient leading to false positive results of serological tests for Lyme disease (Mackenzie, 2013; Royal College of Pathologists of Australasia, 2019). This can also be the case for other spirochaetal infections patients have been exposed to, for example syphilis, leptospirosis or relapsing fever (Shapiro and Gerber (2000) in Mackenzie, 2013) and in cases of recent primary infection with varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, Herpes simplex type 2 virus, and *Rickettsia rickettsia* (Feder (1991) in Mackenzie, 2013). Additionally, bacterial endocarditis, Anaplasmosis or *Heliobacter pylori* infection may cause false positive results (Lindsay et al., 2014).

Antibodies in the serum of patients with autoimmune disorders may also cause false positive results in the ELISA. Indeed, Waddell et al. (2016), in their systematic review of accuracy of diagnostic tests for Lyme disease in North America, noted that in some studies patients with diseases that have similar signs and symptoms to Lyme disease or have humoral responses that overlap with Lyme disease and are known to cross-react (e.g. rheumatoid arthritis, systemic lupus erythematosus, syphilis, autoimmune disorders, leptospirosis, periodontitis, relapsing fever, tularaemia, Southern Tick-associated Rash Illness (STARI), multiple sclerosis, and Epstein-Barr virus infection) were included as controls to more precisely define test specificity (Waddell et al., 2016).

Immunocompromised patients

Above we noted that NICE advised patients of the possible reduction in accuracy of tests that assess for the presence of antibodies, in cases where the person has reduced immunity, for example, in people on immunosuppressant treatments which might affect the development of antibodies (National Institute for Health and Care Excellence, 2018j).

While not entirely specific to Lyme disease, McManus and Cincotta (2015) noted evidence is also accumulating which suggests that immune dysregulation induced by *Borrelia* (and other tick-borne infections) can impact the sensitivity of serological diagnostics (and interpretation of indirect diagnostics of Borreliosis), by down regulation of both the innate and antibody responses in patients. Key points raised in this paper included: serology testing of Borreliosis patients can result in false negatives (ELISA and Western blot) due to production of low affinity IgG subclasses and reduced total IgG; and prolonged IgM response observed could be due to relapsing fever *Borrelia* infection or inhibition of isotype switching prevention of the IgG response. As such, McManus and Cincotta stated that 'indirect tests that rely on an immune response are contraindicated in immunocompromised individuals' (McManus & Cincotta, 2015, p. 87).

Dessau et al. (2018), in their position paper for ESGBOR-ESCMID, also noted there are reports of immunocompromised Lyme disease patients without a detectable antibody response, but commented that this may be coincidental and a larger series of samples would be required to establish if a lower test sensitivity would apply in these special groups of patients.

4.6. Diagnostic testing for Lyme disease in Australia

The considerations in making a differential diagnosis and diagnosing Lyme disease in Australia was covered in 3.5.8 Diagnosis of Lyme disease in Australia.

Despite multiple studies which have thoroughly searched for it in Australian ticks and patients, the organisms that cause Lyme disease have not, to date, been identified in Australia. In a country such as Australia, where Lyme disease is not endemic and there is a low prevalence population, there are additional challenges in diagnosing Lyme disease where Lyme disease is not commonly seen in clinical practice. It is not possible to reliably diagnose Lyme disease on clinical symptoms and signs alone. Laboratory testing is essential, as many other infectious and non-infectious diseases can have similar features to Lyme disease and all stages of Lyme disease have features that mimic other medical conditions (Lum et al., 2015; Royal College of Pathologists of Australasia, 2019).

As discussed earlier, in their 2019 review of European and American guidelines (16 guidelines from seven countries) for the diagnosis of Lyme disease, Eldin et al. found all guidelines indicated that the diagnosis of Lyme disease is currently based on a two-tier serology at all stages of infection, except for the early localised dermatological presentation known as EM (Eldin et al., 2019). In the review of Eldin et al., 15 of the 16 international guidelines recommended no serology testing in the case of EM suspicion due to early serology not being sensitive enough (40 – 60 per cent) to confirm Lyme diagnosis at the EM stage (Eldin et al., 2019). However, in Australia, where Lyme disease in not endemic, diagnostic testing is recommended.

In Australia, laboratory diagnostic testing for Lyme disease is required for two reasons.

- Unless the clinician is familiar with the pathognomonic EM rash, it is clinically safer to obtain supportive evidence of infection through diagnostic testing (culture or PCR of the tissue or more usually antibody testing on a convalescent sample).
- Diagnostic laboratory support is preferred for patients presenting with non-specific signs and symptoms of a disease syndrome, notwithstanding the limitations of the tests (Lum et al., 2015).

A diagnosis of Lyme disease in Australia requires:

- a careful medical history
- a history of overseas travel to areas where Lyme disease is endemic; a patient must have been exposed to ticks however, a history of documented tick bite is not essential as many tick bites go unnoticed
- objective clinical findings, and
- appropriate in vitro diagnostic tests undertaken by NATA/RCPA accredited laboratories (Royal College of Pathologists of Australasia, 2019).

As discussed in 3.5.8 Diagnosis of Lyme disease in Australia, in areas not endemic for Lyme disease [for example Australia], the positive predictive value of the serology test will be low (Chalada et al., 2016; Collignon et al., 2016; Royal College of Pathologists of Australasia, 2019). Tests should only be requested if there is a well-founded suspicion of Lyme disease and not in situations of low pre-test probability, in order to minimise risk of a false positive result (Moore et al. (2106) in Collignon et al., 2016).

There is established Australian guidance for diagnostic laboratory testing for Lyme disease (Lum et al., 2015; Royal College of Pathologists of Australasia, 2019). Diagnostic testing for

Lyme disease should only be initiated following advice from appropriate experts such as a consultant physician practising in his or her speciality of infectious diseases or a specialist microbiologist and should only be undertaken in Australia in a pathology laboratory accredited by NATA/RCPA to conduct such testing.

If Lyme disease is being considered, patients should be referred for Lyme disease serology to the GPs' regular Approved Pathology Practitioner (APP) (Lum et al., 2015).

Confirmed diagnosis

A confirmed case of Lyme disease in Australia requires laboratory evidence AND clinical evidence AND epidemiological evidence.

The Royal College of Pathologists of Australasia notes that caution is important in dealing with specimens for Lyme disease testing and in the interpretation of positive or indeterminate laboratory results, and advises that medical microbiologists should add explanatory comments to all such reports to assist the referring doctor to interpret the laboratory findings correctly (Royal College of Pathologists of Australasia, 2019).

No confirmed diagnosis of Lyme disease and symptoms resolve

If the immunoblot test for Lyme disease is negative and symptoms have resolved, NICE advises medical professionals to explain to the patient that no treatment is required (National Institute for Health and Care Excellence, 2018j).

No confirmed diagnosis of Lyme disease and symptoms persist

If the immunoblot test for Lyme disease is negative (regardless of the ELISA result) but symptoms persist, NICE recommends considering a discussion with, or referral to, a specialist appropriate to the patient's history and symptoms (for example, adult or paediatric ID physician, rheumatologist or neurologist) to:

- review whether further testing may be required for suspected Lyme disease (for example, synovial fluid aspirate, or biopsy, or lumbar puncture for cerebrospinal fluid analysis); OR
- consider alternative diagnoses including both infectious (including other tick-borne diseases) and non-infectious diseases (National Institute for Health and Care Excellence, 2018j).

4.6.1. NATA/RCPA Accreditation and Accredited Laboratories

Above, we reported that Brown (2018) found in his analysis of submissions made by patients who identified as having Lyme disease or DSCATT to the Senate Inquiry that nearly three-quarters (508 patients; 72.8 per cent) of the 689 submitters reported data on 'any' diagnostic laboratory testing. Of the 137 submissions that disclosed a NATA/RCPA accredited diagnostic pathology test, only 14 patients; (10.2 per cent) reported a positive serology (representing 2.8 per cent of all that reported pathology and 2.0 per cent of all submissions). The majority (454 patients; 89.4 per cent) who reported data on diagnostic testing reported positive serology from a non-NATA/RCPA accredited laboratory. This represented 65 per cent of all submissions (Brown, 2018).

RCPA notes that sometimes laboratory specimens are sent by referring doctors to non-NATA/RCPA accredited laboratories in Australia and overseas (mainly US and Germany) for Lyme disease testing. RCPA issued the following advice.

> *Many of the tests performed by such laboratories, according to Australian expert pathologists, have not been validated for use to diagnose Lyme disease, based on consensus documents published by expert European (citing Beaman 2016) and North American (citing Dessau et al. 2018) professional bodies. Until the latter two consensus documents advise otherwise, no confidence can be attached to the results of such unvalidated tests. The referring doctor (and their patients) must be advised "caveat emptor" ("let the buyer beware") (Royal College of Pathologists of Australasia, 2019, p. 5)*.

Diagnostic tests conducted overseas are not covered under Australia's Medicare arrangements.

Therefore, for diagnostic testing for Lyme disease in Australia it is essential to use NATA/RCPA-accredited, internationally recognised laboratories. NATA accreditation provides a means of determining, formally recognising and promoting that an organisation is competent to perform testing, inspection, calibration, and other related activities. Accreditation delivers confidence and underpins the quality of results. NATA's accreditation is based on a peer-review process and is based on international standards. Since NATA accreditation is highly regarded both nationally and internationally as a reliable indicator of technical competence, use of the NATA logo and use of a NATA endorsement on reports tells prospective and current clients that the facility has been assessed against best international practice (National Association of Testing Authority, Australia, n.d.).

Additionally, RCPA notes Australia leads the world in laboratory accreditation and advises all pathology laboratories in Australia receiving funding via Medicare must be accredited by the National Association of Testing Authorities (NATA)/RCPA Laboratory Accreditation Program. The Standards are set by the National Pathology Accreditation Advisory Council (NPAAC). The quality management aspects of the NPAAC requirements are based on the international standard ISO 15189 Standard for Medical Laboratories (Royal College of Pathologists of Australasia, n.d.).

NATA/RCPA accredited laboratories follow international best practice in diagnostic testing for Lyme disease

NATA/RCPA Accredited Laboratories can detect tick-borne illnesses.

The current standard laboratory protocol for diagnosing Lyme disease in Australian Diagnostic Laboratories follows international best practice and uses a two-tier serology system, the first stage involving screening with an 'enzyme-immune-assay (ELISA)' and, if positive, followed by an immunoblot assay (Western blot) (Royal College of Pathologists of Australasia, 2019, p. 4). Assays generally incorporate known specific antigens from both European and American strains of *B. burgdorferi* s.l. known to cause Lyme disease. Standard practice has been to confirm a positive EIA with an immunoblot. RCPA advises the number of positive bands seen in the immunoblot and their specificity and clinical significance varies (for example, there are differences in US and European criteria) and must be interpreted with caution, especially in the absence of an Australian *Borrelia* sp. (Royal College of Pathologists of Australasia, 2019).

The issue of discordant results between accredited laboratories in Australia, and non-accredited Australian and overseas laboratories requiring further inquiry was a finding of the Senate Inquiry along with the acknowledgement that the Department of Health had contracted the NRL to conduct a review of serological assays used to diagnose Lyme disease (Senate Community Affairs References Committee, 2016a, 2016b).

In Australia, the NRL review of serological assays to diagnose Lyme disease determined the tests used by accredited laboratories to diagnose Lyme disease had equivalent reliability to tests used in overseas laboratories (National Serology Reference Laboratory Australia, 2017). This means Australian NATA/RCPA accredited laboratories are able to confidently diagnose classical Lyme disease acquired in patients who have travelled to endemic areas and have contracted the infection more than four weeks prior to testing, noting that most patients seroconvert within four to eight weeks of infection (Royal College of Pathologists of Australasia, 2019).

While the NRL report confirmed that Australian laboratories have equivalent reliability to tests used in overseas laboratories, tests for Lyme disease have limitations whether internationally or in Australia (see section 4.5.4 Latest international guidance and advice to health professionals and patients about two-tier testing for Lyme diseases).

The NRL '*Final Report: Investigation of the performance of assays for Lyme disease in Australia*' was published in May 2017. The report noted the project was designed to determine the ability of *in vitro* diagnostic devices IVDs ('tests' uses for testing individuals for Lyme disease) to detect *Borrelia burgdorferi* sensu lato and not other *Borrelia* species. Objectives of the project were:

- to evaluate the IVDs used to test Australian individuals for Lyme disease both in Australian and overseas laboratories to the extent possible within the resources available; and
- to show whether Lyme disease testing performed by Australian laboratories was of high quality (National Serology Reference Laboratory Australia, 2017).

Eight institutions provided serum specimens of sufficient volume to the project, four in Australia and four overseas. In Australia, the institutions were:

- Sullivan and Nicolaides Pathology (SNP);
- Pacific Laboratory Medicine Services at Royal North Shore Hospital (PaLMS);
- Australian Biologics; and
- Australian Red Cross Blood Service (ARCBS).

The overseas laboratories were:

- Rare and Imported Pathogen Laboratory (RIPL), Public Health England (PHE);
- InfectoLab, Germany;
- Armin Labs, Germany; and
- IGeneX Inc. USA.

NRL's conclusions were as follows.

- Results reported by medical testing laboratories using the test kits in Australia were consistent with those from international laboratories. There can be confidence that infections with *Borrelia burgdorferi sl* are appropriately detected or excluded using these tests more than 80 per cent of the time.
- Two-step testing with an immunoassay followed by an immunoblot test on positive results provides the best diagnostic accuracy. Confirmatory immunoblots should be read using scanning software rather than read by eye to limit inconsistency.
- There was reasonable 'test to test' correlation between the different IVDs (a true positive on one test was generally positive on another test).
- Test kits varied in their performance and generally IVDs that use native proteins are less reliable than other IVDs and are best avoided (Department of Health, 2018c; National Serology Reference Laboratory Australia, 2017).

Regarding the relevance of the findings to positive test results for Lyme disease in people who have not travelled to areas where Lyme disease is widespread, the report stated:

> *The investigation was designed to evaluate the tests for Lyme disease. It did not evaluate the use of the test in individual patients. The research confirms that false positive results can occur in individuals who have not been exposed to Borrelia burgdorferi sl. A positive test result in someone who has not travelled to an overseas region with Lyme disease is likely to be a false detection of antibody to Borrelia burgdorferi sl. In these cases, other causes of the symptoms should be sought, or at least the test repeated.*
>
> *For any illness, results from tests must be interpreted in the clinical context of the patient and the test must be performed for the correct indications. When there is discordance between the patient's clinical history and examination and a serology test result, the test result must be considered cautiously (Department of Health, 2018c, p. 2).*

A follow-up paper to the NRL report, published in 2019, noted that in the known negative population, specificities of the immunoassays ranged between 87.7 per cent and 99.7 per cent, and in Australia's low prevalence population, this would translate to a positive predictive value of <4 per cent (Best et al., 2019).

In their paper, Best et al. (2019) aimed to determine the level of agreement in results between commonly used *B. burgdorferi* serology assays in specimens of known status and between results reported by different laboratories when they use the same serology assay. The authors noted that while the spirochaetes of the *B. burgdorferi* s.l. complex have not been identified in Australia, Australian patients exist, some of whom have not left the country, who have symptoms consistent with 'so-called 'chronic Lyme disease'', and when blood specimens from these individuals are tested in specialist Australian laboratories outside Australia or in Australian laboratories, conflicting results are sometimes obtained. Such discrepancies have caused patients to question the results from the Australian laboratories and seek assistance from the Australian Government to clarify why these discrepancies occur (Best et al., 2019).

Best et al. (2019) tested 771 clinical and blood donor specimens in five immunoassays and five immunoblots used in Australia and elsewhere for the detection of IgG antibodies to *B. burgdorferi* s.l. A further 176 blood donor specimens were tested in the five immunoassays

assays only. Positive, negative and equivocal specimens were contributed by participating laboratories located in Lyme disease endemic and non-endemic areas (see NRL report above for participating laboratories). As mentioned previously, the authors noted that while a classical two-tier algorithm for Lyme disease testing may be considered a gold-standard approach (Centers for Disease Control and Prevention (2005) in Best et al., 2019), they also pointed out that differences in the antigens used and quality of the serological assays available for the testing mean that different final interpretations can be obtained when different assays are used for each of the tiers (Ang et al. (2011) in Best et al., 2019).

Best et al. (2019) acknowledged a number of limitations to the study including:

- use of archived specimens and allocating presumed positive and negative status by consensus results between assays
- specimens were contributed by participating laboratories based on their test results which had been generated in many cases in the assays included in the study
- they may have been able to draw further conclusions on assay performance if they had known definitively the relationship between timing of specimen collection and onset and/or type of symptoms were relevant (Best et al., 2019).

Despite these limitations, they found that when using the same assay, discordance between study and clinical laboratories' results occurred less than two per cent of the time, that assays agreed on positive results approximately 80 per cent of the time, and on negative results approximately 90 per cent of the time. The authors noted the findings suggested that discordance in results between laboratories is more likely due to variation in algorithms or in the use of assays with different sensitivities and specificities rather than conflicting results being reported from the same assay in different laboratories (Best et al., 2019).

4.7. Less commonly used laboratory methods for direct detection of *B. burgdorferi* in clinical tissue specimens

4.7.1. Culture

The diagnostic 'gold standard' for specificity of *Borrelia* infection is the isolation of *Borrelia* spp. by culture from patient specimens with subsequent PCR-based or other confirmation of its identity (Lindsay et al., 2014). Cultivable samples include ticks, infected animal (particularly reservoir) tissues and human tissues (EM skin biopsy, blood, synovial tissue and CSF) (Royal College of Pathologists of Australasia, 2019).

The culture of *Borrelia* bacteria is difficult, culture is expensive and requires special media and laboratory expertise, the number of spirochaetes in clinical specimens is low and culture is used/attempted usually only in reference laboratories (Borchers et al., 2015; Collignon et al., 2016, 2016; Halperin, 2015; Moore et al., 2016; National Institute for Health and Care Excellence, 2018b; Royal College of Pathologists of Australasia, 2019).

While RCPA notes culture has no clinical application and serves only as an important research tool (especially in the Australian context), it advises clinicians should discuss with reference laboratories before sending specimens for culture, with the best specimen advised to be probably a biopsy of the skin rash in early, acute Lyme disease (Royal College of Pathologists of Australasia, 2019).

Sensitivity for culture for EM is 40 per cent to 70 per cent, but is only approximately 3 per cent to 17 per cent for CSF samples, and very low for synovial fluid or tissue samples; therefore, negative results do not exclude the diagnosis of Lyme disease (Borchers et al., 2015). The NICE review (2018) [C: diagnostic tests] found *B. burgdorferi* culture, which also functioned as a reference standard in the review, showed poor results when compared to clinical diagnosis (National Institute for Health and Care Excellence, 2018b).

Culture is not routinely performed and is rarely used as a reference standard in clinical studies (Borchers et al., 2015; National Institute for Health and Care Excellence, 2018b). As results are not available for two to six weeks, it is not compatible with providing a rapid diagnostic result and therefore has limited utility in the clinical setting and is not useful for clinical decision making (Borchers et al., 2015; National Institute for Health and Care Excellence, 2018b; Royal College of Pathologists of Australasia, 2019).

In Australia, Chalada et al. (2016), in their review of the evidence regarding culture from patients, reported that while biopsies of EM had been taken from numerous Australian patients for histology or PCR, there has only been one published report of an Australian *Borrelia* culture being successful (Hudson et al. (1998) in Chalada et al., 2016). Chalada et al. noted that although the disease appeared to follow the tick bite contracted in New South Wales, the patient had also travelled to three Lyme disease endemic countries in Europe 17 months before the onset of symptoms and that while this published case demonstrated a culture confirmed Lyme Borreliosis causing *Borrelia* isolate in an Australian patient, Australian acquisition could not be confirmed (Chalada et al., 2016).

4.7.2. Molecular (DNA and RNA sequence analysis) 'PCR'

The same samples as used for culture may be also be tested by molecular techniques (Royal College of Pathologists of Australasia, 2019). In Australia, PCR for overseas *Borrelia* spp. can be done in Australian Reference Laboratories (Royal College of Pathologists of Australasia, 2019). Molecular investigations are valuable for clinical research investigations but are of limited clinical utility at present (Royal College of Pathologists of Australasia, 2019). PCR for detection of *B. burgdorferi* DNA in Lyme disease patient samples is affected by many of the same limitations as culture with the exception that results may be obtained faster and PCR may be more sensitive in samples with a low concentration of *B. burgdorferi* (Waddell et al., 2016).

PCR on DNA extracted from tissue or fluid specimens is useful for the confirmation of *Borrelia* infection, particularly in the synovium of patients with Lyme arthritis and also in cases of diagnostic uncertainty (Borchers et al., 2015). Collignon et al. noted that while PCR targeting various gene targets (flaB, 16SrRNA, recA, p66, ospA, 5SrRNAe23SrRNA gene spacer region) can provide highly specific evidence of *B. burgdorferi* nucleic acid, the very low organism load means that even the sensitivity of PCR in this context 'is not great' (Aguero-Rosenfeld et al. (2005) in Collignon et al., 2016, p. 1). Further, if too many PCR cycles are undertaken, specificity is lost and there is also the possibility of contamination (Collignon et al., 2016).

The variability of methodologies, gene targets and primers from study to study continue to impact the interpretation of the PCR results (Liveris et al. (2012), Eshoo et al. (2012), and Nocton et al. (1996) in Waddell et al., 2016). Borchers et al. (2015) similarly noted none of the large variety of methods used are standardised and therefore distinct methods yield divergent results. Sensitivity of PCR in EM is 75 to 80 per cent, 15 to 30 per cent in CSF and 60 to 85 per cent in synovial fluid samples. As with culture of *Borrelia*, negative findings do not exclude the diagnosis of Lyme disease (Borchers et al., 2015).

Regarding accuracy, the NICE review found that PCR, which also functioned as a reference standard in the review, showed poor results when compared to clinical diagnosis (National Institute for Health and Care Excellence, 2018b). In their systematic review and meta-analysis of diagnostic tests for Lyme disease, Waddell et al. (2016) found the sensitivities of PCR studies conducted in North America to be lower than those that employed a two-tier serology diagnostic protocol (Liveris et al. (2012), Eshoo et al. (2012), and Nocton et al. (1996) in Waddell et al., 2016).

Within the Australian context, Beaman noted a single non-accredited laboratory has reported five PCR-positive ECM specimens (Mayne (2012), and Mayne et al. (2014) in Beaman, 2016). Similarly, Chalada et al. noted *Borrelia burgdorferi* s. l. DNA has been detected and sequenced in five Australian patients presenting with Lyme-like illness (Mayne et al. (2014), Mayne (2012), and Mayne (2015) in Chalada et al., 2016). Limitations raised by Chalada et al. of these three studies included the primer sequences not being published, some patients having travelled overseas, non-specific amplification possibly leading to a false positive PCR reaction, and at the time of Chalada et al.'s paper being submitted, a laboratory having not shared their primer sequences or any DNA or isolates with researchers for independent verification (Chalada et al., 2016).

4.8. Commercially available laboratory testing methods to be avoided

As highlighted in 4.5.6 Considerations, limitations and important variables in serology testing for Lyme disease, concerns were raised in the literature about unvalidated commercially available laboratory testing methods, and incorrect diagnoses (and treatment) based on these methods. This section reports on the recommendations, guidelines and guidance from international authorities and Australian and international clinical professional bodies about commercially available laboratory testing methods to be avoided.

Measurement of CD57 lymphocytes (by flow cytometry) and PCR for Lyme disease on urine samples are not recommended in the laboratory diagnosis of Lyme disease in Australian laboratories (Royal College of Pathologists of Australasia, 2019). IDSA/AAN/ACR concurred in 2019, advising that some commercially available laboratory testing methods including non-standard serology interpretation, urine antigen or DNA testing, or the use of lymphocyte transformation tests or a quantitative CD57 lymphocyte assay should be avoided for clinical use due to lack of systematic, independent, reproducible validation studies (Lantos et al., 2019).

Likewise, current guidance from the CDC (Centers for Disease Control and Prevention, 2018) on laboratory tests that are not recommended for Lyme disease due to the accuracy and clinical usefulness not having been adequately established, is similar to those highlighted by IDSA/AAN/ACR and Australian laboratory guidance. Examples of tests that are not recommended by the CDC, based on evidence include:

- Capture assays for antigens in urine
- Culture, immunofluorescence staining, or cell sorting of cell wall-deficient or cystic forms of *B. burgdorferi*
- Lymphocyte transformation tests
- Quantitative CD57 lymphocyte assays
- 'Reverse Western blots'
- In-house criteria for interpretation of immunoblots
- Measurements of antibodies in joint fluid (synovial fluid), and
- IgM or IgG tests without previous ELISA/EIA/IFA (Concerns Regarding a New Culture Method for Borrelia burgdorferi Not Approved for Diagnosis of Lyme disease (2014), Johnson et al. (2014), Notice to readers: caution regarding testing for Lyme disease (2005), and Marques et al. (2009) in Centers for Disease Control and Prevention, 2018).

Published results of urine antigen tests and CD57 tests have been shown to be inaccurate (Klempner et al. (2001), and Marques et al. (2009) in Moore et al., 2016). The authors cautioned that the evidence indicated false positive results for alternative tests or unvalidated interpretation criteria can lead to patient confusion and misdiagnosis (Concerns Regarding a New Culture Method for Borrelia burgdorferi Not Approved for the Diagnosis of Lyme Disease (2014), Nelson et al. (2014), and Nelson, Elmendorf and Mead (2014) in Moore et al., 2016). Aguero-Rosenfeld and Wormser (2015) concurred, noting patients tested by laboratories using unvalidated tests may be erroneously diagnosed as having Lyme disease and then may be prescribed long courses of antibiotics which can lead to severe side effects and even death.

Moore et al. (2016) looked at the evidence on unvalidated tests and interpretation criteria, noting several alternative testing centres use laboratory developed tests, also known as home brew tests, that are not currently subject to FDA regulations and might not be clinically validated (Centers for Disease Control and Prevention (2005), and Nelson et al. (2014) in Moore et al., 2016). Aguero-Rosenfeld and Wormser (2015) also noted these 'in-house' or 'home brew' antibody assays (laboratory developed tests) often use interpretation criteria different from that recommended by the CDC with many of these laboratories offering these tests as per 'customer' requests, including the reporting of 'CDC nonspecific bands' on immunoblots.

Moore et al. (2016) and Aguero-Rosenfeld and Wormser (2015) cited a 2014 study which found a false positive rate of 58 per cent for samples from healthy controls submitted to an alternative testing centre that used 'in-house' developed immunoblots and unvalidated criteria to interpret IgM and IgG immunoblots (Fallon et al. (2014) in Aguero-Rosenfeld and Wormser, 2015; Fallon et al. (2014) in Moore et al., 2016). Waddell et al. (2016), in their systematic review, also looked at validation data from licensed assays and 'in-house' tests used by several laboratories across North America and noted that the performance of 'in-house' tests cannot be validated or critiqued as the composition of the test is not always publicly available or evaluated in the peer-reviewed literature (Waddell et al., 2016, p. 18). Thus, the performance of these 'in-house' assays and some of the older commercial assays have not been evaluated against well characterised panels of serum from patients with the full spectrum of Lyme disease clinical symptoms, with appropriate numbers of healthy controls and patients with look-alike diseases (Molins et al. (2014) in Waddell et al., 2016, p. 18).

Of the laboratories that are using unvalidated tests, some are offering a variety of coinfection panels including testing for pathogens that have not been proven to be transmitted by the ticks that transmit Lyme disease (Aguero-Rosenfeld & Wormser, 2015).

Concerns about unvalidated laboratory testing also included new methods for culturing *Borrelia* that have been demonstrated to be unreliable (Auwaerter et al., 2011; Moore et al., 2016; Waddell et al., 2016). Moore et al. (2016) highlighted that an evaluation of published results from a laboratory claiming to have a new *Borrelia* culture method demonstrated these results to be highly suspicious for laboratory contamination (Nelson et al. (2014), Johnson et al. (2014) in Moore et al., 2016). Earlier, Auwaerter et al. highlighted the use of an unconventional culture method by a former president of International Lyme and Associated Diseases Society (ILADS) reported positive blood cultures for *B. burgdorferi* in more than 90 per cent of a group of patients who had previously received antibiotic treatment for Lyme disease (Phillips et al. (1998) in Auwaerter et al., 2011). However, Auwaerter et al. noted the findings of Phillips et al. could not be replicated by others, who also showed the novel culture medium was lethal for *Borrelia* species (Marques et al. (2000) in Auwaerter et al., 2011).

Similar to the tests not recommended by the CDC, Auwaerter et al. (2011) highlighted problems with diagnostic tests that are or have been advocated by some 'Lyme literate' medical doctors and 'chronic Lyme disease' activists, and commented that despite continued warning from the FDA and the CDC about the potential unreliability of unvalidated diagnostic tests for Lyme disease, many 'Lyme literate' medical doctors continue to use such assays (Auwaerter et al., 2011).

The authors cited

- the CDC MMWR 2005 which reported no clinical validation for the following diagnostic tests: flow cytometry; immunofluorescence for L-forms of *Borrelia;* urine reverse Western blot; and urine dot blot (Notice to readers: caution regarding testing for Lyme disease (2005) in Auwaerter et al., 2011)
- Klempner et al. (2003), that had shown the Lyme urine antigen test to be unreliable (Klempner et al (2003) in Auwaerter et al., 2011)
- Marques et al. (2009), that had reported the CD57 cell count test had found no specific association with *B. burgdorferi* infection (Marques et al. (2009) in Auwaerter et al., 2011)
- Dumler (2001) which had reported for PCR variable sensitivity in the plasma, urine and CSF and no clinical validation (Dumler (2001) in Auwaerter et al., 2011)
- Zoschke et al. (1991) which showed for Lymphocyte transformation low specificity and no clinical validation (Zoschke et al. (1991) in Auwaerter et al., 2011).

Auwaerter et al. commented Lyme specialty laboratories are favoured by some Lyme disease activists and 'Lyme literate' medical doctors because their non-standard testing methods and interpretation criteria often lead to more positive results than other laboratories that rely on validated methods (Shah et al. (2007) in Auwaerter et al., 2011). Two immunological tests, a T-cell assay and measurement of the CD57 cell count were noted by Auwaerter et al. to be favoured by some 'Lyme literate' medical doctors to indicate the presence of *B. burgdorferi* infection; both of these tests were noted by Auwaerter et al. to be considered unreliable (Marques et al. (2009), and Zoschke et al. (1991) in Auwaerter et al., 2011).

In addition to concerns about the unvalidated tests used by Lyme specialty laboratories, Auwearter et al. (2011) highlighted concerns about ownership, affiliation with Lyme organisations and lawsuits from incorrect diagnosis. More recently, Auwaerter et al. noted that in 2009 several residents in Kansas won a $30 million suit against another Lyme disease speciality laboratory for incorrectly diagnosing individuals with Lyme disease (Auwaerter et al., 2011).

Additionally, Eldin et al.'s 2019 review of European and American guidelines for the diagnosis of Lyme borreliosis identified a number of alternative diagnostic tools that had been proposed for Lyme disease in recent years. This included various PCR systems and antigen detection in urine or blood, lymphocyte transformation tests, numeration of CD57 cells, positive natural killer cells, enzyme-linked immune-spot assays (ELISPOT) xenodiagnoses and commercially available *B. burgdorferi* rapid diagnostic tests (RDT) (Eldin et al., 2019). Similar to the CDC and IDSA/AAN/ACR advice, Eldin et al. advised these methods have been insufficiently evaluated and as a consequence immunohistochemical detection of *Borrelia* from tissues, lymphocyte transformation tests, detection of specific cytokines (CXCL 13) or circulating immune-complex, CD57 cells, *Borrelia* antigens from patients' samples, and detection of *Borrelia* in samples by light microscopy are not recommended in most guidelines (Wormser et al. (2006), British Infection Association (2011), Hofman et al. (2017), Delaere(2016), and Huppertz et al. (2012) in Eldin et al., 2019).

Eldin et al. (2019) noted that the German Borreliosis Society guidelines are the only ones to recommend lymphocyte transformation tests in almost all stages of Lyme disease but do not specify sensitivity or specificity values for this test. As noted previously, Eldin et al. identified six German guidelines with five of these issued by academic societies and available on the

website of the Association of Scientific Medical Societies in Germany. The guideline by the German Borreliosis Society was reported by Eldin et al. (2019, p. 122) to be defined as a 'transdisciplinary medical association of physicians and researchers working on Lyme and tick-borne diseases' and is not officially recognised by the German authorities as an academic society. It was also the one guideline to have discordant recommendations when compared to the other guidelines and had the lowest quality score (score point of 1) (Eldin et al., 2019).

4.9. International developments and recommendations in testing for Lyme disease

Waddell et al. (2016) pointed out that in addition to future work to continually improve the sensitivity of all tests particularly for early Lyme disease and the ability to distinguish between active and previous infections, ongoing work into new immuno-assay techniques and combinations of antigen targets that may help inform disease stage is also occurring. They also noted development of point-of-care tests that do not require highly specialised technical skills and subjective interpretation of the results would help address some criticisms of immunoblot techniques.

4.9.1. CDC

The CDC (Centers for Disease Control and Prevention, 2019c) supports the development of new tests for diagnosing Lyme disease, advising that new tests may be developed as alternatives to one or both steps of the two-step process currently recommended. Before the CDC will recommend a new test, it must be cleared by the FDA. The CDC provides links to the HHS Federal research updates on Lyme disease diagnostics (Centers for Disease Control and Prevention, 2019a), and the Updated CDC Recommendation for Serologic Diagnosis of Lyme disease (Mead et al., 2019).

Updated CDC Recommendation for Serologic Diagnosis of Lyme disease (2019)

The CDC in its MMWR advised, on 29 July 2019, FDA had cleared several Lyme disease serologic assays with new indications for use based on a modified two-test methodology (Food and Drug Administration (2019) in Mead et al., 2019). The modified methodology uses a second EIA in place of a Western immunoblot assay.

The indications for public health practice were that, when cleared by FDA for this purpose, serologic assays that utilise a second ELISA in place of Western immunoblot are acceptable alternatives for the serologic diagnosis of Lyme disease. The updated CDC recommendation was that clearance by FDA of the new Lyme disease assays indicates that test performance has been evaluated and is 'substantially equivalent to or better than' a legally marketed predicate test (Mead et al., 2019, p. 73). Based on the criteria established at the 1994 Second National Conference on Serologic Diagnosis of Lyme Disease, clinicians and laboratories should consider serologic tests cleared by FDA as CDC-recommended procedures for Lyme disease serodiagnosis (Mead et al., 2019).

4.9.2. National Institutes of Health

The National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) funds research to develop better methods of diagnosing, treating and preventing Lyme disease (National Institute of Allergy and Infectious Diseases, 2019). NIAID advised in its 'Current efforts in Lyme disease research, 2019 Update report it has long highlighted the need for improved Lyme disease diagnostics, particularly the need for new diagnostic tests that can detect the earliest stages of infection. NIAID also noted, as has been discussed previously, the limitation that the current two-tier standard test is open to subjective interpretation. Currently, no point-of-care diagnostic test is available for Lyme disease that can substitute for laboratory-based testing. NIAID noted a point-of-care diagnostic that accurately detects Lyme disease early in infection would enable physicians to make more informed treatment decisions when a patient in a Lyme disease endemic area presents with symptoms consistent with Lyme disease.

NIAID efforts towards developing improved diagnostics include:

- developing and testing a new cytokine-based immunoassay for Lyme diagnosis which, if successful, could allow for earlier and more rapid diagnosis of Lyme disease
- metabolic biomarkers and biosignatures for improved diagnostics are being identified and characterised. These studies may contribute to new methods for detecting Lyme disease, including diagnosis of early-stage of disease, accurate staging of disease, or indications of successful treatment. For example, researchers are exploring ways of detecting small molecule metabolites in urine of early Lyme disease patients, which would be very helpful in the clinic since urine is an easily obtainable sample
- rapid, point-of-care Lyme diagnostic tests using lateral flow technologies
- research on multiplex qPCR assays to simultaneously detect Lyme disease and coinfections such as babesiosis (National Institute of Allergy and Infectious Diseases, 2019).

Research funded by NIH into development of point of care tests for Lyme disease was reported by NIH (National Institutes of Health, 2019) in October 2019 to be providing promising results.

5. RESEARCH QUESTION 4: WHAT ARE THE TREATMENT MODALITIES THAT HAVE BEEN PROVIDED TO PATIENTS (INCLUDING SUBGROUPS OF PATIENTS) WITH DSCATT IN AUSTRALIA AND WHAT IS THE EVIDENCE BASE TO SUPPORT THESE TREATMENT MODALITIES?

5.1. Overview and key findings

This section provides the findings of the literature reviewed to answer research question 4:

What are the treatment modalities that have been provided to patients (including subgroups of patients) with DSCATT in Australia and what is the evidence base to support these treatment modalities?

5.1.1. Key findings

There are no published peer-reviewed publications of clinical studies on the treatment and treatment outcomes in patients experiencing the symptoms associated with DSCATT.

The available information on the treatment modalities that have been provided to patients experiencing DSCATT in Australia comes from self-reported information provided by patients, anecdotal information provided by patient advocacy groups or anecdotal information and medical opinion from treating medical professionals, as reported to the Senate Inquiry, and The House of Representatives Standing Committee on Health.

A large number of patients [not further defined] who identified as suffering from Lyme disease or DSCATT told the Senate Inquiry that 'Lyme literate' practitioners often prescribe a course of treatment that may include antibiotics and/or natural therapies that are not supported by Medicare or the Pharmaceutical Benefits Scheme (PBS). Evidence from a 'Lyme literate' doctor to the Senate Inquiry confirmed that patients with Lyme-like illness are being provided with antibiotics, including long-term antibiotics and long-term IV antibiotic therapy. Self-reported evidence collected by the Lyme Disease Association of Australia (LDAA) (2012) and provided to the Senate Inquiry found treatment regimens reported by patients to LDAA included natural supplements, antibiotics, diet, salt and Vitamin C combination, adrenal treatment, hormone treatment and heavy metal chelation treatment.

An analysis of the patient submissions to the Senate Inquiry found 49.9 per cent of submitters reported having received antibiotics, with 45.7 per cent having received oral antibiotics and 16.6 per cent reporting having received IV/IM antibiotics. One in four submitters had seen a 'Lyme literate' doctor for diagnosis and treatment and 17.2 per cent reported having been treated overseas. The author's conclusion suggested patients may have sought alternative and potentially non-evidence-based diagnoses and treatments.

The appropriateness of treatments provided to patients who experience DSCATT was a major concern raised to the Senate Inquiry and The House of Representatives Standing Committee on Health by Australian medical authorities and medical professional associations.

<u>Overseas-acquired Lyme disease</u>

The majority of international guidelines recommend one course of antibiotics for all presentations of Lyme disease with approaches to therapy being generally similar on both sides of the Atlantic.

Lyme disease is treated with antimicrobials from several classes with activity against *B. burgdorferi,* including doxycycline, penicillin, amoxicillin, cefuroxime, ceftriaxone and azithromycin, with the goals of treatment being the resolution of objective signs and symptoms of infection with prevention of relapsed active infection or new complications of infection. Under most circumstances, oral therapy is effective and preferred over intravenous therapy due to equivalent efficacies, tolerability, and cost.

Treatment recommendations, based on available RCTs published by US professional bodies such as the IDSA, the American Academy of Paediatrics and a variety of national and supranational associations in Europe (EUCALB) indicate that the approaches to therapy are generally similar on both sides of the Atlantic with some minor differences in the recommended dosage and treatment duration.

The majority of international guidelines, including IDSA, NICE and IDSA/AAN/ACR, recommend one course of antibiotic therapy for all presentations of Lyme disease.

There is a strong body of evidence and international authority recommendations that do not support ongoing and long-term treatment of Lyme disease with antibiotics.

Prolonged intravenous or oral antibiotic therapy for Lyme disease did not significantly improve outcomes in studies performed in North America and Europe and can be associated with significant adverse effects. A 2016 RCT on longer term therapy for symptoms attributed to Lyme disease found longer term antibiotic therapy did not provide additional benefit or better outcomes compared to shorter-term antibiotics.

There are therapeutic modalities not recommended for the treatment of Lyme disease.

Therapeutic modalities *not recommended* for treatment of patients with any manifestation of Lyme disease include combinations of antimicrobials, long-term antibiotic therapy, hyperbaric oxygen, fever therapy, intravenous immunoglobulin, ozone, cholestyramine, energy and radiation-based therapies, vitamins and nutritional managements, magnesium and bismuth injections, chelation and heavy metal therapies, and stem cell transplants. Lack of biological plausibility, lack of efficacy, absence of supporting data or the potential for harm underpin this advice.

Known Australian tick-borne diseases

There is official Australian guidance for the treatment of known Australian tick-borne diseases.

Treatment recommendations for known Australian tick-borne diseases are provided by Therapeutic Guidelines Ltd and, additionally for Q fever, by the Communicable Diseases Network Australia Guidelines for Public Health Units. QTT, FISF and ASF, and Q fever are all treated with doxycycline.

For QTT, early initiation of doxycycline is considered critical, as a delay in appropriate antibiotic therapy is associated with increased likelihood of progression to severe disease and complications.

The Q fever CDNA guidelines specify that if Q fever is suspected clinically (in people with appropriate symptoms AND who are at high risk of contracting Q fever, empirical treatment should be commenced without waiting for laboratory tests.

5.1.2. Literature reviewed

Australian Government reports	(House of Representatives Standing Committee on Health, 2016; Senate Community Affairs References Committee, 2016a, 2016b)
Australian Department of Health reports, reports to, and guidance	(Allen + Clarke, 2019; Communicable Diseases Network Australia, 2018; Department of Health, 2018b, 2020; TMS Consulting Pty Ltd, 2018a)
(Inter)national authority and intergovernmental reports, evidence-reviews, guidelines and guidance	(Centers for Disease Control and Prevention, 2019b, 2020; Marzec et al., 2017; National Institute for Health and Care Excellence, 2018c, 2018d, 2018e, 2018f, 2018a, 2018g, 2018h, 2018i, 2018j)
Guidelines (International and Australian) by clinical and professional bodies	(Cameron et al., 2014; Communicable Diseases Network Australia, 2018; Deutsche Borreliose-Gesellschaft, 2010; Lantos et al., 2010, 2019; Therapeutic Guidelines, n.d., 2019; Wormser et al., 2006)
Systematic Reviews (with/without meta-analysis)	(Cadavid et al., 2016; Lantos et al., 2014)
Narrative literature reviews and reviews	(Auwaerter et al., 2011; Beaman, 2016; Borchers et al., 2015; Collignon et al., 2016; Klempner et al., 2013; Stewart et al., 2017)
Randomised controlled trials	(Berende et al., 2016)
Prospective cohort studies	
Observational studies	(Brown, 2018)
Other	(Lyme Disease Association of Australia, 2012)

5.1.3. Quality of the evidence

Treatment modalities with which patients identifying as having DSCATT in Australia have been provided

The quality of the available information on the treatment modalities that patients identifying as having DSCATT in Australia have been provided is low.

While the Senate Community Affairs References Committee reports and the DSCATT Forum report were scored as 'high' with the AACODS tool, the information provided about treatments by patients and their treating doctors within these reports was self-reported and anecdotal.

While the veracity of this information is not being questioned in this literature review, the self-reported and anecdotal nature of the information means that, from an academic point of view, it is of low reliability and at high risk of bias and therefore does not meet the scientific quality required to underpin an evidence-based clinical pathway. The information is, however, useful in informing a DSCATT Clinical Pathway, as it can provide an understanding for medical and allied health professionals of the types of treatments patients may have been

previously provided, or may request or expect, when presenting to primary care and questioning whether their symptoms may be attributed to DSCATT.

Evidence base to support treatments for Lyme disease, known Australian tick-borne diseases

The evidence base to support the treatment of Lyme disease, and known Australian tick-borne diseases is robust and meets the scientific quality to underpin and evidence-based clinical pathway.

The majority of the advice and recommendations regarding the treatment of Lyme disease came from international authorities, and guidelines and guidance from international professional clinical bodies. We drew heavily on two recent international guidelines on Lyme disease, both of which were underpinned by evidence-based reviews of the literature. Evidence reviewed also included an RCT. For the treatment of known Australian tick-borne diseases, the evidence reviewed was more limited but is robust and included high-level Australian national guidance from CDNA and reference to Therapeutic Guidelines Ltd.

5.2. Treatment modalities that have been provided to patients (including subgroups of patients) with DSCATT in Australia

There are no published peer-reviewed publications of clinical studies on the treatment and treatment outcomes of DSCATT in Australia.

The available information on the treatment modalities that have been provided to patients with DSCATT in Australia comes from self-reported information provided by patients, anecdotal information provided by patient advocacy groups or anecdotal information and medical opinion from treating medical professionals, as reported to the Senate Inquiry, and The House of Representatives Standing Committee on Health Case study on tick-borne and Lyme-like diseases. Additionally, Brown (2018) reported on treatments received by patients in his analysis of patient submissions made to the Senate Inquiry.

Review of available information

5.2.1. Antibiotics

The Senate Inquiry heard concerns from a large (not further defined in the report) number of patients who identified as suffering from Lyme disease or DSCATT that 'Lyme literate' practitioners often prescribe a course of treatment that may include antibiotics that are not supported by Medicare or the Pharmaceutical Benefits Scheme (PBS) (Senate Community Affairs References Committee, 2016a).

An analysis of 698 first person submissions to the Senate Inquiry from Australian people who identified as suffering from Lyme disease or Lyme-like illness identified that one in four (n= 291; 41.7 per cent) submitters had seen a 'Lyme literate' doctor for diagnosis and treatment of their illness and 17.2 per cent reported having been treated overseas (Brown, 2018). Half (348; 49.9 per cent) of submitters reported they had received antibiotics, 45.7 per cent reported they had received oral antibiotics and 16.6 per cent reported having received IV/IM antibiotics. No further information was provided by Brown as to whether submitters had received both oral and IV/IM antibiotics.

The available information on treatments provided by medical professionals in Australia to patients with Lyme-like illness comes from the Australian Chronic Infectious and Inflammatory Disease Society (ACIIDS), and from evidence from Dr Richard Schloeffel given to the Senate Inquiry and The House of Representatives Standing Committee on Health (House of Representatives Standing Committee on Health, 2016).

The Senate Inquiry heard that some Australian medical practitioners, such as those associated with ACIIDS, argue that if classical Lyme disease is not treated, it can become chronic, and that such practitioners argue that treatment for 'chronic Lyme disease' is different to classical Lyme disease and recommend the use of long-term antibiotics (Senate Community Affairs References Committee, 2016a). 'Lyme literate' practitioners told the Senate Inquiry that the use of long-term antibiotics was evidence-based and in many cases [not further defined] assisted patients to get better (Senate Community Affairs References Committee, 2016a). Further, Dr Schloeffel told the Inquiry into Chronic Disease Prevention and Management in Primary Health Care that ILADS recommends a longer period of antibiotic treatment than the CDC (House of Representatives Standing Committee on Health, 2016).

Evidence from Dr Schloeffel to the Senate Inquiry indicates that patients identifying as having Lyme disease or DSCATT are being provided with antibiotics, including long-term antibiotics, including long-term IV antibiotic therapy. Dr Schloeffel stated:

> *We have treated 4,000 patients in five years. We are currently treating only 1,500 patients. Of the other 2,500 patients we have treated, most are better. They are getting better because they are having an appropriate diagnosis and appropriate treatment, sometimes with long- term antibiotics – oral in the main. But because we have so many sick patients we are doing a lot of intravenous therapies as well, including intravenous antibiotics for long periods of time, which is leading to a positive outcome, but under the same rigor that any intensive therapy would require, and we are doctors who are extremely qualified to do this work (Senate Community Affairs References Committee, 2016a, p. 40)*

Dr Schloeffel also told The House of Representatives Standing Committee on Health 'I have many antibiotic protocols, because every patient is different,' (House of Representatives Standing Committee on Health, 2016, p. 145) (see section below on other treatment modalities for full quote), but also emphasised the importance of not 'bombarding' with doses of antibiotics that are too high.

In addition, the LDAA told The House of Representatives Standing Committee on Health that infectious diseases specialists commonly follow the IDSA but that this is 'seen as vastly inadequate by any medical professional that is educated and experienced in treating Lyme-like disease' (House of Representatives Standing Committee on Health, 2016, p. 144). LDAA also submitted that longer term treatment is required in cases where patients have co-infections, and that international Lyme experts and Lyme-treating doctors in Australia agree that 'four weeks is simply not long enough' (House of Representatives Standing Committee on Health, 2016, p. 146).

The Senate Inquiry heard in the submission from ACIIDS that ACIIDS doctors refer to the guidelines laid down by ILADS when treating patients in Australia for Lyme-like illness. ACIIDS doctors also advised the Senate Inquiry through their submissions that they refer to the German guidelines to inform the treatment of patients with Lyme-like illness in Australia (Senate Community Affairs References Committee, 2016a). We understand that this ACIIDS guideline is now obsolete, although it remains on the internet. As such, the now-obsolete ACIIDS guideline is not discussed further in this literature review. Our search did not reveal a current publicly available ACIIDS guideline, although the Senate Inquiry was of the understanding that Dr Schloeffel, together with colleagues Dr Peter Dobie and Dr Hugh Derham, were in the process of drafting new evidence-based guidelines for diagnosis and treatment of tick-borne illness in Australia (Senate Community Affairs References Committee, 2016a).

5.2.2. Other treatment modalities

The House of Representatives Standing Committee on Health heard from the LDAA that the complexity of treatment pathways is due to the 'different infections and different manifestations' (House of Representatives Standing Committee on Health, 2016, p. 145) patients in Australia present with' with Dr Schloeffel stating, regarding the complexity of treating tick-borne or Lyme-like diseases:

> *The type of treatment that we do is not just about throwing antibiotics at patients….It is about management and giving the patient an understanding of their illness, making a proper diagnosis, sorting out their mental state and making sure they have carers and community support. It is about providing them with advice about how they should change their diet or improve their eating patterns, providing adequate supplementation for foods and for things that they may require as part of the treatment but also as a result of treatment. So they will be on vitamins and supplements and other things, which they have often already started because they have already seen six or seven naturopaths before they see you. Then depending on their diagnosis, very gently and slowly, there is an antibiotic protocol. I have many antibiotic protocols, because every patient is different (House of Representatives Standing Committee on Health, 2016, p. 145)*.

The Senate Inquiry heard concerns from a large (not further defined in the report) number of patients who identified as suffering from Lyme disease or DSCATT that 'Lyme literate' practitioners often prescribe a course of treatment that may include other natural therapies (in addition to antibiotics as mentioned above) that are not supported by Medicare or the Pharmaceutical Benefits Scheme (PBS) (Senate Community Affairs References Committee, 2016a).

The LDAA submitted to the Senate Inquiry their report Lyme disease: Australian patient experience in 2012, which included self-reported information collected via a survey, on how patients are treated once they have a diagnosis of Lyme disease. Natural supplements, antibiotics and diet were, in 2012, the most common treatments for patients undergoing treatment for Lyme disease (Lyme Disease Association of Australia, 2012). All of the survey respondents (n=224) answered the question 'Are you currently undergoing treatment? The majority (n=193, 86 per cent) reported they were currently undergoing treatment for Lyme disease. Participants in the survey were also asked to describe their treatment regimens and were provided with a list of common treatments, as detailed in the table that follows. In the present report, the data has been reorganised in decreasing order of prevalence. Natural supplements, antibiotics and diet were, in 2012, the most common treatments for patients undergoing treatment for Lyme disease (Lyme Disease Association of Australia, 2012).

Table 14: Treatment regimens

What does your treatment regimen include?	Count
Natural supplements	147
Antibiotics	137
Diet	122
Salt and Vitamin C combination	28
Adrenal treatment	25
Hormone treatment	21
Heavy metal chelation treatment	16

Source: LDAA, Lyme disease: Australian patient experience in 2012, November 2012, page 28.

In addition to the common treatments specified in the table above, participants were able to select a category of 'other treatments' they were currently undergoing. The additional treatments and therapies are reproduced from the LDAA 2012 report and reported in the table that follows.

Table 15: Additional treatments and therapies

Other treatments in use	Count
Herbs/herbal treatment	5
Vitamin B/C/D	5
Detoxification (FIR sauna, Mud packs, Epsom salts bath)	3
Exercise	3
Probiotics	3
RIFE	2
Homeopathy	2
Anti-inflammatory drugs/food	1
Antivirals, anti-fungal lozenges	1
Anxiety medication	1
Bicillin injections	1
Blood thinners	1
Colonics	1
Hyperbaric O_2 therapy	1
Holistic dentistry	1
IV Vitamin C and IV Glutathione	1

Other treatments in use	Count
Lymphatic drainage and massage	1
Marshall Protocol	1
Opiates	1
Osteopathy	1
Ozone/oxygen therapy	1
Physiotherapy/chiropractic support	1
Traditional Chinese Medicine (TCM)	1

Source: LDAA, Lyme disease: Australian patient experience in 2012, November 2012, page 28.

While this LDAA document is not peer-reviewed and is based on self-reported information collected by a patient advocacy organisation via a survey, the findings are included in this literature review as they can provide an understanding for medical and allied health professionals of the types of treatments patients may have been previously provided, or may request or expect, when presenting to primary care and questioning whether their symptoms may be attributed to DSCATT.

In addition to the range of other treatment modalities identified in the LDAA 2012 patient experience survey above, the Senate Inquiry heard concerns from patients that in some cases prescribed treatments are not available in Australia, for example, 'hyperthermia treatment' in a clinic in Germany where the body is heated to kill off bacteria, or ozone therapy (Senate Community Affairs References Committee, 2016a). Dr Schloeffel told The House of Representatives Standing Committee on Health that in addition to antibiotic treatment, he is also involved in treatment using hyperthermia, a method he advised was used in Germany in which the body is heated for nine hours to 41.7 degrees in an intensive care unit. Dr Schloeffel stated that over 1,000 Australians have travelled to Germany to receive this particular treatment, which 'seems to be very effective' (House of Representatives Standing Committee on Health, 2016, p. 146).

5.3. Concerns about evidence base to support treatment modalities provided to patients with DSCATT in Australia

5.3.1. Evidence for treatments provided for patients experiencing DSCATT

There are no published peer-reviewed studies of clinical treatments provided to patients in Australia experiencing symptoms associated DSCATT and the outcomes of those treatments. Despite 'Lyme literate' practitioners having told the Senate Inquiry they have treated over 4000 patients in five years, and attested that of the 2,500 treated most are better, these doctors have not published any peer-reviewed clinical studies of their treatments or treatment outcomes.

5.3.2. Appropriateness of treatments and medical harm

The appropriateness of treatments provided to patients who experience DSCATT was a major concern raised to the Senate Inquiry and The House of Representatives Standing Committee on Health. The Senate Community Affairs References Committee heard concerns including:

- from medical authorities, about some of the treatments offered by 'Lyme literate' practitioners, such as side-effects from antibiotics, infections from intravenous catheters (such as PICC lines) and potential toxins from unregulated medications (citing Royal Australasian College of Physicians), with these authorities (citing NSW Health; Royal Australasian College of Physicians; Victorian Department of Health and Human Services; Australasian Society for Infectious Disease) arguing that these treatments are not evidence-based and risk causing harm to patients
- the use of long-term antibiotics to address symptoms ascribed to Lyme-like illness, as there is no evidence to support the use of combination antibiotics, immunoglobulin, hyperbaric oxygen, specific nutritional supplements or prolonged courses of antibiotics for the management of Lyme disease (citing WA Department of Health) and that the consequences of long-term antibiotic use can have negative effects for both the individual and the broader community, because it promotes the proliferation of multi-drug resistant organisms (citing the Royal College of Pathologists of Australasia) (Senate Community Affairs References Committee, 2016a).

The Australian Medical Association (AMA), the RACGP and the Australian Government Department of Health have addressed the question of evidence-based treatment recommendations with respect to DSCATT. The AMA explained to the Senate Inquiry that doctors have a responsibility to rely on evidence to determine a diagnosis and treatment plan and

> *In the absence of a conclusive aetiology for an indigenous vector of Lyme disease or a Lyme-like disease, diagnosis remains difficult and patients are frustrated when their illness is not diagnosed or treated. The AMA understands that this sentiment is genuine and that a failure to reach a conclusive diagnosis can be stressful, however the medical profession's role is to make clinically appropriate treatment recommendations based on the best available evidence. It is ethically and legally appropriate for doctors to refuse demands by patients, patients' family members or other third parties for tests, treatments or procedures that are not clinically appropriate (Senate Community Affairs References Committee, 2016b, p. 33).*

Similarly, the RACGP stated that, as it advocates for evidence-based practice, it 'cannot support many of the treatments currently being used or advocated', regardless of 'whatever success individual doctors have with their patients' (House of Representatives Standing Committee on Health, 2016, p. 146).

In addition to the serious concerns raised about overuse and long-term use of antibiotic treatment and antimicrobial resistance for Australian patients receiving treatment for DSCATT raised to the Senate Inquiry and The House of Representatives Standing Committee on Health, concerns regarding the dangers of IV therapy and antibiotic overuse and misuse were found in the published literature about DSCATT (Beaman, 2016; Brown, 2018; Collignon et al., 2016). As noted previously, Brown found in his analysis of 698 patients submissions made to the Senate Inquiry that 41.7 per cent had seen a 'Lyme literate' doctor, half (49.9 per cent) had received antibiotics and one in six (16.6 per cent) reported they had received IM/IV antibiotics. Brown commented that the vast majority of antibiotics prescribed to respondents are likely to have been inappropriate with potential to cause harms from both side effects and antimicrobial resistance. Brown noted the documented harms associated with long-term antibiotic treatment of 'chronic Lyme disease', including from unnecessary intravenous access, which has been associated with deaths (Patel et al. (2000), and Holzbauer et al. (2010) in Brown, 2018).

A study on Lyme disease in a British referral clinic had identified other potential hazards of taking antibiotics unnecessarily include their toxicity, potential hypersensitivity reactions, anaphylaxis (allergy) and predisposition with *Clostridium difficile* and antibiotic-resistance bacteria (Cottle et al. (2012) in Collignon et al., 2016). Collignon et al. commented that while the questions of whether persistent *B. burgdorferi* infection occurs and classical Lyme disease exists in Australia can be debated, there are clear risks associated with receiving IV antibiotics including infections by bacteria and fungi, which in many cases are fatal (Collignon et al. (1994), Ong et al. (2013), and Patel et al. (2000) in Collignon et al., 2016), including, as Brown above mentioned, in some people treated for "Lyme disease" (Patel et al. (2000) in Collignon et al., 2016). Additionally, Collignon et al. highlighted that while many people who believe they have Lyme disease or DSCATT, along with some of their medical practitioners, believe that prolonged antibiotic therapy including intravenous antibiotics, may cure their disease, citing the ILADS guidelines. Collignon et al. noted that evidence from the US and Europe,

where classical Lyme disease is endemic, do not confirm this view, with prolonged intravenous antibiotic therapy (longer than one month) having been shown in randomised control trials not to significantly improve symptoms (Berende et al. (2016), Klempner et al. (2001), Krupp et al. (2003), and Fallon et al. (2008) in Collignon et al., 2016).

In addition to the experiences of Australian patients reported by Brown (2018) above, Beaman (2016) also noted Australian experiences reported through the national ASID-OzBug bulletin board include patients paying many thousands of dollars for non-specialist consultations, and transportation of specimens for testing at overseas laboratories using non-approved protocols that have resulted in misdiagnoses associated with experimental treatments that have caused serious complications including line sepsis, pancreatitis, and pseudomembranous colitis (Beaman, 2016).

As such Collignon et al. (2016) noted, and of relevance to developing the DSCATT Clinical Pathway, Australian medical practitioners are faced with a difficult dilemma, as growing numbers of patients, their supporters, and some integrative medical practitioners are demanding diagnoses and treatment according to the 'chronic Lyme disease' school of thought. However, as Collignon et al. also noted, evidence of a local aetiological agent and a competent vector that together could cause classical Lyme disease in Australia has yet to be reported, and it is only after links between specific tick-borne pathogens and patients who present with this constellation of chronic, non-specific symptoms have been established (through further research using next generation sequencing and metagenomics) that effective, evidence-based management protocols can be safely developed (Collignon et al., 2016). In the meantime, Beaman (2016) advised that after excluding alternative causes of rheumatological disease or fatigue syndromes and other infectious diseases known to be transmitted by tick-bites in Australia, and until those links (noted by Collignon et al. above) have been established, symptomatic treatment including psychological support should be offered (Marques (2008) in Beaman, 2016).

5.3.3. Social and financial harms of treatment modalities

Patients identifying as having Lyme disease or Lyme-like illness in Australia have suffered social and financial costs and harms from the treatments provided to them (Brown, 2018; Senate Community Affairs References Committee, 2016a). While this section sits somewhat outside the research question on the evidence base to support the treatment modalities provided to patients with DSCATT in Australia, it is relevant to the development of a DSCATT Clinical Pathway, particularly the management of patients with persistent symptoms who remain undiagnosed, and the practice of harm minimisation by medical professionals.

A large number (not further defined) of patients diagnosed with Lyme-like illness expressed concerns to the Senate Inquiry about the accessibility and high cost of treatments prescribed by 'Lyme literate' practitioners, including:

- a course of treatment that may include antibiotics and other natural remedies that are not supported by Medicare or the Pharmaceutical Benefits Scheme (PBS)
- treatments often costing hundreds of dollars per week, with one submitter claiming to have spent over $100,000 on treatment since diagnosis, and as a result of the high costs, a number of submitters, particularly those receiving welfare or pension payment, stated they had not been able to afford the prescribed treatments (Senate Community Affairs References Committee, 2016a).

The high cost of treatment is in addition to the high cost of consulting 'Lyme literate' practitioners and obtaining a diagnosis, prior to treatment. Patients reported to the Senate Inquiry, such practitioners allegedly charge between $300 and $900 for consultations, with the diagnostic tests used by 'Lyme literate' practitioners also involving significant expense (for example, $800 for tests in Australia and $2,000 for tests from overseas laboratories) (Senate Community Affairs References Committee, 2016a).

In his analysis of 698 publicly available patient submissions made by patients who identified as suffering from Lyme disease or DSCATT, Brown (2018) noted the social and financial harms associated with diagnosis and treatment of DSCATT, with over half of submissions (56.2 per cent) reporting significant financial hardship with a median cost of treatment of $30,000, with a wide range from $750 to $700,000 (Brown, 2018). As discussed above, 41.7 per cent of patients in the analysis had seen a 'Lyme literate' doctor, half (49.9 per cent) reported they had received antibiotics and one in six (16.6 per cent) reported they had received IM/IV antibiotics. As such, Brown concluded that not only had these patients experienced social and financial harms, they are also at risk of nosocomial harms and that negative medical interactions and the media may contribute to patients seeking alternative and potentially non-evidence-based diagnoses and treatments (Brown, 2018).

5.4. Evidence base for treatment of Lyme disease in people returning to Australia from Lyme disease endemic countries

To inform the development of the DSCATT Clinical Pathway, we searched for the most recently published international treatment guidelines and international authority guidance for Lyme disease. We identified and reviewed the 2018 NICE guideline on Lyme disease from the UK and the 2019 IDSA/AAN/ACR draft clinical treatment guideline from the US, both of which were underpinned by systematic reviews of the evidence. We included guidance from the CDC and publications in the CDC's Morbidity and Mortality Weekly Reviews. We also identified and reviewed systematic reviews, including a Cochrane systematic review, an RCT and several reviews.

The 2006 IDSA guideline, while outside the date inclusion range for the literature review, is the recommended guideline for treating a confirmed case of classical Lyme disease acquired overseas in an endemic area in the Australian Department of Health's 2015 guidelines on the diagnosis of Lyme disease (Wormser et al. (2006) in Lum et al., 2015). The 2006 guideline was also noted by the Senate Inquiry (Senate Community Affairs References Committee, 2016a). At the time of publication of the guideline by Lum et al., the IDSA guideline was the current guideline on the diagnosis and treatment of Lyme disease for IDSA. The 2006 IDSA guideline on Lyme disease has now been archived, while the 2019 draft IDSA/AAN/ACR clinical practice guideline for Lyme disease is being finalised.

An investigation to determine whether the IDSA violated antitrust laws in the promulgation of the IDSA's 2006 Lyme disease guidelines mentioned above was initiated in November 2006 by Connecticut Attorney General Richard Blumenthal. In April 2008, the Connecticut Attorney General reached an agreement to end the investigation, with the IDSA agreeing to convene an independent review panel to determine whether the 2006 Lyme disease guidelines were based on sound medical and scientific evidence and whether these guidelines should be changed or revised (Lantos et al., 2010).

Lantos et al. concluded:

> *The Review Panel finds that the 2006 Lyme disease guidelines were based on the highest-quality medical and scientific evidence available at the time and are supported by evidence that has been published in more recent years. The Review Panel did not find that the 2006 guidelines authors had failed to consider or cite relevant data and references that would have altered the published recommendations. In addition to the review by this panel, the recommendations in the 2006 IDSA guidelines are further corroborated by guidelines and statements by other independent bodies from the United States and Europe. It is expected that the IDSA will review the 2006 Lyme disease guidelines on a regular basis to consider any new evidence that would warrant a substantive change to the current recommendations" (Lantos et al., 2010, p. 5).*

This research question also addresses the evidence base on treatments prescribed to patients with ongoing symptoms attributed to Lyme disease and patients diagnosed with 'chronic Lyme disease', both in Australia and internationally. As noted earlier, while some Australians and healthcare providers believe that a form of 'chronic Lyme disease' exists, globally, 'chronic Lyme disease' is a disputed diagnosis which lacks sufficient supporting evidence. As such, in addition to the guidelines mentioned above, we reviewed the 2014 ILADS Lyme

disease treatment guidelines and the German guidelines DBG (2010) to identify consistency or discordance with the most recent guidelines from NICE and the IDSA/AAN/ACR (draft).

5.5. Overview of international guidelines and international authority guidance on the treatment of Lyme disease

5.5.1. IDSA/AAN/ACR (2019) Draft Lyme disease Clinical Practice Guideline

The 2019 IDSA/AAN/ACR Draft Lyme disease clinical practice guidelines are an evidence-based guideline for treatment of Lyme disease (in addition to prevention and diagnosis of Lyme disease) prepared by a multidisciplinary panel representing the IDSA, the American Academy of Neurology, and the American Academy of Rheumatology (Lantos et al., 2019). The 2019 draft clinical practice guideline was developed following the processes in the IDSA Handbook of Clinical Practice Guideline Development and is informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options. The systematic review included 359 references. The clinical questions were set out as PICO questions. All recommendations were labelled as either "strong" or "weak" according to the GRADE approach. The scope of the guideline includes treatment of early Lyme disease, its neurologic, cardiac, and rheumatological complications and Lyme disease complicated by coinfection. The guideline is intended primarily for medical practitioners in North America, although IDSA/AAN/ACR note many recommendations will be applicable to patients in Europe and Asia. Additionally, recognising that Lyme disease is evaluated and treated by physicians from different subspecialties in varied clinical settings, the IDSA/AAN/ACR note the guideline has official representation from numerous organisations including scientific, primary care, and medical specialties (Lantos et al., 2019). The draft guideline is being finalised currently.

5.5.2. NICE (2018) Lyme disease guideline

The NICE Lyme disease guideline, published in April 2018, covers diagnosing and managing Lyme disease, and aims to raise awareness of Lyme disease should it be suspected and ensure people have prompt and consistent diagnosis and treatment. The recommendations aim to standardise antibiotic treatment and to provide a consistent framework for good practice in managing Lyme disease (National Institute for Health and Care Excellence, 2018j).

The guideline recommendations for antibiotic therapy are presented for a range of Lyme disease presentations, including Lyme disease without focal symptoms and Lyme disease with focal symptoms, with recommendations for each presentation informed by evidence reviews.

The 2018 NICE guidelines were underpinned by multiple evidence-based reviews including:

- Management of EM (National Institute for Health and Care Excellence, 2018c)
- Management of non-specific symptoms related to Lyme disease (National Institute for Health and Care Excellence, 2018d)
- Management of neuroborreliosis (National Institute for Health and Care Excellence, 2018e)
- Management of Lyme arthritis (National Institute for Health and Care Excellence, 2018f)
- Management of Acrodermatitis chronica atrophicans (National Institute for Health and Care Excellence, 2018a), and

- Management of Lyme carditis (National Institute for Health and Care Excellence, 2018g).

5.5.3. CDC

The CDC provides guidance on treatment of Lyme disease on its website (Centers for Disease Control and Prevention, 2020). The CDC advises people treated with appropriate antibiotics in the early stages of Lyme disease usually recover rapidly and completely. Antibiotics commonly used for oral treatment include doxycycline, amoxicillin, or cefuroxime axetil. People with certain neurological or cardiac forms of illness may require intravenous treatment with antibiotics such as ceftriaxone or penicillin.

The CDC guidance on treatment is presented for localised (early) Lyme disease and for (late) Lyme disease. Treatment regimens for early Lyme disease include doxycycline, cefuroxime axetil and amoxicillin and range in duration from 10-21 days depending on the antibiotic used. The CDC notes that recent publications suggest the efficacy of shorter courses of treatment for early Lyme disease. For late Lyme disease, the CDC refers to two 2016 publications, by Hu, and by Sanchez et al. (Hu (2016) and Sanchez et al. (2016) in Centers for Disease Control and Prevention, 2020). The CDC advises the regimens are guidelines only and may need to be adjusted depending on a person's age, medical history, underlying health conditions, pregnancy status, or allergies. Additionally, the CDC specifically notes that for people intolerant of amoxicillin, doxycycline, and cefuroxime axetil, the macrolides azithromycin, clarithromycin, or erythromycin may be used, although they have a lower efficacy. People treated with macrolides should be closely monitored to ensure that symptoms resolve (Centers for Disease Control and Prevention, 2020)

The CDC notes that the National Institutes of Health (NIH) has funded several studies on the treatment of Lyme disease that show most people recover when treated within a few weeks of antibiotics taken by mouth. In a small percentage of cases, symptoms such as fatigue and muscle aches can last for more than 6 months. This condition is known as "Post-treatment Lyme Disease Syndrome", although the CDC notes it is often called 'chronic Lyme disease.' For details on research into 'chronic Lyme disease' and long-term treatment trials sponsored by NIH, the CDC refers to the National Institutes of Health Lyme Disease website (National Institutes of Health Lyme Disease https://www.niaid.nih.gov/diseases-conditions/lyme-disease in Centers for Disease Control and Prevention, 2020).

5.5.4. ILADS (2014)

The 2014 ILADS guideline 'Evidence assessments and guideline recommendations in Lyme disease: the clinical management of known tick bites, EM rashes and persistent disease' were developed by the International Lyme and Associated Diseases Society (ILADS) (Cameron et al., 2014). The guideline replaced those issued by ILADS in 2004. The guideline cited 213 references and is intended to assist clinicians by presenting evidence-based treatment recommendations which follow the Grading of Recommendations Assessment, Development and Evaluation system. The authors also note 'ILADS guidelines are not intended to be the sole source of guidance in managing Lyme disease and they should not be viewed as a substitute for clinical judgment nor used to establish treatment protocols' (Cameron et al., 2014, p. 1103).

Unlike the NICE 2018 guideline and IDSA/AAN/ACR draft clinical practice guideline, which provide treatment guidance on all manifestations of Lyme disease, the 2014 ILADS guideline only addresses three clinical questions:

- the usefulness of antibiotic prophylaxis for known tick bites;
- the effectiveness of EM treatment; and
- the role of antibiotic retreatment in patients with persistent manifestations of Lyme disease (Cameron et al., 2014).

Cameron et al. found there to be limited evidence regarding the treatment of known tick bites, EM rashes and persistent disease. Their grading of Recommendations Assessment, Development and Evaluation-based analyses found this evidence to be of very low quality, due to limitations in trial designs, imprecise findings, outcome inconsistencies and non-generalisability of trial findings (Cameron et al., 2014).

ILADS recommendations for antibiotic treatment for patients with EM are provided in 5.6.1 Treatment of erythema migrans. ILADS treatment recommendations for patients who have persistent manifestations of Lyme disease are provided in 5.8.10 ILADS (2014).

5.5.5. German Guidelines (DBG) (2010)

The German guidelines were published in April 2008, with a revised second addition in December 2010 (Deutsche Borreliose-Gesellschaft, 2010). The guideline includes 162 references.

The guidelines state:

> *This guideline, 'Diagnosis and Treatment of Lyme borreliosis' was prepared with great care. However, no liability whatever can be accepted for its accuracy, especially in relation to dosages, either by the authors or by the German Borreliosis Society (Deutsche Borreliose-Gesellschaft, 2010, p. III)*.

The guidelines advised the scientific basis for antibiotic treatment is still inadequate at this time, with the exception of the localised early stages (EM). The authors made the following statements:

> *There are now a few studies available which provide evidence of the positive effect and the safety of long term antibiotic therapy (Deutsche Borreliose-Gesellschaft, 2010, p. 12)*.
>
> *Additional factors are involved in vivo which lie in the capability of Borrelia to evade the immune system specifically under the influence of various antibiotics (Deutsche Borreliose-Gesellschaft, 2010, p. 12)*.

The Deutsche Borreliose-Gesellschaft guidelines advised the treatment of Lyme borreliosis can be conducted either as a monotherapy or with a synchronous combined therapy and that:

> *The efficiency of a combined antibiotic therapy has not been scientifically attested to date; this form of treatment is based on microbiological findings and on empirical data that have not so far been systematically investigated (Deutsche Borreliose-Gesellschaft, 2010, p. 13)*.

5.6. Overview of international guideline treatment recommendations

Treatment recommendations, based on available RCTs published by American professional bodies such as the IDSA (Wormser et al. (2006) in Borchers et al., 2015), the American Academy of Pediatrics (American Academy of Pediatrics (2012) in Borchers et al., 2015) and a variety of national and supranational associations in Europe (EUCALB) (Mygland et al. (2010), British Infection Association (2011), and www. eucalb.com in Borchers et al., 2015) indicates the approaches to therapy are largely similar on both sides of the Atlantic with some minor differences in the recommended dosage and treatment duration (Borchers et al., 2015). Borchers et al. noted, in contrast to the American professional associations and EUCALB, ILADS guidelines for the management of Lyme disease advocate more aggressive and longer treatment courses for patients with persistent symptoms or refractory disease, and commented 'but much of the terminology is poorly defined, and the cited "evidence" is largely restricted to older studies that support such an approach and ignores much of the other available evidence' (Borchers et al., 2015, p. 92). We do note Borchers et al. were referring to the 2004 ILADS Lyme disease treatment guidelines when making these comments; however, we also note that ACIIDS doctors submitted the 2004 ILADS guidelines, along with the 2014 ILADS guidelines to the Senate Inquiry, as guidelines they refer to for the treatment of Australian patients with Lyme disease or those experiencing DSCATT.

The majority of international guidelines, including IDSA (Lantos et al., 2010; Wormser et al., 2006) and NICE (2018j) and IDSA/AAN/ACR (draft) (Lantos et al., 2019), recommend one course of antibiotic therapy for all presentations of Lyme disease.

Lyme disease is treated with antimicrobials from several classes with activity against *B. burgdorferi,* including doxycycline, penicillin, amoxicillin, cefuroxime, ceftriaxone and azithromycin, with the goals of treatment being the resolution of objective signs and symptoms of infection with prevention of relapsed active infection or new complications of infection (Lantos et al., 2019). Under most circumstances oral therapy is effective and preferred over intravenous therapy due to equivalent efficacies, tolerability and cost (Lantos et al., 2019). IDSA/AAN/ACR advises there is no clinical evidence to support regimens intended to treat fastidious states of *B. burgdorferi* infection, such as morphologic variants (aka "cyst" forms, "round" bodies, or "L-forms"), or to treat biofilms (Lantos et al. (2014) in Lantos et al., 2019).

The latest guidelines from NICE (2018) recommend antibiotic therapy of 21 days for all presentations except Lyme arthritis (28 days) (2018j). Subsequent to NICE recommendations, IDSA/AAN/ACR (draft) recommended patients with EM be treated with either a 10-day course of doxycycline or a 14-day course of amoxicillin, cefuroxime axetil or phenoxymethylpenicillin rather than longer treatment courses (**strong recommendation; moderate quality of evidence**) (Lantos et al., 2019).

In the following subsections we have provided an overview of the treatment recommendations for the various manifestations of Lyme disease from the IDSA/AAN/ACR 2019 Draft Lyme disease clinical practice guideline, the 2018 NICE Lyme disease guideline, the 2014 ILADS guideline and the findings of a Cochrane systematic review on the treatment of Lyme neuroborreliosis. We note the IDSA/AAN/ACR Lyme disease clinical practice guideline is draft. We recommend the reader refer to the IDSA/AAN/ACR final Lyme disease guidelines when these are published by IDSA. We also refer the reader to the respective guidelines for full details of treatment regimens.

5.6.1. Treatment of erythema migrans

The IDSA/AAN/ACR and NICE both recommend short courses of oral antibiotics of between 10 and 21 days for treatment of EM, although the two organisations differ on the duration of the antibiotic therapy. In contrast, ILADS recommends four to six weeks of antibiotic therapy for EM, to prevent development of 'chronic Lyme disease', a diagnosis disputed by IDSA/AAN/ACR and NICE.

IDSA/AAN/ACR (draft 2019)

For patients with EM, IDSA/AAN/ACR recommends treatment with either a 10-day course of doxycycline or a 14-day course of amoxicillin, cefuroxime axetil or phenoxymethylpenicillin rather than longer treatment courses (**strong recommendation; moderate quality of evidence**) (Lantos et al., 2019). The summary of evidence noted different durations of antibiotic therapy have been evaluated in the treatment of Lyme disease ranging from a short five day course to more than 21 days and no difference in outcomes has been associated with duration of therapy from several studies comparing the same antibiotic used for different durations. IDSA/AAN/ACR reported the rationale for their recommendation was that shorter durations of antibiotics may reduce adverse effects and cost (Lantos et al., 2019).

NICE (2018)

The 2018 NICE Lyme disease guideline recommends that 21 days of treatment should be offered as standard antibiotic treatment for EM (2018j). The recommendation was underpinned by the Evidence-review Management of EM (National Institute for Health and Care Excellence, 2018c). Twenty studies were included in the evidence review; 18 being RCTs and two being non-randomised comparative studies. The latter two studies were included as no RCT evidence could be found for the comparison of different doses of doxycycline in adults and azithromycin with amoxicillin in children.

In interpreting the evidence, the committee considered cure (resolution of symptoms), reduction in symptoms, symptom relapse, and quality of life as *critical* outcomes to decision making. Adverse events were considered to be *important* to decision making.

The rationale for the recommendation included that a number of studies examined antibiotic treatment of Lyme disease with EM using different antibiotics, doses and durations of treatment; and many of the studies did not reflect current prescribing practices and the evidence was of poor quality. There was evidence that doxycycline is more clinically effective than some other antibiotics. However, the evidence showed no clear difference in effectiveness between doxycycline, an amoxicillin/probenecid combination, and azithromycin. The evidence also showed no benefit of intravenous or intramuscular cephalosporin over doxycycline. It was noted that doxycycline and amoxicillin are able to penetrate the blood–cerebrospinal fluid barrier and pass into the central nervous system, whereas azithromycin cannot. This may be important to prevent the development of further symptoms. Doxycycline can also be taken in a single daily dose, which may help with adherence. Additionally, current guidelines give ranges for treatment duration, generally between 10 and 21 days, without guidance on when to use a longer or shorter course. The committee agreed that this is not clear enough for generalists. The evidence for treatment duration was limited. The committee decided that longer courses of 21 days of treatment should be offered as standard because of their concern at low cure rates in some studies and the lack of clear evidence for shorter courses. They also agreed that a longer course may be reassuring for people being treated for Lyme disease who continue to have symptoms. The

evidence showed adverse event rates were not increased for longer courses (National Institute for Health and Care Excellence, 2018c).

Additional advice included:

- to ask women (including young women under 18) if they might be pregnant before offering antibiotic treatment for Lyme disease; and
- if symptoms worsen during treatment for Lyme disease, assess for an allergic reaction to the antibiotic, and be aware that a Jarisch-Herxheimer reaction may cause exacerbation of symptoms but does not usually warrant stopping treatment (National Institute for Health and Care Excellence, 2018c, 2018j).

ILADS (2014)

ILADS (Cameron et al., 2014) recommendations for the treatment of EM were:

Recommendations 2a,b,d,e: Courses of antibiotic treatments of 10 or 20 days depending on antibiotic are not recommended for patients with EM rashes because failure rates in the clinical trials were unacceptably high. Failure to fully eradicate the infection may result in the development of a chronic form of Lyme disease, exposing patients to its attendant morbidity and costs, which can be quite significant. Clinicians should prescribe amoxicillin, cefuroxime or doxycycline as first-line agents for the treatment of EM. Initial antibiotic therapy should employ four to six weeks of amoxicillin 1500–2000 mg daily in divided doses, cefuroxime 500 mg twice daily or doxycycline 100 mg twice daily or a minimum of 21 days of azithromycin 250–500 mg daily. Clinicians should continue antibiotic therapy for patients who have not fully recovered by the completion of active therapy. Clinicians should re-treat patients who were successfully treated initially but subsequently relapse or have evidence of disease progression. The authors noted the recommendations were based on very low-quality evidence (Cameron et al., 2014).

5.6.2. Treatment of Lyme neuroborreliosis

IDSA/AAN/ACR (2019 draft)

For patients with Lyme disease-associated meningitis, cranial neuropathy, radiculoneuropathy or with other peripheral nervous system (PNS) manifestations, IDSA/AAN/ACR recommend using parenteral ceftriaxone, cefotaxime, or penicillin, or oral doxycycline over other antimicrobials (**Strong recommendations; moderate quality evidence**). Decisions about the choice of antibiotic recommended should primarily be made based on individual factors unrelated to effectiveness. The preferred antibiotic duration is 14 to 21 days (Lantos et al., 2019).

In patients with Lyme disease-associated parenchymal involvement of the brain or spinal cord IDSA/AAN/ACR recommend using parenteral over oral antibiotics (**strong recommendation; moderate quality evidence**). This manifestation is extremely rare, treatment in this population has not been systematically studied. IDSA/AAN/ACR noted typically, 2 to 4-week courses of antibiotics have been used successfully in these patients (Lantos et al., 2019).

NICE (2018)

For the management of Lyme neuroborreliosis, the NICE 2018 guideline recommends as first treatment, antibiotics taken orally for 21 days for the management of Lyme disease affecting the cranial nerves and peripheral nervous system, and antibiotics administered intravenously for 21 days for the management of Lyme disease affecting the central nervous system. Care of children and young people under 18 should be discussed with a specialist (National Institute for Health and Care Excellence, 2018j).

The NICE recommendations were underpinned by the Evidence Review Management of neuroborreliosis (National Institute for Health and Care Excellence, 2018e). In the systematic review, seven randomised studies involving 450 European participants with Lyme neuroborreliosis were identified published between 1989 and 2016; no trials conducted in the US were found. The committee agreed on a 21-day course of intravenous ceftriaxone 4g daily as the initial treatment for adults and young people (aged 12 and over) with Lyme disease affecting the central nervous system, with a 21-day course of doxycycline 400mg daily recommended as an alternative treatment. The higher dose (4g) is the recommended dose for bacterial meningitis. For Lyme disease affecting the cranial nerves or the peripheral nervous system, the committee agreed on a 21-day course of doxycycline 200mg daily as the initial treatment for adults and young people (aged 12 and over), with amoxicillin recommended as an alternative treatment (National Institute for Health and Care Excellence, 2018e).

Cochrane Review of antibiotics for the neurological complications of Lyme disease

In 2016, Cadavid et al.'s review of antibiotics for the neurological complications of Lyme disease was published in the Cochrane Library Cochrane Database of Systematic Review (Cadavid et al., 2016). The review included seven randomised studies involving 450 European participants with Lyme neuroborreliosis; no trials conducted in the US were found. The authors concluded there is mostly low- to very low-quality clinical evidence from a limited number of mostly small, heterogeneous trials with diverse outcome measures, comparing the relative efficacy of central nervous system-penetrant antibiotics for the treatment of LNB. These randomised studies provided some evidence that doxycycline, penicillin G, ceftriaxone, and cefotaxime are efficacious in the treatment of European Lyme neuroborreliosis. No evidence of additional efficacy was observed when, in one study, an initial antibiotic treatment with intravenous ceftriaxone was followed by additional longer treatment with oral amoxicillin. The authors concluded it was therefore not possible to draw firm conclusions on the relative efficacy of accepted antibiotic drug regimens for the treatment of Lyme neuroborreliosis. The majority of people are reported to have good outcomes, and symptoms resolve by 12 months regardless of the antibiotic used. A minority of participants did not improve sufficiently, and some were retreated (Cadavid et al., 2016).

5.6.3. Treatment of Lyme carditis

IDSA/AAN/ACR (2019 draft)

In outpatients with Lyme carditis, IDSA/AAN/ACR suggests using oral antibiotics over IV antibiotics (**weak recommendation; very low quality evidence**) (Lantos et al., 2019).

In the hospitalised patient with Lyme carditis, IDSA/AAN/ACR suggests initially using ceftiazone over oral antibiotics until there is evidence of clinical improvement, then switching to oral antibiotics to complete treatment (**weak recommendation; very low quality evidence**).

For the treatment of Lyme carditis, IDSA/AAN/ACR suggests 14 to 21 days of total antibiotic therapy over longer durations (**weak recommendation; very low quality evidence**) (Lantos et al., 2019).

NICE (2018)

For the management of Lyme carditis, the NICE 2018 Lyme disease guidelines recommended course of antibiotic treatment is 21 days. Care of children and young people under 18 with Lyme disease and focal symptoms such as carditis should be discussed with a specialist (National Institute for Health and Care Excellence, 2018j). The recommendations were underpinned by the Evidence review Management of Lyme carditis (National Institute for Health and Care Excellence, 2018g). The review did not identify any relevant RCTs and cohort studies comparing the effectiveness of antibiotics and steroids versus each other or placebo as treatment for people with carditis related to Lyme disease. As no studies of antibiotic treatment for heart problems caused by Lyme disease were identified; therefore, the committee reviewed the evidence available for treating other symptoms of Lyme disease and used this, their experience of current practice, and their knowledge of care for people with heart problems, to develop the recommendations (National Institute for Health and Care Excellence, 2018g).

5.6.4. Treatment of Lyme arthritis

IDSA/AAN/ACR (2019 draft)

For patients with Lyme arthritis, IDSA/AAN/ACR recommends using oral antibiotic therapy for 28 days (**strong recommendation; moderate-quality evidence**) (Lantos et al., 2019).

The rationale for the recommendation included that oral antibiotics are easier to administer than IV antibiotics, are associated with fewer complications, and are less expensive.

IDSA/AAN/ACR also provided treatment guidance for patients in whom Lyme arthritis has not completely resolved. In patients with Lyme arthritis with partial response (mild residual joint swelling), IDSA/AAN/ACR made no recommendation for a second course of antibiotic versus observation after a first course (**No recommendation, knowledge gap).** In patients with Lyme arthritis with no or minimal response (moderate to severe joint swelling with minimal reduction of the joint effusion) to an initial course of antibiotic, IDSA/AAN/ACR suggests a two-to-four week course of IV ceftriaxone over a second course of oral antibiotics (Lantos et al., 2019).

NICE (2018)

For the management of Lyme arthritis, the NICE 2018 Lyme disease guidelines recommends oral antibiotic therapy for 28 days, noting that longer courses of treatment (28 days) are appropriate when treating Lyme arthritis, because it is difficult for antibiotics to penetrate to the synovium and synovial fluid. Care of children and young people aged under 18 years with Lyme disease and non-erythema migrans presentations should be discussed with a specialist (National Institute for Health and Care Excellence, 2018j).

The recommendations were underpinned by the Evidence review Management of Lyme arthritis (National Institute for Health and Care Excellence, 2018f). The systematic review included three RCTs published between 1985 and 1994. The quality of the evidence was appraised as low to very low, due to risk of bias, imprecision and indirectness, lack of blinding which could have had an effect on subjective outcomes, such as signs and symptoms which could not be measured by objective tests (National Institute for Health and Care Excellence, 2018f).

5.6.5. Management of patients with acrodermatitis chronica atrophicans

IDSA/AAN/ACR (2019 draft)

For patients with acrodermatitis chronica atrophicans, IDSA/AAN/ACR suggests oral antibiotic therapy for 21 to 28 days over shorter durations **(weak recommendation, low-quality evidence).** The rationale for the recommendation was that antibiotic therapy is recommended both for the resolution of acrodermatitis chronica atrophicans and to prevent further progression of infection to other tissues (Lantos et al., 2019).

NICE (2018)

For management of acrodermatitis chronica atrophicans, the NICE 2018 Lyme disease guideline recommendations are the same as for Lyme arthritis and a 28 day course of antibiotic treatment. Care of children and young people under 18 with Lyme disease and non-erythema migrans presentations should be discussed with a specialist (National Institute for Health and Care Excellence, 2018j).

The recommendation was underpinned by the Evidence Review Management of Acrodermatitis chronica atrophicans (National Institute for Health and Care Excellence, 2018a). The review included only one cohort study, which was published in 1996. No RCTs were identified. The quality of evidence was assessed as very low quality due to the non-randomised study design, risk of bias and imprecision. There were particular concerns about the selection of people, the general lack of blinding to the treatment allocation, and inadequately defined outcomes. As the evidence for antibiotics was very limited, the committee also took into account evidence for other presentations of Lyme disease and their experience and knowledge of current practice (National Institute for Health and Care Excellence, 2018a).

5.6.6. Management of patients with non-focal (non-specific) symptoms of Lyme disease

NICE (2018)

In patients with non-focal symptoms of Lyme disease (symptoms such as fever, sweats and muscle pain, which are not specific to an organ system) the NICE Lyme disease 2018 guideline recommends that patients should be given the same treatment as people with EM (National Institute for Health and Care Excellence, 2018j).

This recommendation was underpinned by the Evidence Review Management of non-specific symptoms related to Lyme disease (National Institute for Health and Care Excellence, 2018d). NICE found no relevant RCTS and cohort studies that assessed the effectiveness of antimicrobial therapy in people with solely non-specific symptoms and no prior antibiotic treatment of Lyme disease (National Institute for Health and Care Excellence, 2018d).

5.6.7. Management of women with Lyme disease during pregnancy and their babies

In the earlier section on clinical epidemiology, two patients had reported in submissions to the Senate Inquiry that they had acquired Lyme disease or DSCATT congenitally (Brown, 2018).

The NICE recommendations for management for women with Lyme disease during pregnancy and their babies were informed by an evidence review for person-to-person transmission (National Institute for Health and Care Excellence, 2018i).

The committee acknowledged that mother-to-baby transmission of Lyme disease is possible in theory. There was an **absence of evidence**, but the risk appears to be very low. The committee decided that women could be reassured that pregnancy and their baby are unlikely to be affected and highlighted the importance of completing treatment. It was also agreed that pregnant women should be treated following usual practice but using antibiotics suitable in pregnancy.

Given the absence of evidence and the lack of a standard approach to care, the committee agreed that care of babies born to mothers with Lyme disease during pregnancy should be discussed with a paediatric infectious disease specialist if the mother has concerns about her baby. In addition, to ensure that babies with Lyme disease do not go untreated, the committee agreed that babies should receive treatment if they have serology showing IgM antibodies specific to Lyme disease or symptoms that might be caused by Lyme disease (National Institute for Health and Care Excellence, 2018i).

No evidence was found for transmission of Lyme disease through sexual contact or blood products and the committee agreed that they could not make recommendations in these areas (National Institute for Health and Care Excellence, 2018i).

5.6.8. Relevance of international guidelines to the Australian setting

Recommendation:

International treatment guidelines may not be entirely applicable in the Australian health care setting, even in patients who have a travel history overseas to an endemic area.

Treatment for Lyme disease in the Australian health care context should only be initiated based on the expert advice of either a consultant physician practising in his or her speciality of infectious disease or a specialist microbiologist. This advice will be based upon results of confirmatory testing conducted in a NATA/RCPA accredited laboratory and/or other clinical findings relevant to informing a treatment decision.

5.7. Therapeutic modalities not recommended for treatment of patients with any manifestation of Lyme disease

There is no evidence to support the use of combination antibiotics, immunoglobulin, hyperbaric oxygen, specific nutritional supplements or prolonged courses of antibiotics for the management of Lyme disease (Borchers et al., 2015; Klempner et al., 2013; Lantos et al., 2019; Lantos, Shapiro, et al., 2015; Marzec et al., 2017; Wormser et al., 2006)

IDSA does not recommend the following therapeutic modalities for treatment of patients with any manifestation of Lyme disease because of a lack of biologic plausibility, lack of efficacy, absence of supporting data, or the potential for harm to the patient:

- first-generation cephalosporins, fluoroquinolones, carbapenems, vancomycin, metronidazole, tinidazole, amantadine, ketolides, isoniazid, trimethoprim-sulfamethoxazole, fluconazole, benzathine penicillin G,
- combinations of antimicrobials,
- pulsed-dosing (i.e., dosing on some days but not others),
- long-term antibiotic therapy,
- empirical antibabesiosis therapy in the absence of documentation of active babesiosis
- anti-*Bartonella* therapies,
- hyperbaric oxygen,
- fever therapy (with or without malaria induction),
- intravenous immunoglobulin,
- ozone,
- cholestyramine,
- intravenous hydrogen peroxide,
- vitamins and nutritional managements, and
- magnesium or bismuth injections (Wormser et al., 2006).

The strength of recommendation and quality of evidence for this IDSA recommendation for the therapeutic modalities listed above was E-III (**Strongly against; Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees**) (Wormser et al., 2006).

More recently, a 2015 study to identify websites of clinics that marketed non-antimicrobial therapies for Lyme disease identified more than 30 alternative treatments (Lantos, Shapiro, et al., 2015). A review of the medical literature by the authors did not substantiate efficacy or in most cases any rationale for the advertised treatments which fell into following several broad categories: oxygen and reactive oxygen therapy; energy and radiation-based therapies; nutritional therapy; chelation and heavy metal therapy; biological and pharmacological therapies ranging from certain medication without recognised therapeutic effects on *B. burgdorferi* to stem cell transplantation (Lantos, Shapiro, et al., 2015).

A report by Marzec et al. was published in the CDC MMWR (Marzec et al., 2017). Marzec (2017) advised treatment offered for the diagnosis of 'chronic Lyme disease', such as

prolonged antibiotic or immunoglobulin therapy, lacks data supporting effectiveness and as such treatment can result in serious complications, it is therefore not recommended. Marzec (2017) noted the various treatments prescribed to patients given a diagnosis of 'chronic Lyme disease', and for which there is often no evidence of effectiveness, include extended courses of antibiotics (lasting months to years), IV infusions of hydrogen peroxide, immunoglobulin therapy, hyperbaric oxygen therapy, electromagnetic frequency treatments, garlic supplements, colloidal silver, and stem cell transplants (Feder et al. (2007), and Lantos et al. (2015) in Marzec et al., 2017). Additionally, the CDC, in Marzec's report, noted at least five randomised, placebo-controlled studies have shown that prolonged courses of IV antibiotics in particular do not substantially improve long-term outcome for patients with a diagnosis of 'chronic Lyme disease' and can result in serious harm, including death (Feder et al. (2007), Wormser et al. (2006), Berende et al. (2016), and CDC guidance https://www.cdc.gov/lyme/treatment/prolonged/index.html.) in Marzec et al., 2017).

Five cases were described to illustrate complications resulting from unproven treatments, including septic shock, *Clostridium difficile* colitis, osteodiscitis, abscess and death (Marzec et al., 2017).

The five cases described to the CDC over the past several years [not further defined] are reproduced below to demonstrate the severity and scope of adverse effects that can be caused by unproven treatments for Lyme disease.

Table 16: Complications resulting from unproven treatments for "chronic Lyme disease", from Marzec et al. (2017)

Case studies
Patient A A woman in her late 30s with fatigue and joint pain received a diagnosis of chronic Lyme disease, babesiosis, and *Bartonella* infection by a local physician. Despite multiple courses of oral antibiotics, her symptoms worsened, and a peripherally inserted central catheter (PICC) was placed for initiation of IV antibiotic treatment. After three weeks of treatment with IV ceftriaxone and cefotaxime, the patient's joint pain continued, and she developed fever and rash. She became hypotensive and tachycardic and was hospitalised in an intensive care unit, where she was treated with broad spectrum IV antibiotics and required mechanical ventilation and vasopressors. Despite maximal medical support, she continued to worsen and eventually died. The patient's death was attributed to septic shock related to central venous catheter–associated bacteremia.
Patient B An adolescent girl sought medical advice regarding years of muscle and joint pain, backaches, headaches, and lethargy. She had received a diagnosis of chronic fatigue syndrome, but sought a second opinion from an alternative medicine clinic and was told she had chronic Lyme disease. The patient was treated with oral antibiotics, including rifampin, trimethoprim-sulfamethoxazole, and doxycycline, for three months. These were discontinued because of abnormal liver enzyme test results. Three months later, a PICC was placed to administer IV antibiotics, including ceftriaxone. After receiving both IV and oral antibiotic therapy for five months without improvement, the antibiotics were discontinued, but the PICC was not removed. One week after antibiotics had been discontinued, the patient developed pallor, chills, and fever to 102.9°F (39.4°C). After consultation with the alternative medicine clinic, she was given another dose of ceftriaxone through the PICC. Later that day she was evaluated in an emergency department with fever to 105.3°F (40.7°C), hypotension, and tachycardia consistent with septic shock. Blood and PICC tip cultures grew *Acinetobacter* spp. She was hospitalised in an intensive care unit and required vasopressors as well as broad-spectrum antibiotics to treat the infection. The PICC was removed, and the patient was eventually discharged after several weeks of hospitalisation.

Case studies
Patient C A woman in her late 40s received multiple arthropod bites and subsequently developed a flu-like illness with pain in her arms, legs, and back. One year after her symptoms began, she received a diagnosis of Lyme disease using the recommended two-tiered serologic test (positive enzyme immunoassay test result followed by positive immunoglobulin G Western immunoblot). She was treated with two four-week courses of oral doxycycline. The patient developed fatigue, cognitive difficulties, and poor exercise tolerance, and two years after her initial diagnosis she received a diagnosis of chronic Lyme disease based on the results of unvalidated tests. She was treated with intramuscular penicillin for approximately five weeks without improvement, then IV ceftriaxone for four months, followed by IV azithromycin for six months administered via a tunneled IV catheter. One year later, she received additional IV ceftriaxone via a new IV catheter, plus oral doxycycline, tinidazole (an antiparasitic medication), and azithromycin for approximately four weeks. The patient developed back pain, shortness of breath, and malaise, and was hospitalised. The catheter was removed, and blood and catheter tip cultures yielded *Pseudomonas aeruginosa*. She was treated with aztreonam for four weeks; however, her back pain worsened, and she was readmitted to the hospital. A computed tomography scan indicated destruction of both the 9th and 10th thoracic vertebrae, and magnetic resonance imaging of her spine confirmed osteodiscitis. A bone biopsy and culture grew *P. aeruginosa* with the same antibiotic susceptibility profile as her previously diagnosed bacteremia. She was treated for osteodiscitis, and her back pain eventually improved.
Patient D A woman in her 50s developed progressive weakness, swelling, and tingling in her extremities and received a tentative diagnosis of chronic inflammatory demyelinating polyneuropathy. Despite various treatments over a five-year period, her symptoms did not substantially improve, and a diagnosis of amyotrophic lateral sclerosis was made. The patient was subsequently evaluated by another physician and was told she had chronic Lyme disease, babesiosis, and Rocky Mountain spotted fever. Initial treatment with herbs and homeopathic remedies had no effect. She was treated with IV ceftriaxone and oral trimethoprim-sulfamethoxazole, acyclovir, fluconazole, and tinidazole. After seven months of intensive antimicrobial treatment, her pain improved, but the weakness worsened. She discontinued treatment after developing *C. difficile* colitis that caused severe abdominal cramps and diarrhoea. The *C. difficile* infection became intractable, and her symptoms persisted for over two years, requiring prolonged treatment. The patient subsequently died from complications of amyotrophic lateral sclerosis.
Patient E A woman in her 60s with autoimmune neutropenia, mixed connective tissue disease, and degenerative arthritis received a diagnosis of chronic Lyme disease neuropathy, for which she received IV immunoglobulin every three weeks via a tunnelled venous catheter with an implanted subcutaneous port. After undergoing treatments for more than ten years, she developed fevers and neck pain and was hospitalised; the catheter was removed, and blood and catheter tip cultures yielded methicillin-sensitive *Staphylococcus aureus*. She was treated with IV antibiotics via a newly placed PICC. Although the patient was advised to have the PICC removed once the antibiotic course finished, she chose to keep it for further IV immunoglobulin therapy. Two months later, she was readmitted for recurrent fevers. The PICC was removed, and cultures of the tip grew coagulase negative *Staphylococcus*; blood cultures were negative. She was treated with IV antibiotics and discharged. The patient subsequently received a new implanted subcutaneous venous catheter and restarted IV immunoglobulin therapy, after which she was readmitted for fever and back pain. Blood cultures were positive for methicillin-sensitive *S. aureus*, and magnetic resonance imaging indicated inflammation of the lumbar facet joints, epidural space, and paraspinal muscles, consistent with infection. Despite appropriate antibiotic treatment, her back pain worsened, and she required surgical drainage of a paraspinal abscess.

The CDC cautioned that in addition to the dangers associated with inappropriate antibiotic use, such as selection of antibiotic resistant bacteria, these treatments can lead to injuries related to unnecessary procedures, bacteraemia and resulting metastatic infection, venous thromboses, and missed opportunities to diagnose and treat the actual underlying cause of the patient's symptoms. CDC advice was that patients and their healthcare providers need to be aware of the risks associated with treatments for 'chronic Lyme disease' (Marzec et al. 2017).

5.8. Management of prolonged or ongoing symptoms following treatment of Lyme disease

In 5.2.1 Antibiotics we reported 'Lyme literate' practitioners told the Senate Inquiry that the use of long-term antibiotics was evidence-based and in many cases [not further defined] assisted patients to get better (Senate Community Affairs References Committee, 2016a). Additionally, Dr Richard Schloeffel provided evidence to the Senate Inquiry that patients with Lyme-like illness in Australia are being provided with antibiotics, including long-term antibiotics, including long-term IV antibiotic therapy (Senate Community Affairs References Committee, 2016a).

5.8.1. Use of long-term antibiotics is not supported by evidence or recommended and has the potential to cause harm

There is a strong body of evidence and international authority recommendations that do not support ongoing and long-term treatment of Lyme disease with antibiotics (Auwaerter et al., 2011; Berende et al., 2016; Borchers et al., 2015; Centers for Disease Control and Prevention, 2019b; Klempner et al., 2013; Lantos et al., 2019; Marzec et al., 2017; National Institute for Health and Care Excellence, 2018h, 2018j; Therapeutic Guidelines, 2019; Wormser et al., 2006).

Evidence cited by many authors and international authorities includes five clinical RCTs that have shown prolonged antibiotic therapy has no clear and lasting benefit in relieving post treatment Lyme disease symptoms. Some authors and clinicians hold a counter view, including ILADS.

5.8.2. RCTs

Berende et al.'s RCT on longer-term therapy for symptoms attributed to Lyme disease found longer-term antibiotic therapy did not provide additional benefit or better outcomes compared to shorter-term antibiotics (Berende et al., 2016).

Berende et al's randomised, double-blind, placebo-controlled trial conducted in Europe involving patients with persistent symptoms attributed to Lyme disease investigated the health-related quality of life of patients using the RAND-36 Health Status Inventory (RAND SF-36). Patients (281 patients, 89 per cent of whom had previously received antibiotic treatment for the diagnosis of Lyme disease) were randomised to receive a 12-week oral course of either doxycycline, clarithromycin plus hydroxychloroquine, or placebo. All study groups received IV ceftriaxone for two weeks prior to randomised regimen being initiated. Health-related quality of life was assessed at baseline, at the end of the treatment period at week 14 (after the 2-week course of IV ceftriaxone and the 12-week course of the randomised study drug or placebo had been completed), at 26 weeks, at 40 weeks and at 52 weeks after the start of the treatment period.

At the final observation point, 52 weeks after initiation of therapy, health-related quality of life scores did not differ significantly among the three groups. In all study groups the SF-36 physical component summary score increased significantly from base-line to the end of the treatment period ($p < .001$). The authors concluded that 14 weeks of antimicrobial therapy did not provide benefit beyond that with shorter term treatment among patients who present with fatigue, or musculoskeletal, neuropsychological, or cognitive disorders that are

temporally related to prior Lyme disease or accompanied by positive *B. burgdorferi* serologic findings.

Regarding safety, overall, 205 patients (73.2 per cent) reported at least one adverse event. Nine patients (3.2 per cent) had a serious adverse event, four of which were thought to be related to drug use that occurred during the two-week ceftriaxone phase. No serious drug-related adverse event occurred during the 12-week randomised phase. 19 patients (6.8 per cent) had an adverse event that led to discontinuation of the study drug. The rates of adverse events were similar among the study groups (Berende et al., 2016).

5.8.3. NICE (2018)

NICE reviewed the the evidence for the management of ongoing symptoms related to Lyme disease and made the following recommendations.

- For managing ongoing symptoms of Lyme disease after a course of antibiotics, patients should not be routinely offered more than two courses of antibiotics, because of a lack of evidence of benefit.
- The importance of considering alternative diagnoses to prevent inappropriate antibiotic treatment and misdiagnosis and that discussion with a specialist or referral should be considered for some people if a different tick-borne disease is possible.
- That healthcare professionals help people with long-term symptoms related to Lyme disease to access support if needed (National Institute for Health and Care Excellence, 2018j).

The NICE recommendations for the management of ongoing symptoms were underpinned by the Evidence Review Management of ongoing symptoms (National Institute for Health and Care Excellence, 2018h). NICE considered six studies in its evidence review (Berende et al. (2016), Cameron (2008), Fallon et al. (2008), Klempner et al. (2001), Kaplan et al. (2003), and Krupp et al. (2003) in National Institute for Health and Care Excellence, 2018h).

NICE advised that current treatment of Lyme disease is a single course of antibiotics; however, people who have had treatment for Lyme disease sometimes report ongoing symptoms, the cause of which is often not clear and includes reinfection, or organ damage caused by Lyme disease which may take a long time to heal or may even be permanent (National Institute for Health and Care Excellence, 2018j).

The term ‘ongoing symptoms’ was preferred for the guideline as it does not attribute cause of symptoms; terms such as ‘chronic Lyme disease’ imply possible chronic infection and may be misleading (National Institute for Health and Care Excellence, 2018j).

5.8.4. CDC

The CDC, in its advice about Post-Treatment Lyme Disease Syndrome (PTLDS) (Centers for Disease Control and Prevention, 2019b), notes that although most cases of Lyme disease can be cured with a two-to-four-week course of oral antibiotics, patients can sometimes have symptoms of pain, fatigue, or difficulty thinking that lasts for more than six months after they finish treatment. The reason why some patients experience PTLDS is not known (Centers for Disease Control and Prevention, 2019b). The CDC advises some experts believe that *Borrelia burgdorferi* can trigger an “auto-immune” response causing symptoms that last well after the infection itself is gone. Auto–immune responses are known to occur following other

infections, including campylobacter (Guillain-Barré syndrome), chlamydia (Reiter's syndrome), and strep throat (rheumatic heart disease). Other experts hypothesise that PTLDS results from a persistent but difficult to detect infection. Finally, some believe that the symptoms of PTLDS are due to other causes unrelated to the patient's *Borrelia burgdorferi* infection (Centers for Disease Control and Prevention, 2019b).

CDC has issued the following advice:

- Patients with PTLDS usually get better over time, but it can take many months to feel completely well.
- If a patient has been treated for Lyme disease and still feels unwell, see their healthcare provider to discuss additional options for managing symptoms with their healthcare provider.
- If a patient is considering long-term antibiotic treatment for ongoing symptoms associated with a Lyme disease infection, they should talk to their healthcare provider about the possible risks of such treatment (Centers for Disease Control and Prevention, 2019b).
- The CDC advised there is no proven treatment for PTLDS. It notes that while short-term antibiotic treatment is a proven treatment for early Lyme disease, studies funded by the National Institutes of Health (NIH) have found that long-term outcomes are no better for patients who received additional prolonged antibiotic treatment than for patients who received placebo (citing NIAID-NIH https://www.niaid.nih.gov/diseases-conditions/lyme-disease-antibiotic-treatment-research). The CDC advised long-term antibiotic treatment for Lyme disease has been associated with serious, sometimes deadly complications, and provided eight links to studies to support this advice (Strizova et al. (2019), Goodlet and Fairman (2018), Marzec et al. (2017), De Wilde et al. (2017), Marks et al. (2016), Lantos et al. (2015, Holzbauer et al. (2010), and Patel et al. (2000) in Centers for Disease Control and Prevention, 2019b). The paper by Marzec et al. 2017 was discussed above in 5.7 Therapeutic modalities not recommended for treatment of patients with any manifestation of Lyme disease.

5.8.5. IDSA/AAN/ACR 2019 (draft)

In their review of the evidence on prolonged symptoms following treatment of Lyme disease, IDSA/AAN/ACR noted that the prevalence of persistent symptoms following standard treatment of Lyme disease is a matter of uncertainty, and estimates depend in large part on the patient population and methods of long-term assessment (Lantos et al., 2019). Longitudinal studies (Shadick et al. (1994), and Nowakowski et al. (2003) in Lantos et al., 2019) had found patients appropriately diagnosed with or treated for Lyme disease had described either persisting or recurrent fatigue, musculoskeletal pain, neurocognitive and other non-specific subjective symptoms in 10-20 per cent or more one year after treatment, although studies indicate these symptoms appear to subside over time (Wormser et al. (2015), Wormser et al. (2015), Wills et al. (2016) in Lantos et al., 2019).

IDSA/AAN/ACR noted, importantly, findings from prospective controlled trials, several being recently conducted, where healthy controls were identified at the same time that patients with Lyme disease were being treated and both groups were followed over the ensuing months or years, the frequency of this symptom complex was the same in controls as in treated patients. These findings raised the possibility that this phenomenon may represent

anchoring bias whereby commonly occurring non-specific symptoms are inaccurately linked to prior diagnosis of Lyme disease (Cerar et al. (2010), Seltzer et al. (2000), Dersch et al. (2016, Bechtold et al. (2017), and Stupica et al. (2018) in Lantos et al., 2019).

IDSA/AAN/ACR considered whether patients with persistent symptoms following standard treatment for Lyme disease should receive additional antibiotics. They recommended against additional antibiotic therapy for patients who have persistent or recurring non-specific symptoms such as fatigue, pain, or cognitive impairment following treatment for appropriately diagnosed Lyme disease, but who lack objective evidence of reinfection or treatment failure (**Strong recommendation; moderate quality evidence**) (Lantos et al., 2019).

IDSA/AAN/ACR noted that evidence of persistent infection or treatment failure would include objective signs of disease activity such as arthritis, meningitis or neuropathy. The recommendation placed high value on avoiding harm due to unnecessary antibiotic exposure or to unnecessary IV access devices (Lantos et al., 2019). IDSA/AAN/ACR noted the risks of these interventions were not matched by convincing evidence that antibiotics improved patients' symptom experience or quality of life any better than a placebo (Lantos et al., 2019). To support this recommendation IDSA/AAN/ACR cited several clinical trials, noted by other authors and guidelines in this literature review, that had investigated antibiotic treatment of patients with disabling symptoms that had persisted months after standard treatment for documented Lyme disease (Klempner et al. (2001), Kaplan et al. (2003), Krupp et al. (2003), Klempner et al. (2013), Fallon et al. (2008) Berende et al. (2016) in Lantos et al., 2019).

IDSA/AAN/ACR noted that, in all studies, subjects improved; however, the improvement was also experienced by placebo-treated subjects. IDSA/AAN/ACR also noted numerous adverse events were reported in all studies including complications attributed to the antibiotic, with one serious antibiotic allergic reaction occurring in both the Fallon and Krupp studies (Lantos et al., 2019). Three patients in the Fallon study had IV line complications, as did three in the Krupp study. IDSA/AAN/ACR reported one patient on the Fallon study required cholecystectomy for ceftriaxone-associated gall bladder pseudolithiasis, while 43 per cent of the patients receiving ceftriaxone reported diarrhoea in the Krupp study. IDSA/AAN/ACR commented that 'despite these studies many patients receive prolonged IV antibiotic therapy for these symptoms - a practice that has been associated with a number of documented deaths' (Patel et al. (2000), and Holzbauer et al. (2010) in Lantos et al., 2019, p. 63).

Of their review of the evidence on additional antibiotic treatment for patients with persistent symptoms following standard treatment of Lyme disease, IDSA/AAN/ACR concluded the current body of clinical literature does not support the hypothesis that persistent symptoms should be interpreted as clinical infection or that antibiotic treatment is safe and effective (Klempner et al. (2001), Kaplan et al. (2003), Krupp et al. (2008), Klempner et al. (2013), Fallon et al. (2008) in Lantos et al., 2019). The authors noted a body of literature conducted in animal models has raised hypotheses of microbiological persistence but that the studies are highly heterogeneous and of limited generalisability to natural human infection. Moreover, animal models cannot reproduce the human experience of fatigue and pain experience, thus reducing the reliability of any insight into the biology of humans experiencing such symptoms following treatment of Lyme disease (Lantos et al., 2019).

Additionally, and as noted earlier, the IDSA/AAN/ACR 2019 draft guideline reported there was no clinical evidence to support regimens intended to treat fastidious states of *B. burgdorferi* infection, such as morphologic variants (aka "cyst" forms, "round" bodies, or "L-

forms"), or to treat biofilms (Lantos et al. (2014) in Lantos et al., 2019). This systematic review is described below in 5.8.8 Systematic reviews.

Regarding antibiotic therapy for patients having been given the diagnosis of 'chronic Lyme disease', IDSA/AAN/ACR note that no higher quality studies have addressed the question whether patients with these highly heterogenous symptoms but no alternative diagnoses should be treated as if they had Lyme disease, and (in the opinion of some) treated for an extended period of time (Lantos et al., 2019). The authors note that by definition these patients often have no compelling clinical or laboratory support for the diagnosis of ongoing Lyme disease and the clinical trials, as reviewed above by IDSA/AAN/ACR, have found that prolonged antimicrobial therapy is not helpful for persistent symptomology after treatment of verified Lyme disease. IDSA/AAN/ACR advice was that prolonged antibiotic treatment is unlikely to benefit individuals who lack a verifiable history of Lyme disease while at the same time exposing them to significant risk (Lantos et al., 2019).

5.8.6. Therapeutic Guidelines Ltd

In Australia, Therapeutic Guidelines Ltd also reviewed the evidence and advised that prolonged intravenous or oral antibiotic therapy for Lyme disease did not significantly improve outcomes in studies performed in North America and Europe, and can be associated with significant adverse effects (Therapeutic Guidelines, 2019).

5.8.7. IDSA (2010)

The voluntary review of the IDSA 2006 guidelines in 2008 also reviewed the evidence regarding post-Lyme disease syndromes, noting the controversial and public profile nature of this subject (Lantos et al., 2010). The Review Panel reviewed numerous sources of evidence including large volumes of case reports, case reports submitted by ILADS, journal correspondence, patient testimony and the available randomised, placebo-controlled, clinical trials of long-term antibiotic therapy for symptoms attributed to Lyme disease and made the following conclusions:

- The prospective, controlled clinical trials of extended antibiotic treatment of Lyme disease have demonstrated considerable risk of harm, including potentially life-threatening adverse events, attributable both to antibiotic treatment and to intravascular access devices. Such events include intravenous catheter infection, including septicemia (line sepsis), venous thromboembolism, drug hypersensitivity reactions, and drug induced cholecystitis. Minor adverse events, such as diarrhoea and candidiasis, were also more common among antibiotic treated patients (Fallon et al. (2008), Klempner et al. (2001), Krupp et al. (2003), Oski et al. (2007), and Kaplan et al. (2003) in Lantos et al., 2010). In a recent cohort of 200 patients, catheter-associated adverse events, such as thrombosis and infection, occurred a mean of 81 days into therapy, underscoring the cumulative risk of adverse events with increasing time.
- Prospective, controlled clinical trials have demonstrated little benefit from prolonged antibiotic therapy. Nearly all primary outcome measures failed to demonstrate an advantage to prolonged antibiotic therapy. Statistically significant improvements in treatment groups were not demonstrated across studies, were nonspecific, were of unclear clinical importance, and in one case, were not sustained at the end of the trial.

- The risk/benefit ratio for prolonged antibiotic therapy discourages prolonged antibiotic courses for Lyme disease. Several presenters in the hearing argued that patients with symptoms attributed to 'chronic Lyme disease' confer considerable societal cost. This argument, however, was not accompanied by quantitative evidence from controlled trials that prolonged antibiotic therapy could even partly reduce this cost. The Review Panel concluded that a societal benefit was at best hypothetical based on current evidence (Lantos et al., 2010).

The Review Panel reviewed six studies to inform the above statements (Fallon et al. (2008), Klempner et al. (2001), Krupp et al. (2003), Oski et al. (2007), Kaplan et al. (2003), and Stricker et al. (2010) in Lantos et al., 2010).

5.8.8. Systematic reviews

As noted in 5.8.5 IDSA/AAN/ACR 2019 (draft), IDSA/AAN/ACR cited the systematic review of Lantos et al. (2014) in its draft 2019 guideline and reported they found no clinical evidence to support regimens intended to treat fastidious states of *B. burgdorferi* infection, such as morphologic variants (aka "cyst" forms, "round" bodies, or "L-forms"), or to treat biofilms (Lantos et al., 2019).

This systematic review was of the medical and scientific literature to evaluate whether morphologic variants of *B. burgdorferi* play a role in human Lyme disease, whether they have been associated with illnesses compatible with 'chronic Lyme disease' and whether there is evidence to support antibiotic choices meant to eradicate these morphologic variants concluded:

> *There is little evidence that supports a role of B. burgdorferi morphologic variants in the pathogenesis of Lyme disease and no evidence that they influence treatment outcomes. The presence of round morphologic variants in vivo has been described only in a small number of case reports and case series. As different terminology and laboratory methods were used in these studies, it is difficult to be sure that in aggregate they describe similar structures. We found no convincing scientific evidence that these morphologic variants are associated with chronic B. burgdorferi infection, or with the sometimes disabling and protracted symptoms that are often the pretext for a chronic Lyme disease diagnosis (Lantos et al., 2014, p. 668).*

5.8.9. Reviews

Three reviews that considered the evidence on long-term antibiotic therapy in the treatment of Lyme disease found evidence did not support the practice and introduced harm (Borchers et al. 2015; Klempner et al. (2013); Auwaerter et al. (2011).

Borchers, in their 'rigorous review' of diagnostic criteria and treatment for Lyme disease concluded "Antibiotics are routinely and typically used to treat patients with Lyme disease, but there is no evidence that prolonged or recurrent treatment with antibiotics change the natural history of Lyme disease".

We had previously noted their comparison of guidelines by IDSA and EUCALB indicated the approaches to therapy are largely similar on both sides of the Atlantic but that ILADS guidelines (2004) advocate more aggressive and longer treatment courses for patients with persistent symptoms or refractory disease.

Borchers et al. also reviewed the treatment of Post Lyme disease syndrome (PLDS). They noted, as other authors have done, treatment was associated with several serious or even life-threatening adverse events (Klempner et al. (2001), Fallon et al. (2008), Krupp et al. (2003), Cameron (2008), Fallon et al. (2012), Kaplan et al. (2003) in Borchers et al., 2015). Borchers et al. commented the interpretation of the results of the cited studies and the question of whether or not patients with disabling subjective symptoms should be retreated with (yet another course of) antibiotics remains the subject of intense controversy, but what seems to be lacking from this discussion is the well-known fact that antibiotic therapy can induce considerable disturbances in the intestinal microflora (Borchers et al., 2015). They pointed out that there has been no study of the microbiome in patients with Lyme disease despite increased evidence of the importance of the microbiome in immune mediated pathology, and thus, it may be time to finally consider the role of repeated and prolonged antibiotic treatment itself in the symptoms of patients with PLDS (Borchers et al., 2015).

The 2013 paper by Klempner et al. (Klempner et al., 2013) was cited by many authors in the body of literature reviewed under this research question. Klempner et al. (2013) revisited the National Institutes of Health (NIH)-sponsored antibiotic treatment trials of patients who had persistent unexplained symptoms despite previous antibiotic treatment, and that had determined that retreatment provides little if any benefit and carries significant risk (Klempner et al. (2001), Kaplan et al. (2003), Krupp et al. (2003), and Fallon et al. (2008) in Klempner et al., 2013).

Klempner et al. revisited these trials following the publication of two reassessments of these trials (DeLonmg et al. (2012), and Fallon et al. (2012) in Klempner et al., 2013). Klempner et al. noted DeLong et al. had concluded from their analysis of these studies that retreatment can be beneficial and that the study findings are consistent with continued infection, but Klempner et al. highlighted DeLong et al.'s conclusions were based on questionable assumptions and the authors failed to disclose their support of long-term treatment with antibiotics as well as alternative treatments for Lyme disease (Stricker et al. (2011) in Klempner et al., 2013). Of the paper by Fallon et al. 2012, Klempner et al. noted that the authors had provided their own "reappraisal" of these studies including the 2008 study for which Dr Fallon was the lead investigator (Fallon et al. (2008) in Klempner et al., 2013). Klempner et al. reported Fallon et al.'s 2012 interpretation of these studies is that IV ceftriaxone is moderately efficacious for chronic fatigue following treatment for Lyme disease and that such therapy might be prescribed following a careful discussion with the patient of the risks involved (Klempner et al., 2013).

Klempner et al. reported they carefully considered the points raised by these groups of authors (DeLong et al., 2012, and Fallon et al., 2012), along with their own critical review of the treatment trials. Klempner et al. stated *"Based on this analysis, the conclusion that there is a meaningful clinical benefit to be gained from retreatment of such patients with parenteral antibiotic therapy cannot be justified".* Klempner et al. additionally stated in their conclusion,

> *"DeLong et al. fail to provide credible and convincing evidence that the methodology, findings and conclusions of the Klempner et al. studies are invalid or that the other NIH-sponsored retreatment trials show any evidence that post-treatment symptoms of Lyme disease are due to persistent infection. Neither of the analyses provided by DeLong et al. or Fallon et al. justify a conclusion that there is a meaningful clinical benefit to be gained from retreatment with parenteral antibiotic therapy"(Klempner et al., 2013, p. 7).*

Auwaerter et al. in their article, 'Antiscience and ethical concerns associated with Lyme disease', commented some Lyme disease activists propose that the disease causes mainly non-specific symptoms that can be treated only with long-term antibiotics and other unorthodox and unvalidated treatments (Auwaerter et al., 2011). Auwaerter and colleagues noted concepts about Lyme disease that are unproven or proven to be inaccurate regarding treatment that are obtained from popular Lyme disease websites, and from public statements and presentations made by some 'Lyme literate' medical doctors and 'chronic Lyme disease' activists include:

- Usual doses and durations of antibiotics are insufficient; open ended treatment with multiple antibiotics needed
- Combinations of antibiotics are needed to eradicate *B. burgdorferi* (Auwaerter et al., 2011).

Auwaerter et al. noted that accompanying subjective manifestations, such as fatigue, are often improved but not completely resolved at the conclusion of antibiotic treatment, but evidence from clinical trials (Wormser et al. (2006), Dattwyler et al. (2005), Oski et al. (2007), and Wormser et al. (2003) in Auwaerter et al., 2011) shows that prolonging the initial course of antibiotic treatment does not accelerate the rate of resolution of such symptoms. They cited, as other international guidelines and authors have done, the four National Institutes of Health (NIH-sponsored) double-blind, randomised, placebo-controlled treatment trials have been done to examine whether persistent (for ≥ 6 months) subjective symptoms were improved by retreatment with antibiotics after standard courses or oral or IV treatment for Lyme disease (Wormser et al. (2006), Klempner et al. (2001), Krupp et al. (2003), and Fallon et al. (2008) in Auwaerter et al., 2011). Auwaerter et al. noted data from the two largest studies (Klempner et al. (2001) in Auwaerter et al., 2011), indicated no benefit from re-treatment with 90 days of additional antibiotic therapy, with results from the other two studies (Krupp et al. (2003), and Fallon et al. (2008) in Auwaerter et al., 2011) reporting at most equivocal evidence for benefit. Auwaerter et al. highlighted that none of the investigators of the four studies concluded that the possible risk and unconfirmed benefits of antibiotic treatment outweighed their risks, which were substantial in the two smaller trials (for example, admission to hospital for intravenous catheter sepsis) (Klempner et al. (2001), Krupp et al. (2003), and Fallon et al. (2008) in Auwaerter et al., 2011) and consistent with these findings, there was also no microbiological evidence for persistent of *B. burgdorferi* despite rigorous examination of several body fluid samples, including culture and molecular diagnostic assays (Wormser et al. (2006), Klempner et al. (2001), and Fallon et al. (2008) in Auwaerter et al., 2011).

Despite evidence from these trials, Auwaerter et al. noted that many activists believe that patients whose subjective manifestations of Lyme disease have resolved after antibiotic treatment are still chronically infected (Auwaerter et al., 2011).

Guidelines that hold a counter view

5.8.10. ILADS (2014)

ILADS (2014) Lyme disease guideline is incongruent with other international guidelines and guidance.

ILADS argued that hypothetically the persistence of *Borrelia* is attributed to its residency within the cell and to the development of biologically less active permanent forms (sphaeroplasts, encystment) among other things (Cameron et al., 2014). Note IDSA/AAN/ACR (Lantos et al., 2019) and Lantos et al. (2014) found no clinical evidence to support regimens intended to treat fastidious states of *B. burgdorferi* infection, such as morphologic variants (aka "cyst" forms, "round" bodies, or "L-forms"), or to treat biofilms (IDSA/AAN/ACR 2019 Draft Lyme disease Guidelines).

ILADS recommends that for patients who have persistent manifestations of Lyme disease, if antibiotic retreatment is undertaken, clinicians should initiate treatment with 4–6 weeks of the selected antibiotic. ILADS also recommends injectable and IV antibiotics (Cameron et al., 2014). The recommendations below are reproduced from the ILADS (2014) guidelines Executive summary of treatment recommendations (Cameron et al., 2014).

> *Recommendation 3a: Clinicians should discuss antibiotic retreatment with all patients who have persistent manifestations of Lyme disease. These discussions should provide patient-specific risk–benefit assessments for each treatment option and include information regarding C. difficile infection and the preventative effect of probiotics (although none of the subjects in the retreatment trials developed C. difficile infection). (**Strong recommendation; very low-quality evidence**) p.1109*
>
> *Recommendation 3b: While continued observation alone is an option for patients with few manifestations, minimal QoL impairments and no evidence of disease progression, in the panel's judgment, antibiotic retreatment will prove to be appropriate for the majority of patients who remain ill. Prior to instituting antibiotic retreatment, the original Lyme disease diagnosis should be reassessed and clinicians should evaluate the patient for other potential causes of persistent disease manifestations. The presence of other tick-borne illnesses should be investigated if that had not already been done. Additionally, clinicians and their patients should jointly define what constitutes an adequate therapeutic trial for this particular set of circumstances. When antibiotic retreatment is undertaken, clinicians should initiate treatment with 4–6 weeks of the selected antibiotic; this time span is well within the treatment duration parameters of the retreatment trials. Variations in patient-specific details and the limitations of the evidence imply that the proposed duration is a starting point and clinicians may, in a variety of circumstances, need to select therapeutic regimens of longer duration. Treatment options are extensive and choices must be individualized. Each of these options would benefit from further study followed by a GRADE assessment of the evidence and consideration of associated risks and benefits, but until this information is available, clinicians may act on the currently available*

*evidence. In choosing between regimens, clinicians should consider the patient's responsiveness to previous treatment for Lyme disease, whether the illness is progressing and the rate of this progression; whether untreated co-infections are present; whether the patient has impaired immune system functioning or has received immunosuppressant corticosteroids and whether other co-morbidities or conditions would impact antibiotic selection or efficacy. Clinicians should also weigh the extent to which the illness interferes with the patient's QoL, including their ability to fully participate in work, school, social and family-related activities and the strength of their initial response against the risks associated with the various therapeutic options. Antibiotic selection should also consider medication tolerability, cost, the need for lifestyle adjustments to accommodate the medication and patient preferences. For patients with mild impairments who had a strong-to moderate response to the initial antibiotic, repeat use of that agent is favoured. Patients with moderate impairments or only a modest response to the initial antibiotic may benefit from switching to a different agent or combination of agents. For patients with significant impairments and/or a minimal or absent therapeutic response, a combination of oral antibiotics, injectable penicillin G benzathine or iv. ceftriaxone (with the latter two used alone or in combination with other agents) is preferred. For patients who experienced disease progression despite earlier therapy, treatment with injectable penicillin G benzathine or iv. ceftriaxone, alone or in combination with other antibiotics, is advisable. Additionally, minimal or absent responses and disease progression require a re-evaluation of the original diagnosis (**Recommendation**; **very low-quality evidence**). p.1109*

Recommendation 3c: Clinicians should re-assess patients immediately following the completion of the initial course of retreatment to evaluate the effectiveness of retreatment and the need for therapeutic adjustments. Reassessment may need to be done much earlier and with greater scrutiny in patients with severe disease or when the therapeutic intervention carries substantial risk. For patients who improve yet continue to have persistent manifestations and continuing QoL impairments following 4–6 weeks of antibiotic retreatment, decisions regarding the continuation, modification or discontinuation of treatment should be based on several factors. In addition to those listed in Recommendation 3b, the decision to continue treatment may depend on the length of time between the initial and subsequent retreatment, the strength of the patient's response to retreatment, the severity of the patient's current impairments, whether diagnostic tests, symptoms or treatment response suggest ongoing infection and whether the patient relapses when treatment is withdrawn. In cases where the patient does not improve after 4–6 weeks of antibiotic retreatment, clinicians should reassess the clinical diagnosis as well as the anticipated benefit. They should also confirm that other potential causes of persistent manifestations have been adequately investigated prior to continuing antibiotic retreatment. Decisions regarding the continuation,

> *modification or discontinuation of treatment should consider the factors noted above as well as the definition of an adequate therapeutic trial. Whenever retreatment is continued, the timing of subsequent follow-up visits should be based on the level of the therapeutic response, the severity of ongoing disease, the duration of current therapy and the need to monitor for adverse events.* ***(Recommendation, very low-quality evidence)*** *p.1110*

While the ILADS 2004 guideline is out of the date range of this literature review, Borchers and colleagues highlighted that guideline advocated, as does the 2014 ILADS guideline recommendations above, much more aggressive and longer treatment courses for patients with persistent symptoms or refractory disease (Borchers et al., 2015).

5.9. Initial management of known Australian tick-borne diseases

5.9.1. Queensland Tick Typhus (QTT)

Early recognition and treatment is important in QTT. Early initiation of doxycycline is critical as a delay in appropriate antimicrobial therapy is associated with increased likelihood of progression to severe disease and complications (Stewart et al., 2017).

Doxycycline should be administered orally in mild-to-moderate infection and intravenously in severe infection. There are no published data on the importance of antibiotics in mild *R. australis* infection although early administration probably prevents hospitalisation and morbidity (Stewart et al., 2017).

Patients usually show marked clinical improvement after 48 hours of antimicrobial therapy (Graves & Stenos, 2017; Stewart et al., 2017).

Refer to *Therapeutic Guidelines: Antibiotic* (Therapeutic Guidelines, n.d.) for treatment recommendations.

5.9.2. Flinders Island Spotted Fever (FISF)

Patients with FISF are treated with doxycycline.

Refer to *Therapeutic Guidelines: Antibiotic* (Therapeutic Guidelines, n.d.) for treatment recommendations.

5.9.3. Australian Spotted Fever (ASF)

Patients with ASF are treated with doxycycline.

Refer to *Therapeutic Guidelines: Antibiotic* (Therapeutic Guidelines, n.d.) for treatment recommendations.

5.9.4. Q fever

If Q fever suspected clinically (appropriate symptoms AND at high risk epidemiologically), commence empirical treatment while waiting for laboratory tests.

While achieving a timely, definitive diagnosis of Q fever is challenging, early treatment is beneficial and empirical antibiotic therapy should be considered if the presentation and clinical history suggest a zoonotic disease (Eastwood et al., 2018). The Q fever CDNA guidelines for Public Health Units specify that if Q fever is suspected clinically, empirical treatment should be commenced without waiting for laboratory tests (Communicable Diseases Network Australia, 2018).

Treatment recommendations are:

- A two-week course of oral doxycycline is generally used to treat Q fever
- Trimethoprim + sulfamethoxazole is recommended for pregnant women until 32 weeks gestation, even if recovered, to prevent maternal and fetal complications (Communicable Diseases Network Australia, 2018).

Also refer *Therapeutic Guidelines: Antibiotics* (Therapeutic Guidelines, n.d.).

5.10. Ongoing management of known Australian tick-borne diseases

5.10.1. Queensland Tick Typhus

Delay in correct antimicrobial therapy is associated with increased likelihood of progression to severe disease and complications. However, some individuals, for unknown reasons, progress to severe disease and sepsis despite early doxycycline therapy, with concurrent comorbidities, *Rickettsia* inoculum size and inherent virulence in Rickettsial strains being suggested factors (Stewart et al., 2017).

The literature indicates there is little systematic evidence on the outcomes of acute *R. australis* infection, particularly in non-hospitalised patients; however, where severe hospitalised cases with complications have been documented, a full recovery following acute illness is expected. Additionally, there is no evidence of chronic infection; however, a post infective syndrome of lethargy, malaise and muscle pains persisting for several months or more after acute infection has been described (Stewart et al., 2017).

5.10.2. Q fever

After treatment of *C. burnetii* primary infection, CDNA guidance includes the following recommendations

- screening for risk factors of chronic Q fever, including pre-existing valvular heart disease/valvular prosthesis, vascular aneurysms/vascular grafts and immunosuppression
- undertaking a cardiac assessment to assess whether there are underlying abnormalities of the heart valves
- monitoring serologically and clinically at 3,6,9,12,18 and 24 months those who, after acute infection, are at higher risk of chronic Q fever (Communicable Diseases Network Australia, 2018).

Chronic Q fever requires prolonged treatment with antibiotics. Expert advice from an infectious diseases physician and other specialist physicians should be sought as appropriate (Communicable Diseases Network Australia, 2018).

6. RESEARCH QUESTION 5: WHAT CURRENT GUIDELINES AND APPROACHES TO INVESTIGATION AND ONGOING SYNDROMIC MANAGEMENT OF SYMPTOMS ASSOCIATED WITH DSCATT HAVE BEEN FOUND EFFECTIVE INTERNATIONALLY?

6.1. Overview and key findings

This section provides the findings of the literature reviewed to answer research question 5:

What current guidelines and approaches to investigation and ongoing syndromic management of symptoms associated with DSCATT have been found effective internationally?

6.1.1. Key findings

Patients with MUS are at risk of potentially harmful additional testing and are often subjected to repeated diagnostic investigations, and unnecessary and costly referrals and interventions.

People experiencing debilitating symptoms attributed to ticks, without any definitive diagnosis, could be considered to fall within the definition of MUS. International evidence indicates patients with MUS are at risk of potentially harmful additional testing and are often subjected to repeated diagnostic investigations, and unnecessary and costly referrals and interventions. In managing MUS in general practice, balancing the iatrogenic risk of investigation with the therapeutic risk of missing something important, is a challenge for GPs.

An analysis of patient submissions to the Senate Inquiry noted that, in patients who identified as having Lyme disease or DSCATT, the non-specific symptoms, female preponderance and lack of confirmatory laboratory testing suggested patients were more likely to be experiencing a MUPS disorder (such as CFS) than an active or latent infection. Additionally, they experience social and financial harms and are at risk of nosocomial harms and may also have sought alternative and potentially non-evidence-based diagnoses and treatments.

A 2017 review of MUS guidelines in Europe estimates that 3-11 per cent of patients visiting general practice repeatedly consult their GP for MUS. However, this finding might not be entirely applicable to Australia. MUS exist along a continuum ranging from self-limiting symptoms to recurrent and persistent symptoms through to symptom disorders.

Advice from the RACGP and a review of the international MUS guidelines summarising guidelines from the Netherlands, Denmark, UK and Germany is consistent: patients with MUS often feel stigmatised and not taken seriously. To manage these concerns, all guidelines make the following recommendations.

- Highlighting the importance of paying attention to the doctor-patient relationship.
- Providing an individualised approach that recognises the patient's illness and taking the patient's symptoms seriously.
- Demonstrating empathy with consultations aiming to validate the patient's distress.
- Highlighting the importance of providing an explanation in the patient's language about the possible causes of their symptoms (patients benefit from an explanation that makes sense, removes blame from the patient, generates ideas on how to manage the symptoms. The 2011 UK guidance, published by the Royal College of General Practitioners in the UK, advises that GPs should be explicit about their thoughts, uncertainties, and expectations of referrals to specialist care).
- Caution that patients with persistent [MUS] suffer from their symptoms, are functionally impaired, and are at risk of potentially harmful additional testing and treatment.

The stepped care approach is recommended internationally to manage symptom severity in patients with MUS.

International and Australian guidelines on MUS recommend a stepped care approach to address three levels of symptom severity, which lack clear cut-off points. They also advise that it is important that one care provider, preferably the GP, keeps control and co-ordinates the care process.

The stepped care model of care is internationally recognised and familiar to and widely used by GPs in Australia in all aspects of patient care. Stepped care is an evidence-based, staged system comprising a hierarchy of interventions, from the least to the most intensive, matched to the individual's needs. Within a stepped care approach, an individual will be supported to transition up to higher intensity services or transition down to lower intensity services as their needs change.

In addition to being recommended as an approach for managing care for people with MUS, the stepped care service model has been shown in RCTs to be effective for the management of chronic pain, for the management of depression and anxiety, and in the assessment and management of anxiety and depression in adult cancer patients. Stepped care models are widely used in England, Scotland, US, New Zealand, and Australia.

6.1.2. Literature reviewed

Australian Government reports	(House of Representatives Standing Committee on Health, 2016; Senate Community Affairs References Committee, 2016a, 2016b)
Australian Department of Health reports, reports to, and guidance	(Allen + Clarke, 2019; Department of Health, 2019; TMS Consulting Pty Ltd, 2018b)
(Inter)national authority and intergovernmental reports, evidence reviews, guidelines and guidance	
Guidelines and guidance (International and Australian) by clinical and professional bodies	(Butow et al., 2015; Chitnis et al., 2011; Olde Hartman et al., 2013; Royal Australian College of General Practitioners, 2016; Royal College of Psychiatrists, 2017; Stone, 2015)
Systematic Reviews (with/without meta-analysis)	
Narrative literature reviews and reviews	(Beaman, 2016; Collignon et al., 2016; Olde Hartman et al., 2017)
Randomised controlled trials	
Prospective cohort studies	
Observational studies	(Brown, 2018; Xue et al., 2007)
Qualitative studies	(Ali et al., 2014)

Other	(Australian Commission on Safety and Quality in Health Care, nd, 2018; Cancer Council Australia, 2015; Choosing Wisely Australia, n.d.; General Practice Mental Health Standards Collaboration, 2019; National Health and Medical Research Council, 2014; National Prescribing Service MedicineWise, 2019; US Department of Veterans Affairs, 2009; Williamson, M. et al., 2008)

6.1.3. Quality of the evidence

The evidence base used in this literature review to support the management of patients with MUS is robust. We drew heavily on recommendations, guidelines and guidance from Australian and international clinical professional associations. Additionally, advice and recommendations came from prestigious and respected Australian organisations such as the Australian Commission on Quality and Safety in Health Care, National Health and Medical Research Council (NHMRC), Choosing Wisely Australia and Cancer Council.

6.2. Patients experiencing DSCATT and the potential for medically unexplained symptoms

We noted in 3.8.2 Medically unexplained symptoms, when discussing likely differential diagnoses, that Brown (2018), noted the most commonly reported symptoms by patients (fatigue, disordered thinking, or 'brain fog', sensory disturbance, arthralgia and myalgia, and headache), coupled with submissions showing a 'striking female preponderance' (80.3 per cent when reported), were prominent components of both fibromyalgia and CFS, the two most prominent MUPS disorders (Brown, 2018, p. 424). Brown had further commented that the non-specific symptoms, female preponderance, and lack of confirmatory laboratory testing suggested patients were more likely to be experiencing a MUPS disorder (such as CFS) than an active or latent infection, as had been found by investigators of 'chronic Lyme disease' in the US, which reached the same conclusion by actively comparing healthy, CFS and 'alternatively diagnosed Lyme' groups (Patrick et al. (2015) in Brown, 2018).

Brown (2018) also highlighted patients who made submissions to the Senate Inquiry experience social and financial harms, are at risk of nosocomial harms and may also have sought alternative and potentially non-evidence-based diagnoses and treatments.

We also noted in this earlier section that people experiencing debilitating symptoms attributed to ticks, without any definitive diagnosis could be considered to fall within the definition of MUS.

6.3. Initial and ongoing management of patients with persistent symptoms and who remain undiagnosed

Where there is no diagnosis and the patient is experiencing symptoms that are medically unexplained, it is especially important to ensure that person-centred care is provided that validates, addresses and manages their symptoms as well as possible.

The Australian Commission on Safety and Quality in Health Care advises that 'Person centred-care' involves:

- seeking out and understanding what is important to the patient
- fostering trust
- establishing mutual respect, and
- working together to share decisions and plan care (Australian Commission on Safety and Quality in Health Care, 2018).

Key dimensions include respect, emotional support, physical comfort, information and communication, continuity and transition, care coordination, access to care, and partnerships with patients, carers and family in the design and delivery of care (Australian Commission on Safety and Quality in Health Care, 2018, p. 1).

Patients should be treated symptomatically and are also encouraged to consider the potential for harm with complementary medicines for which there is no evidence in those with comorbidities. All people with MUS, (including those experiencing DSCATT) can be assisted to have an improved quality of life with good care in a partnership between patient and the health care team.

International and Australian guidelines provide evidence-based, practical and consistent recommendations for people that can be applied to patients with DSCATT. Good communication and empathy are important. Patients' concerns need to be taken seriously and their symptoms acknowledged and alleviated.

The most common unexplained symptoms reported by patients experiencing DSCATT include fatigue, disordered thinking, sensory disturbance, arthralgia, headache (Brown, 2018). These symptoms can have multiple different causes, depending on the particular symptoms, cluster, and time frame of symptom(s).

6.3.1. Medically Unexplained Symptoms

People experiencing debilitating symptoms attributed to ticks, without any definitive diagnosis could be considered to fall within the definition of MUS. A review of MUS guidelines in Europe (Olde Hartman et al., 2017) estimates that between 3-11 per cent of patients visiting general practice repeatedly consult their GP for MUS. However, this finding might not be entirely applicable to Australia. MUS exist along a continuum ranging from self-limiting symptoms to recurrent and persistent symptoms through to symptom disorders.

Advice from the RACGP (Stone, 2015) and the review of the international MUS guidelines (Olde Hartman et al., 2017), summarising guidelines from the Netherlands, Denmark, UK and Germany (two of which provide evidence graded recommendations), is consistent. Patients with MUS often feel stigmatised and not taken seriously.

To manage these concerns, all guidelines recommend:

- highlighting the importance of paying attention to the doctor-patient relationship
- providing an individualised approach that recognises the patient's illness and taking the patient's symptoms seriously
- demonstrating empathy with consultations aiming to validate the patient's distress
- highlighting the importance of providing an explanation in the patient's language about the possible causes of their symptoms. (Patients benefit from an explanation that makes sense, removes blame from the patient, generates ideas on how to manage the symptoms. The 2011 UK guidance published by the Royal College of General Practitioners in the UK, advises that GPs should be explicit about their thoughts, uncertainties and expectations of referrals to specialist care (Chitnis et al., 2011)); and
- caution that 'patients with persistent MUS suffer from their symptoms, are functionally impaired, and are at risk of potentially harmful additional testing and treatment' (Olde Hartman et al., 2017, p. 1).

A qualitative study into the experiences of patients identifying with 'chronic Lyme disease' reported on the importance of actively engaged and sympathetic clinical encounters. They showed that where patient concerns are fully acknowledged and addressed, they experience greater satisfaction with their healthcare (Ali et al., 2014).

Having any chronic medical condition of any cause increases the likelihood of mental health conditions, which in turn can lead to poorer outcomes. An article on managing medically unexplained illness in general practice published by RACGP (Stone, 2015) notes that acknowledging the difficulty of chronic symptoms and supporting the important mental health strategies is vital to person centred care in chronic disease. Additionally, all patients with MUS need support to manage distressing symptoms and the disability that accompanies them (Stone, 2015). Helping patients understand that the mind and body are interconnected in complex ways and that holistic care is often essential to improve health is important. Reattribution, the technique of shifting the focus away from only physical symptoms and biomedical diagnoses to a more holistic understanding of illness, was noted as a useful technique in primary care (Stone, 2015).

6.3.2. Practice Harm Minimisation

International evidence indicates patients with MUS suffer from their symptoms, are functionally impaired, are at risk of potentially harmful additional testing (Olde Hartman et al., 2017) and are often subjected to repeated diagnostic investigations, and unnecessary and costly referrals and interventions (Royal College of Psychiatrists, 2017).

Of relevance to DSCATT, and as previously noted in 5.2 Treatment modalities that have been provided to patients (including subgroups of patients) with DSCATT in Australia, the Senate Committee (2016a) heard concerns from a large (not further defined in the report) number of patients who identified as suffering from Lyme disease or DSCATT that 'Lyme literate' practitioners often prescribe a course of treatment that may include antibiotics that are not supported by Medicare or the Pharmaceutical Benefits Scheme (PBS). In his analysis of submissions to the Senate Inquiry from patients who identified as having Lyme disease or DSCATT, Brown (2018) identified the social and financial harms associated with diagnosis and treatment of DSCATT, with over half of submissions (56.2 per cent) reporting a median

cost of treatment of $30,000. Nearly half (45.7 per cent) reported they had received oral antibiotics and one in six (16.6 per cent) reported they had received IM/IV antibiotics (Brown, 2018).

As noted in 3.5.6 Potential to misdiagnose potentially treatable illnesses as Lyme disease, the potential to misdiagnose potentially treatable illness while diagnosing Australian patients with debilitating symptom complexes as having Lyme disease was a major cause of concern raised in the Senate Inquiry reports (2016a, 2016b) including by the Medical Board of Australia (MBA), Australian Health Practitioner Regulation Agency (AHPRA) and Medical Council of New South Wales. Concerns raised included the use of unconventional diagnostic techniques such as kinesiology, the reliance on non-accredited laboratories to diagnose Lyme disease, not referring patients with complex diagnoses to specialists where this would have been appropriate, not managing other co-existing medical conditions, and misdiagnosing cancers as DSCATT.

Also as reported earlier, in 5.3.1 Evidence for treatments provided for patients experiencing DSCATT and 5.3.2 Appropriateness of treatments and medical harm, serious concerns have been raised about overuse and long-term use of antibiotic treatment and antimicrobial resistance for Australian patients receiving treatment for DSCATT, including in the Senate Inquiry (2016a, 2016b) by professionals, medical professional bodies and medical professional regulatory bodies and in published papers. Concerns regarding the hazards of taking antibiotics unnecessarily include their toxicity, potential hypersensitivity reactions, anaphylaxis (allergy) and predisposition with *Clostridium difficile* and antibiotic-resistance bacteria (Collignon et al., 2016). Australian evidence reported through the national ASID-OzBug bulletin board found testing at overseas laboratories using non-approved protocols have resulted in misdiagnoses associated with experimental treatments that have caused serious complications including line sepsis, pancreatitis, and pseudomembranous colitis (Beaman, 2016).

Regarding the risks posed from unnecessary and long-term use of antibiotics in patients with DSCATT, as above, Choosing Wisely Australia and the National Prescribing Service (NPS) advise that prescribing a routine course of antibiotics significantly increases the likelihood of an individual carrying a resistant bacterial strain. Resistant bacteria can be spread to family, friends and the broader community. To minimise antibiotic resistance, Australian guidelines recommend that an antibiotic should only be prescribed:

- when benefits to the patient are likely to be substantial
- with the narrowest spectrum to treat the likely pathogen
- at the appropriate dose and for the appropriate duration (Choosing Wisely Australia, n.d.).

In managing MUS in general practice, balancing the iatrogenic risk of investigation with the therapeutic risk of missing something important is a challenge for GPs (Stone, 2015).

Table 17: Recommendations for managing MUS

Avoid
• Repeated diagnostic testing. - Harms include worry that there is still something to be found that hasn't been tested for yet, repeated investigations and treatment, multiple primary care practitioners increased likelihood of false positives, and the finding of minor, non-significant abnormalities in test results that increase anxiety.
• Use of non-accredited laboratories for diagnostic testing and use of unconventional diagnostic techniques (e.g. kinesiology). - Harms include false positives and wrong diagnosis.
• Unnecessary referrals and interventions. - Harms include repeating and extending unnecessary testing and iatrogenic harm as well as financial costs.
• Treatments with known harm and no benefit (e.g. long-term antibiotics, extreme diets, miracle mineral solution, hyperbaric oxygen treatments). - Harms include toxicity, hypersensitivity reactions, predisposition to Clostridium difficile infection, development of antibiotic resistance, line sepsis, severe and persistent vomiting and diarrhoea, and large financial cost without benefit.
Encourage
• Discussion of intended 'natural' or alternative therapies for evaluation of individualised harms versus benefits. - An awareness of the evidence base and side effects to be aware of can assist patients in choosing alternative therapies wisely and avoiding unnecessary out of pocket costs and unintended harms.
• Periodic re-evaluation of symptoms and new symptoms to determine an identifiable cause and efficacy of treatment. - Small changes over time may not be noticed by patients. Review allows encouragement regarding improvements, detection of deterioration, and evaluation of new symptoms arising**.**
• Discussion of possible causes of and treatments for symptoms that have been found on the internet or recommended by friends. - Not having a diagnosis is difficult for patients in many ways and leads to a vulnerability to looking for a cause of their symptoms. The internet, social media and social contacts can be spreaders of both good and poor information. Remaining open to a patient discussing what they have found allows for education, exploration of misinformation, identification of reliable sources and identification of potential treatments to trial.
• Enlistment of other members of a multidisciplinary team.
• Consideration of mental health strategies.

NPS MedicineWise reports that a poll conducted in 2018 shows almost 7 million Australians take some form of complementary medicine every day (National Prescribing Service MedicineWise, 2019). Without a full understanding of patients' health practices, including their use of complementary therapies, it is difficult for clinicians to provide safe and patient-centred health care.

In addition to the alternative and complementary therapies in section 5.7 (many of which were reported by patients to the Senate Inquiry (2016a) to have been recommended to them), refer to NHMRC and Therapeutic Goods Administration (TGA) for information on complementary and alternative medicines in Australia.

A useful resource, *Talking with your patients about Complementary Medicines* (National Health and Medical Research Council, 2014) notes that many Australians report using complementary medicine but do not disclose this information to their clinicians (Williamson et al. (2008) in National Health and Medical Research Council, 2014). One of the most common reasons patients have not discussed their use of complementary medicines is that their clinician has not asked them about it (Xue et al., 2007). The RACGP advises that it is important for GPs to ask patients about their use of complementary therapies and to be aware of the evidence basis, or lack thereof. GPs should also have the knowledge to provide patients with balanced information about potential benefits and risks in order to enable informed decision making (Royal Australian College of General Practitioners, 2016).

The NHMRC (2014) resource recommends that:

- clinicians should be sensitive to the variety of other reasons for patients not disclosing complementary medicines use. These reasons include:
    - a belief that complementary medicines products and therapies are 'natural' and 'safer' than conventional medicine
    - a feeling of dissatisfaction with conventional medicine
    - a lack of awareness of the risk of unintended drug interactions
    - awareness of the clinician's attitude to or knowledge of complementary medicines
    - discomfort in raising the topic, and
    - fear of the practitioner's response
- when clinicians initiate discussions about complementary medicines with their patients, it is important to use an approach that increases collaboration and trust
- clinicians should encourage patients to make treatment decisions based on evidence and can ask their patients if they would like help identifying and interpreting evidence of effectiveness for the complementary therapies they use, and
- clinicians should explain to their patients that all health and treatment decisions involve weighing up potential benefits and potential risks and that this process can help patients to decide whether a treatment is appropriate for them.

Many consumers are not aware of the side effects of some complementary medicine products and their potential interactions with conventional medicines, which may put some users at unnecessary risk of harm. Clinicians may need to consider and explain to their patients the risk of adverse reactions (including unintended medicine interactions). Encourage patients

to ask questions about the efficacy, risks, contraindications and costs of the complementary therapies and the qualifications of the practitioner (Cancer Council Australia, 2015).

If considered clinically necessary, GPs may refer their patient to a pharmacist for a Medicare-supported Home Medicines Review to prevent medication-related problems.

Further information about Complementary Therapies

For further information on complementary and alternative medicines in Australia and around the world, refer to:

- NPS MedicineWise

  https://www.nps.org.au/consumers/complementary-medicines-explained
- The Therapeutic Research Center – a US website that has an interaction checker, effectiveness checker and a database of natural therapies
  https://naturalmedicines.therapeuticresearch.com
- Memorial Sloan Kettering Cancer Centre has information about herbs, botanicals and a number of complementary therapies

  https://www.mskcc.org/cancer-care/diagnosis-treatment/symptom-management/integrative-medicine/herbs
- Cochrane Complementary Medicine

  https://cam.cochrane.org/cochrane-reviews-related-complementary-medicine
- Victoria State Government Better Health Channel
  https://www.betterhealth.vic.gov.au/health/ConditionsAndTreatments/complementary-therapies
- the NHMRC
- TGA.

6.3.3. The Stepped Care Model

The challenge for the GP involves managing individual symptoms, but also creating a framework for the chronic care of patients with significant ongoing illness (Stone, 2015).

The stepped care model of care is internationally recognised and familiar to and widely used by GPs in Australia in all aspects of patient care. The model is recommended for use in patients with MUS by international and Australian guidelines.

Stepped care is an evidence-based, staged system comprising a hierarchy of interventions, from the least to the most intensive, matched to the individual's needs. Within a stepped care approach an individual will be supported to transition up to higher intensity services or transition down to lower intensity services as their needs change (General Practice Mental Health Standards Collaboration, 2019).

As background, international guidelines on MUS recommend a stepped care approach to address three levels of severity of symptoms, which lack clear cut-off points. They also advise that it is important that one care provider, preferably the GP, keeps control and coordinates the care process.

In addition to being recommended as an approach for managing care for people with MUS, the stepped care service model has been shown in RCTs to be effective for the management

of chronic pain (US Department of Veterans Affairs, 2009), and for the management of depression and anxiety (Department of Health, 2019) and in the assessment and management of anxiety and depression in adult cancer patients (Butow et al., 2015). Stepped care models are widely used in England, Scotland, US, New Zealand and Australia.

In Australia, the stepped care model of care is familiar to and widely used by GPs in all aspects of patient care. GPs make assessments to determine the best management approach to guide their patients in accessing services appropriate to their level of need, and thus ensure that more intensive and often costly services are directed to patients best able to benefit from them (General Practice Mental Health Standards Collaboration, 2019). While referrals are made to other relevant health practitioners as appropriate, it is important that one care provider, preferably the GP, coordinates care.

Stepped care models aim to:

- offer a variety of support options for people with different levels and types of need, from low intensity to high intensity
- provide clear pathways between these care options as individuals' needs change, and
- improve collaboration and integration between services (General Practice Mental Health Standards Collaboration, 2019).

Central to the stepped care approach is the development of an individualised care plan, developed in discussion with the patient.

International guidelines concur that doctor-patient communication is key. They emphasise the importance of exploring patient's ideas, concerns and expectations, providing acceptable explanations, providing practical and constructive advice that is applicable to their daily lives is important and offering advice on symptom management. Considering the patient's ethical-cultural background in all steps is recommended (Olde Hartman et al., 2013).

Table 18: Overview of Stepped Care approach to managing medically unexplained symptoms (Olde Hartman et al., 2013)

Step 1: For patients with mild functional limitations and who experience one or several symptoms
• Explore symptoms, conduct physical examination and or additional investigations. List the symptoms. • Summarise findings discussing clearly what was found and explicitly mentioning what was not found. • Try to reach a shared definition of the problem. It is important to recognise the symptoms and the fact the patient is troubled by them. Explore and address anxieties and misconceptions. It is very important that the patient's concerns are treated seriously and in a sensitive manner. • Provide the patient with targeted and tangible information about ways to manage symptoms and an individualised care plan.
Step 2: For patients with moderate functional limitations with several symptoms, cluster symptoms or a symptom duration longer than expected
• Continue GP led care as in Step 1 and if the patient is unable to expand his/her level of activity to an acceptable standard, refer to either primary or secondary care practitioners (e.g. physiotherapy, nurse practitioners, specialist GPs, psychotherapy/Cognitive behavioural therapy). • Refer to secondary specialist services as required. • Make regular follow-up appointments if functional limitation persists (e.g. every 4-6 weeks).
Step 3: For patients with severe functional limitations and a large number of symptoms and duration of 3 months or more
• Refer to secondary, tertiary care providers and or multi-disciplinary teams or treatment centres. • Continue to stimulate the expansion of the patient's functioning and monitor for deterioration in function. • It is important that one care provider, ideally a GP, coordinates the care provided. • Limit long term treatments and investigations that are not useful and may even be harmful. • Make regular follow-up appointments during treatment (e.g. 4–6 weeks).

6.3.4. Recommendations for ongoing management of patients with persistent symptoms and who remain undiagnosed

The GP will lead the ongoing review of symptoms and management plan, in consultation with the patient, with regular review of progress in achieving their goals. In the event of persistent dysfunction, evaluate the situation regularly and offer any new treatment options. The review and evaluation of new symptoms may require a change of level in stepped care for the patient.

Management of ongoing symptoms should involve a multidisciplinary approach, incorporating the teamwork of all medical specialties relevant to the individual patient's care. Diagnosis is challenging, and it is important for GPs to seek opinions of experts in vector-borne diseases including specialist microbiologists with diagnostic experience. The management of patients must be a collaborative approach between GPs and specialists. Telehealth can also be used where appropriate.

Consider referring patients who have MUS to appropriate specialists based on best clinical practice and relevant evidence.

7. REFERENCE LIST

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8. APPENDICES

A: Methodology

B: Critical Appraisals

C: AGREE II Scores

D: AACODS Grey literature appraisals

APPENDIX A: METHODOLOGY

Background

The Australian Department of Health (the Department) commissioned Allen + Clarke Policy and Regulatory Specialists Limited (*Allen + Clarke*) to develop an evidence-based clinical pathway and multidisciplinary care model (the Clinical Pathway) for patients suffering from debilitating symptom complexes attributed to ticks (DSCATT) that can be flexibly applied in both private and public health settings.

The Clinical Pathway must be informed by relevant literature and key documents. As the Clinical Pathway will support decision-making on differential diagnosis and referral pathways for patients presenting with either new onset or unresolved debilitating symptoms with or without a history of tick bites and that cannot be attributed to another condition (acute or chronic), this literature review focuses on published evidence to inform an evidence base to underpin the Clinical Pathway. Acknowledging the attribution to ticks in the term DSCATT, this literature review includes consideration of tick-borne diseases (overseas-acquired Lyme disease and known Australian tick-borne diseases) and considerations, including approaches to management of care for patients for whom a diagnosis for their symptoms may not be established.

The Clinical Pathway will contribute to fulfilling the Australian Government's response to Recommendation 5 of the Senate Committee Final Report: *Inquiry into the growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australian patients,* where the Australian Government agreed to consult with key stakeholder groups to develop a cooperative multidisciplinary framework which can accommodate patient and medical needs. The Clinical Pathway will build on the consultation about the concept of multidisciplinary care previously undertaken through consultation forums with medical professionals, state and territory health authorities and patient groups in April and July 2018.

The minimum requirements of the Clinical Pathway are as follows.

- Assist with a differential diagnosis; including the ruling out of obvious diagnosable conditions, including classical Lyme disease, other tick-borne illnesses, and other obvious chronic debilitating conditions.
- Determine the composition of a multidisciplinary care approach or multidisciplinary care team (MDT) in terms of the skill mix required to comprehensively assess patients once obvious diagnosable conditions have been ruled out.
- Provide advice on when a patient should be referred to a multidisciplinary care approach or MDT, for example: the nature/duration of particular symptoms, absence of diagnosis from prior tests, diagnoses previously being considered and excluded prior to referral to MDT.
- Incorporate an agreed primary care management plan for those patients without a diagnosis that includes relevant ongoing support from their GP, allied health, and/or clinical specialists.
- Be flexible enough to be incorporated into existing public and private health care systems.

Objective

The purpose of this literature review is to provide a comprehensive integrative review of the peer-reviewed and grey literature published since January 2008, to inform an evidence-based Clinical Pathway for patients experiencing symptoms or a complex of symptoms referred to collectively as DSCATT. Acknowledging the attribution of ticks in the term DSCATT, this literature review includes the evidence on tick-borne diseases to be considered in the differential diagnosis (overseas-acquired Lyme disease and known Australian tick-borne diseases), evidence-based treatment of these tick-borne diseases, and evidence-based management of ongoing symptoms for patients for whom there is no diagnosis and who are considered to have MUS or 'undifferentiated illness'.

This literature review is not a systematic review. No primary research or pooled analysis was undertaken. Statements about the quality of the evidence included in this literature review have been provided.

Research questions

This literature review explored five research questions.

Question 1: What is the epidemiology of DSCATT in Australia?

Supplementary Questions

- What information is available on the prevalence, demographics and geographic distribution of patients experiencing DSCATT in Australia?
- What information is available on the symptoms and clinical signs that have been associated with DSCATT as reported by Australian patients and treating physicians?

Question 2: What information is available on diseases or disorders Australian patients experiencing DSCATT symptoms have been diagnosed with and what are the most likely differential diagnoses?

Question 3: What are the current issues associated with diagnostic testing for Lyme disease both in Australia and by overseas laboratories?

Question 4: What are the treatment modalities that have been provided to patients (including subgroups of patients) with DSCATT in Australia and what is the evidence base to support these treatment modalities?

Question 5: What current guidelines and approaches to investigation and ongoing syndromic management of symptoms associated with DSCATT have been found effective internationally?

Literature search process and outcomes

A set of key documents was provided by the Department of Health.

The literature search was guided by a Terms of Reference agreed with the Department of Health. The literature search was conducted between mid-March 2019 and mid-June 2020. This search approach enabled *Allen + Clarke* to identify relevant peer-reviewed evidence, (inter)national and Australian authority and internal and Australian clinical professional guidelines and guidance to underpin the development of the evidence-based Draft DSCATT Clinical Pathway for consultation. During stakeholder consultation on the Draft DSCATT Clinical Pathway, conducted between 13 November 2019 and 24 January 2020, to further develop and refine the Draft DSCATT Clinical Pathway, stakeholders provided literature to

Allen + Clarke that they requested we consider for inclusion in the literature review and/or DSCATT Clinical Pathway.

The following databases were initially searched in mid-March 2019 in accordance with the Terms of Reference agreed with the Department of Health.

- Discover (CINAHL Complete, Medline and PsycINFO)
- Cochrane Library database
- National Institute for Health and Clinical Excellence
- PubMed
- ProQuest (including Sociological Abstracts), and
- Guidelines International Network (www.g-i-n.net) guideline library.

To complete a systematic search, we used combinations of subject/index terms where appropriate (e.g. exploded term 'Borrelia infection') in combination with key words, or key words alone depending on the search functionality of each database or website. As a preliminary PubMed search revealed no published academic literature using the term DSCATT, a range of terms formerly used to describe this set of symptoms including 'Lyme-like disease', 'Lyme-like illness', 'chronic Lyme disease', 'Australian Lyme disease' and 'Lyme' were used in the search.

Duplicate citations, false hits/inaccurate returns were removed before all initial returned citations and abstracts were reviewed for relevance to the research questions.

From this first sweep, full texts for all proposed inclusions agreed by the *Allen + Clarke* project team were retrieved and reviewed for relevance to the research questions and inclusion criteria. An appraisal of study design (to determine overall quality) was completed and the bibliography of each included article was reviewed to identify other relevant research that may be of interest. Published articles on human diseases relevant to the requirements of the clinical pathway (overseas-acquired Lyme disease and known Australian tick-borne diseases) that met the highest levels of evidence were prioritised where available (systematic reviews with or without meta-analyses, RCTs). High quality narrative literature reviews and reviews were included. All relevant Australian peer-reviewed literature we identified on tick-borne diseases in humans and on animal studies of ticks that met the inclusion criteria were included.

A full text Google Scholar search was undertaken to identify official Australian and international literature, reports, policies, position statements, guidance and guidelines using search terms: Lyme, Lyme disease, Lyme-like, Lyme-like illness, Tick-Borne, Ticks, MUS, guidelines, diagnostic testing, treatment, (AND Australia) or (AND United States) or (United Kingdom) or (Canada) or (International).

Material that did not relate to the research questions, non-English language sources, and material published before 31 December 2007 (except where it was included in official Australian reports, statements or guidelines) was excluded. Literature investigating the efficacy of specific complementary and alternative therapies (e.g. ozone therapy) were out of scope of this literature review and were excluded.

From the results of the search, literature was prioritised according to the following criteria.

- Published, peer-reviewed literature.

- Official Australian reports and government inquiries.
- Department of Health reports, reports to and guidance.
- (Inter)national authority and intergovernmental reports, guidelines and guidance.
- Guidelines and guidance (International and Australian) produced by clinical and professional bodies.

Narrative reviews and reviews were included if they met the following criteria.

- They were published in high quality, peer-reviewed journals.
- Their authors were established experts in their field, working in reputable institutions.
- They were consistently well referenced.
- They were Australia-specific (due to additional relevance and relative scarcity).

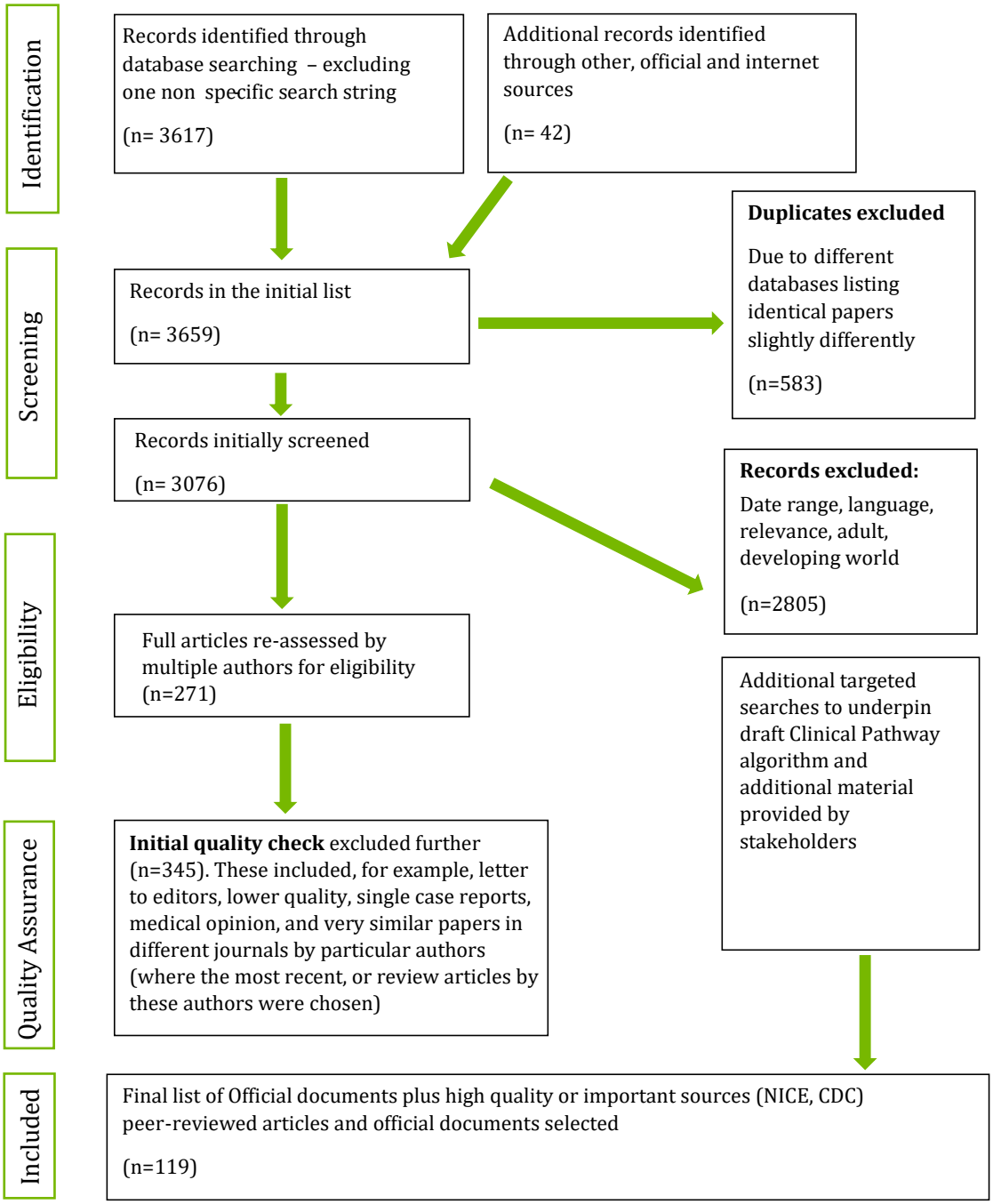
Following the initial searches in mid-March 2019, as described above, we undertook subsequent searches of academic databases (above) and/or Google searches to identify additional material to underpin the algorithm of the Draft DSCATT Clinical Pathway.

We also considered the books, published articles, and websites provided by stakeholders during the Draft DSCATT Clinical Pathway consultation against the research questions and inclusion criteria.

The overall search and selection pathway is shown in the following Literature search flow chart.

A total of 119 items were included in the literature review.

Figure 6: Literature search flow chart



Critical analysis and appraisal

All Australian studies and publications in peer-reviewed journals of relevance to the research questions and DSCATT Clinical Pathway were included, irrespective of quality. Where appropriate, the methodologies of included studies were critically appraised using AMSTAR 2, CASP or COREQ criteria.

- For quantitative research, we used the AMSTAR 2 Systematic Review Checklist; the CASP RCTs checklist; and the CASP Cohort Study Checklist.
- For qualitative research, we used the COREQ (COnsolidated criteria for REporting Qualitative research) Checklist.
- For clinical guidelines, we used the AGREE II Checklist.
- For all grey literature, including Australian Government reports, Department of Health reports, reports to and guidance, (inter)national authority reports and guidance, Australian and international clinical professional body position statements and guidance, and other grey literature, we used the AACODS Checklist.

Narrative literature reviews and reviews, Australian animal studies and Australian case reports of diagnosed cases of Lyme disease were not assessed for quality. Systematic reviews that were not on RCTs, or non-randomised studies of healthcare interventions, were not reviewed using AMSTAR 2. The NICE 2018 Evidence-based reviews on the diagnosis and management of Lyme disease, that underpinned the NICE 2018 Lyme disease guideline were accepted as high quality.

Limitations and assumptions

The Australian Government has chosen to describe this patient group as having Debilitating Symptom Complexes Attributed to Ticks (DSCATT) with this term being only very recently adopted in Australia. A preliminary PubMed search revealed there is no published academic literature using this term.

For this literature review search, we reverted to the terminology most commonly used to describe this set of symptoms in Australia and internationally, including ‘Lyme-like disease’, ‘Lyme-like illness’, ‘chronic Lyme disease’ and ‘Australian Lyme disease’.

In this literature review we have used the term DSCATT but also retained earlier terms including ‘Lyme-like disease’, ‘Lyme-like illness’ and ‘Australian Lyme disease’ where used in the literature.

Australian Government reports, Australian and international authority and medical professional guidance and guidelines, and peer-reviewed published papers were included in this literature review. International authority and Australian and international medical professional guidelines underpinned by evidence-based reviews, systematic reviews, RCTS, and quality literature reviews were prioritised. Australian human epidemiological studies and animal studies of relevance to Lyme disease and known Australian tick-borne diseases were prioritised.

For the research questions on DSCATT (research questions 1, 2, 4 and 5), for conciseness and fairness, we restricted the information included in this literature review to the high-level observations and findings as reported in the relevant Australian Government reports (the Senate Committee Interim and Final Reports (2016) and The House of Representatives Standing Committee on Health Inquiry into Chronic Disease Prevention and Management

(2016)). We included one patient advocacy report, as it provided insights, albeit self-reported, into the range of treatment regimens, including antibiotics, complementary and alternative treatments provided to patients identifying as having Lyme disease or DSCATT in Australia.

Where studies were cited and discussed in evidence-based guidelines, systematic reviews or reviews, we have generally not gone back to the original studies.

We have noted studies and publications cited by the authors of articles included in this literature review and provided these as in text citations. We feel this brings another layer of comprehensiveness to the literature review and several advantages to the reader. By identifying the cited authors in the text and providing the citation in the text, the reader has the opportunity to view at a glance the authors, the recency of the publication and also the consistency/comparability with which papers have been cited by authors to inform their conclusions/recommendations relevant to the various research questions. This approach also acknowledges literature not included in our literature review but published within the inclusion dates for the review. The addition of in text citation provides easy access for readers who may wish to explore an article further.

While the literature review inclusion date is 2008 onwards, we have on occasion included literature that was published prior to 2008. Reasons included where guidelines were cited in official Australian guidance or where there was little appropriate Australian literature on a topic.

This literature review is not a systematic review. No original meta-analysis or other pooled analysis was completed.

APPENDIX B: CRITICAL APPRAISALS

AMSTAR 2 checklist for systematic reviews

AMSTAR 2 is a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions or both.

We assessed four systematic reviews (with or without meta-analyses) with AMSTAR 2, as these reviews assessed healthcare interventions (Cadavid et al., 2016; Cook & Puri, 2016; Leeflang et al., 2016; Waddell et al., 2016).

Three other systematic reviews were included in this literature review (Brunton et al., 2017; Lantos et al., 2014; Lantos & Wormser, 2014). We did not assess these systematic reviews with AMSTAR 2 as they did not relate specifically to healthcare interventions.

Cadavid et al. (2016) Cadavid, D., Auwaerter, P. G., Rumbaugh, J., & Gelderblom, H. (2016). Antibiotics for the neurological complications of Lyme disease. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD006978.pub2

	AMSTAR 2 TOOL QUESTION[3]	Answer	Comment
1	Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	The authors provided specific details about the types of participants, interventions and primary and secondary outcome measures.
2	Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes	The authors reported the review has a published protocol from 2008, but it had been updated in 2014 when it was decided that a meta-analysis was not feasible and the focus of the review was changed to a systematic narrative review.
3	Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Randomised clinical trials of antibiotic treatment of LNB in adults and children that compared any antibiotic treatment, including combinations of treatments, versus any other treatment, placebo, or no treatment. The authors excluded studies of entities considered as post-Lyme syndrome.
4	Did the review authors use a comprehensive literature search strategy?	Yes	The authors stated: On 25 October 2016 we searched the Cochrane Neuromuscular Specialised Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, and Embase. We searched clinical trial registers on 26 October 2016. We reviewed the bibliographies of the randomised trials identified and contacted the authors and known experts in the field to identify additional published or unpublished data. There were no language restrictions when searching for studies.
5	Did the review authors perform study selection in duplicate?	Yes	All review authors checked titles and abstracts identified from the searches to determine which studies met the eligibility criteria. When the review authors could not determine eligibility from the title and abstract, they obtained the full text of all potentially relevant studies for independent assessment. Two review authors

	AMSTAR 2 TOOL QUESTION[3]	Answer	Comment
			independently assessed and decided which of the trials identified from the preliminary searches fitted the inclusion and exclusion criteria and graded the risk of bias of the trials. The review authors resolved disagreements about study inclusion by consensus. Two systematic review specialists conducted a duplicate study selection process. The review authors assessed any discrepancies in comparison with their selection.
6	Did the review authors perform data extraction in duplicate?	Yes	Two review authors independently extracted data from all studies that met the inclusion and exclusion criteria onto a specially designed data extraction form. One of the review authors entered data into the Cochrane Review Manager 5 software (RevMan 2014), and a second review author checked the data extraction. In the case of missing data, the review authors attempted to contact the trial authors. Review authors were not blinded to trial authors, journal, or institution. To assist the review authors, two systematic review specialists conducted an independent data extraction. The CochraneNeuromuscularManaging Editor created analysis tables and added numerical data to the Results using this data extraction. A review author checked the outcome data entry.
7	Did the review authors provide a list of excluded studies and justify the exclusion?	Yes	The excluded studies and the reason for the exclusion was provided in a table.
8	Did the review authors describe the included studies in adequate detail?	Yes	Comprehensive details of included studies were provided.
9	Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	Two review authors independently assessed all of the included studies for risk of bias. In the event of disagreement, all of the review authors achieved consensus through discussion. They used the Cochrane 'Risk of bias' tool to assess risk of bias of the included Studies. To assist the review authors, two systematic review specialists provided by Cochrane conducted an independent 'Risk of bias' assessment, and the review authors addressed any discrepancies in assessments.

	AMSTAR 2 TOOL QUESTION[3]	Answer	Comment
10	Did the review authors report on the sources of funding for the studies included in the review?	Yes	Funding of studies was included in the Characteristics of included studies.
11	If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	N/A	Marked heterogeneity among the seven RCTs prevented meta-analysis.
12	If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	N/A	
13	Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Yes	The author's judgements for each 'Risk of bias' domains in the seven studies were provided along with an appraisal of each study in the text.
14	Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	The authors reported significant heterogeneity of inclusion and exclusion criteria and primary and secondary outcome measures was evident by simple examination. To illustrate this heterogeneity, the authors presented a detailed comparison of the study characteristics in a table.
15	If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	N/A	The authors performed a qualitative synthesis.
16	Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	The declarations of interest stated were: D Cadavid was a full-time paid employee of Biogen during most of the preparatory time for this review. He is currently a full-time employee of Fulcrum Therapeutics. Neither Biogen nor Fulcrum Therapeutics is involved in research on LNB. D Cadavid's work on this review is not related to his employment with Biogen or Fulcrum Therapeutics. PG Auwaerter has served as a medical-legal expert witness regarding Lyme disease; has been reimbursed for travel expenses related to an

	AMSTAR 2 TOOL QUESTION[3]	Answer	Comment
			update of the Lyme Disease Guideline by the Infectious Diseases Society of America, the American Academy of Neurology, and the American College of Rheumatology (IDSA/AAN/ACR); and has been given honoraria for CME courses regarding Lyme disease. J Rumbaugh has been reimbursed for travel expenses related to an update of the Lyme Disease Guideline (IDSA/AAN/ACR). H Gelderblom: none known.

Cook, M., & Puri, B. (2016). Commercial test kits for detection of Lyme borreliosis: A meta-analysis of test accuracy. *International Journal of General Medicine*, *Volume 9*, 427–440. https://doi.org/10.2147/IJGM.S122313

	AMSTAR 2 TOOL QUESTION[4]	Answer	Comment
1	Did the research questions and inclusion criteria for the review include the components of the PICO?	No	There were no research questions or objectives stated. The authors stated: The clinical diagnosis of Lyme borreliosis can be supported by various test methodologies; test kits are available from many manufacturers. Literature searches were carried out to identify studies that reported characteristics of the test kits.
2	Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No	
3	Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Studies were included in the analysis where the following criteria were met: 1. Samples were proven to be positive for LB based on one or more of the following: clinical records of an EM rash; positive serology; culture; samples meeting the CDC criteria (generally being an EM rash or being two-tier positive) or CDC-certified panels with samples characterised by them as positive, negative, or equivocal. Full criteria are available for your reference in Centers for Disease Control and Prevention. 2. The tests were commercially available. 3. The specificity was ≥85%.
4	Did the review authors use a comprehensive literature search strategy?	No	The authors stated: PubMed and Google Scholar were used with the search terms “Lyme disease OR borreliosis AND testing” to identify studies. All papers since 1995 were selected for consideration.
5	Did the review authors perform study selection in duplicate?	Unclear	

	AMSTAR 2 TOOL QUESTION[4]	Answer	Comment
6	Did the review authors perform data extraction in duplicate?	Unclear	
7	Did the review authors provide a list of excluded studies and justify the exclusion?	Yes	A comprehensive table was provided of the studies not meeting inclusion criteria. No PRISMA flow chart was provided.
8	Did the review authors describe the included studies in adequate detail?	Yes	The authors provided a table with details of the included studies.
9	Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	No	
10	Did the review authors report on the sources of funding for the studies included in the review?	No	
11	If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Yes	The authors stated: There was no standardised method for carrying out the evaluations or recording data, and so data were extracted manually from the documents and entered into Microsoft Excel worksheets. This allowed computation and preparation of a standard format giving sample size, positive samples, and percentage of positive results. This was used to define the sensitivity for each stage of disease and for each test method. To compute the overall sensitivity for all studies and for all subgroups, the weighted average of sample size and positive samples of subgroups was used.
12	If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No	
13	Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	No	
14	Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	No	

	AMSTAR 2 TOOL QUESTION[4]	Answer	Comment
15	If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	
16	Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	The authors disclosed they have no financial competing interests and report no conflict of interest in this work.

Leeflang, M. M. G., Ang, C. W., Berkhout, J., Bijlmer, H. A., Van Bortel, W., Brandenburg, A. H., Van Burgel, N. D., Van Dam, A. P. V., Dessau, R. B., Fingerle, V., Hovius, J. W. R., Jaulhac, B., Meijer, B., Van Pelt, W. V., Schellekens, J. F. P., Spijker, R., Stelma, F. F., Stanek, G., Verduyn-Lunel, F., … Sprong, H. (2016). The diagnostic accuracy of serological tests for Lyme borreliosis in Europe: A systematic review and meta-analysis. *BMC Infectious Diseases*, *16*, 1–17. https://doi.org/10.1186/s12879-016-1468-4

	AMSTAR 2 TOOL QUESTION[5]	Answer	Comment
1	Did the research questions and inclusion criteria for the review include the components of the PICO?	Partly	The authors stated they systematically reviewed all available literature to assess the accuracy (expressed as sensitivity and specificity) of serological tests for the diagnosis of the different manifestations of Lyme borreliosis in Europe. Their secondary aim was to investigate potential sources of heterogeneity, e,g,, test-type, whether the test was a commercial test or an in house test, publication year and antigens used.
2	Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No	
3	Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	
4	Did the review authors use a comprehensive literature search strategy?	Yes	The search strategy was included in an Appendix.
5	Did the review authors perform study selection in duplicate?	Yes	
6	Did the review authors perform data extraction in duplicate?	Yes	For each article, two authors independently collected study data.
7	Did the review authors provide a list of excluded studies and justify the exclusion?	Yes	Literature search flow chart included.

	AMSTAR 2 TOOL QUESTION[5]	Answer	Comment
8	Did the review authors describe the included studies in adequate detail?		The authors cited the references for the included 57 case control studies and 18 cross-sectional studies then listed the authors of the studies in tables reporting on their quality assessment.
9	Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	Quality of studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) checklist. This checklist consists of four domains: patient selection, index test, reference standard and flow and timing. Each of these domains has a sub-domain for risk of bias and the first three have a sub-domain for concerns regarding the applicability of study results. The sub-domains about risk of bias include a number of signalling questions to guide the overall judgement about whether a study is highly likely to be biased or not. An appendix on the questions regarding data quality was included.
10	Did the review authors report on the sources of funding for the studies included in the review?	No	
11	If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Yes	The authors stated: We analysed test accuracy for each of the manifestations of Lyme borreliosis separately and separately for case-control designs and cross-sectional designs. If a study did not distinguish between the different manifestations, we used the data of this study in the analysis for the target condition “unspecified Lyme”. Serology assays measure the level of immunoglubulins (Ig) in the patient’s serum. IgM is the antibody most present in the early stages of disease, while IgG increases later in the disease. Some tests only measure IgM, some only IgG and some tests measure any type of Ig. In some studies, the accuracy was reported for IgM only, IgG only and for detection of IgG and IgM. In those cases, we included the data for simultaneous detection of both IgG and IgM (IgT). We meta-analysed the data using the Hierarchical Summary ROC (HSROC) model, a hierarchical metaregression method incorporating both sensitivity and specificity while taking into account the correlation between the two.

	AMSTAR 2 TOOL QUESTION[5]	Answer	Comment
12	If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	
13	Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Yes	
14	Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	The authors stated: There is no recommended measure to estimate the amount of heterogeneity in diagnostic accuracy reviews, but researchers are encouraged to investigate potential sources of heterogeneity. The most prominent source of heterogeneity is variation in threshold, which is taken into account by using the HSROC model. Other potential sources of heterogeneity are: test type (ELISA or immunoblot); a test being commercial or not; immunoglobulin type; antigen used; publication year; late versus early disease; and study quality. These were added as covariates to the model to explain variation in accuracy, threshold or shape of the curve. The authors specifically addressed heterogeneity in the results.
15	If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	Yes	The authors undertook an extensive risk of bias assessment, providing two tables of results and a methodological quality graph. They noted the observed heterogeneity and risk of bias complicate the extrapolation of their results to clinical practice.
16	Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	The authors stated: All authors declare: HS and ML received financial support for the submitted work from ECDC; RD has received personal fees from Diarosin and Orian Diagnostica, personal fees and other from Siemens Diagnostica, other from Oxoid Thermofisher and Gilead, outside the submitted work; VF has received honoraria from DiaSorin, Mikrogen, Siemens and Virotech. HZ and WVB are employees of ECDC. All others report no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other

	AMSTAR 2 TOOL QUESTION[5]	Answer	Comment
			relationships or activities that could appear to have influenced the submitted work.

Waddell, L. A., Greig, J., Mascarenhas, M., Harding, S., Lindsay, R., & Ogden, N. (2016). The accuracy of diagnostic tests for Lyme disease in humans, a systematic review and meta-analysis of North American research. *PLoS One*, *11*(12), 1–23. https://doi.org/10.1371/journal.pone.0168613

	AMSTAR 2 TOOL QUESTION[6]	Answer	Comment
1	Did the research questions and inclusion criteria for the review include the components of the PICO?	Yes	Objective was to summarise the North American evidence on the accuracy of diagnostic tests and test regimes used to diagnose Lyme disease in patients presenting with clinical symptoms in North America at various stages of disease and to address the question of whether there is evidence of superior, equivalent or poor performance by the commercial (approved by the FDA and/or HC) and in house laboratory tests captured in this review.
2	Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes	The systematic review was preceded by a scoping review conducted in 2016 (Greig et al. Public Health Agency of Canada, (available on request) in Waddell et al., 2016) to identify, classify and characterise what is the current state of scientific knowledge on surveillance methods, prevention and control strategies, diagnostic tests, risk factors, and societal attitudes and perceptions towards Lyme disease in humans and *B. burgdorferi* in tick vectors and vertebrate reservoirs. The protocol for the scoping review is available on request.
3	Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	The scoping review methodology was designed to characterise the primary literature on Lyme disease in humans or *B. burgdorferi* tick vectors or reservoirs, thus studies not on Lyme disease or *B. burgdorferi* were excluded from the scoping review. Additionally, the primary research had to address one of the following topics: surveillance/monitoring, prevalence, incidence, societal attitudes and perceptions in North America and global prevention and control strategies, diagnosis and risk factors. Research on clinical Lyme disease and treatment were considered outside the scope of this review. Each

	AMSTAR 2 TOOL QUESTION[6]	Answer	Comment
			relevant paper was classified by purpose, study design, location of the study, *B. burgdorferi*, host species investigated, vector species investigated, sampling dates, diagnostic tests used, and whether the paper contained extractable data. Included papers examined the accuracy of diagnostic tests for Lyme disease in North America after 1995, and included studies that compared results of one test using a validated test panel, results of clinical diagnosis, or a gold standard test result or investigated inter-test agreement.
4	Did the review authors use a comprehensive literature search strategy?	Yes	The scoping review search strategy was developed and pretested by three individuals with extensive experience in knowledge synthesis, zoonotic diseases and library science. The following search algorithm was implemented in eight bibliographic databases: BIOSIS (via web of knowledge), CAB abstracts, Scopus, PubMed, PsycINFO, APA PsycNet, Sociological Abstracts, and EconLit with no limitation on the search, this was followed by a comprehensive search for grey literature.
5	Did the review authors perform study selection in duplicate?	Unknown	This information may be in the scoping review protocol available on request.
6	Did the review authors perform data extraction in duplicate?	Yes	The data extraction form captured all pertinent study details and results. The systematic review was managed in DistillerSR (Evidence Partners, Ottawa, ON, Canada) a web-based systematic review management software. Each form was completed by two reviewers working independently and conflicts were resolved by consensus.
7	Did the review authors provide a list of excluded studies and justify the exclusion?	Yes	A flow diagram was provided with the numbers of studies excluded and the reasons for exclusion.
8	Did the review authors describe the included studies in adequate detail?	Partly	The authors reported the studies identified in the scoping review that evaluated diagnostic tests for humans were fully evaluated in this systematic review. A list of the 48 studies included in the review and the list of licensed tests for Lyme disease as of May 2015 were included in

	AMSTAR 2 TOOL QUESTION[6]	Answer	Comment
			an electronic pdf attachment. Additional information about the studies may be in the scoping review protocol available on request.
9	Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes	The authors used a quality assessment form based on the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. The QUADAS-2 tool assessed the four quality domains with respect to patient selection, the diagnostic tests used, the reference standard and flow and timing of the study. An additional section was added to evaluate comparison tests and capture the presence of funding bias.
10	Did the review authors report on the sources of funding for the studies included in the review?	Unclear	The authors added an additional section to evaluate comparison tests and capture the presence of funding bias. This information may be in the scoping review protocol available on request. The authors did report that 28.6 per cent of studies had authors employed or funded by commercial companies that supplied one or more of the tests evaluated and in four studies the risk of funding bias was identified to be very high. The authors identified which studies these were.
11	If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Yes	The authors stated: Each comparison was extracted, grouped and coded according to tests and type of outcome reported. When there were four or more lines of data for a category, meta-analysis was conducted using hierarchical logistic regression and bivariate models in Stata 13 using Metandi and Midas command packages. These models have been designed to account for the correlation between sensitivity and specificity and they overcome the often violated assumptions of a linear regression model. Meta-analytic statistical summaries of sensitivity, specificity, likelihood ratios and diagnostic odds ratio have been summarised where possible in the tables. Model diagnostics including goodness of fit, normality, influential and outlying points, publication bias and heterogeneity were examined where possible. Publication bias was not evaluated when heterogeneity was >60% or there were less than 10 lines of data. Meta-regression using the bivariate model was used to examine whether predetermined covariates explain some of the

	AMSTAR 2 TOOL QUESTION[6]	Answer	Comment
			between-study variation given there was sufficient data to fit the model (>10 data lines per covariate).
12	If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	
13	Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Yes	In two studies it was apparent that the sample population was not appropriately enrolled in the study as the case population and control population were enrolled at different times and places, which could lead to biased (exaggerated) results for test accuracy.
14	Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes	
15	If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	Yes	
16	Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	The paper reported the authors received no funding for the work and have declared that no competing interests exist.

CASP Checklist for Randomised Controlled Trials

RCTs were appraised for quality using the CASP Randomised Controlled Trials checklist available at CASP Randomised Controlled Trials checklist – https://casp-uk.net/wp-content/uploads/2018/01/CASP-Randomised-Controlled-Trial-Checklist-2018.pdf

Berende, A., ter Hofstede, H. J. M., Vos, F. J., van Middendorp, H. van, Vogelaar, M. L., Tromp, M., van den Hoogen, F. H., Donders, A. R. T., Evers, A. W. M., & Kullberg, B. J. (2016). Randomized trial of longer-term therapy for symptoms attributed to Lyme disease. *The New England Journal of Medicine*, *374*(13), 1209–1220. https://doi.org/10.1056/NEJMoa1505425

CASP Randomised Controlled Trial Standard Checklist	
Section A: Is the basic study design valid for a randomised controlled trial?	
Did the trial address a clearly focussed research question?	Yes - whether longer-term antibiotic treatment of persistent symptoms attributed to Lyme disease leads to better outcomes than does shorter-term treatment.
Was the assignment of patients to treatments randomised?	Yes - randomised, double-blind, placebo-controlled trial. Patients were randomly assigned to one of three groups in a 1:1:1 ratio. Randomisation was computerised and balanced by minimisation for age (<40 or ≥40 years), sex, duration of symptoms (<1 or ≥1 year), and baseline Global Health Composite score of the RAND-36 Health Status Inventory (RAND SF-36).The randomisation list consisted of consecutive medication numbers entered into a secured Web-based database by an independent Web manager.
Were all of the patients who entered the trial properly accounted for at its conclusion?	Yes.
Section B: Was the study methodologically sound?	
Were patients, health workers and study personnel 'blind' to treatment?	Yes - All personnel involved in the study (except the Web manager and study pharmacist) and all participants were unaware of the study-group assignments.
Were the groups similar at the start of the trial?	Yes.
Aside from the experimental intervention, were the groups treated equally?	Yes.

CASP Randomised Controlled Trial Standard Checklist	
How large was the treatment effect?	The SF-36 physical component summary score did not differ significantly among the three study groups at the end of the treatment period, with mean scores of 35.0 (95% confidence interval [CI], 33.5 to 36.5) in the doxycycline group, 35.6 (95% CI, 34.2 to 37.1) in the clarithromycin–hydroxychloroquine group, and 34.8 (95% CI, 33.4 to 36.2) in the placebo group (P = 0.69; a difference of 0.2 [95% CI, –2.4 to 2.8] in the doxycycline group vs. the placebo group and a difference of 0.9 [95% CI, –1.6 to 3.3] in the clarithromycin–hydroxychloroquine group vs. the placebo group); the score also did not differ significantly among the groups at subsequent study visits (P = 0.35). In all study groups (including the placebo), the SF-36 physical-component summary score increased significantly from baseline to the end of the treatment period (P<0.001).
How precise was the estimate of the treatment effect?	95% CI crossed the line of no effect in all cases.
Can the results be applied locally?	Potentially.
Were all clinically important outcomes considered?	Yes.
Are the benefits worth the harms and costs?	Out of 281 randomised patients, four suffered serious adverse events.
CONCLUSIONS: In patients with persistent symptoms attributed to Lyme disease, longer-term antibiotic treatment did not have additional beneficial effects on health-related quality of life beyond those with shorter-term treatment.	

CASP Checklist for Cohort studies

Cohort studies were appraised for quality using the CASP Cohort study checklist available at https://casp-uk.net/wp-content/uploads/2018/03/CASP-Cohort-Study-Checklist-2018_fillable_form.pdf

Nigrovic, L. E., Neville, D. N, Balamuth, F., Bennet, J. E., Levas, M. N., & Garro, A. C. (2019). A minority of children diagnosed with Lyme disease recall a preceding tick bite. Ticks and Tick-borne Diseases, 10(3), 694–696. https://doi.org/10.1016/j.ttbdis.2019.02.015

CASP Checklist for cohort studies	
Section A: Are the results of the study valid?	
Did the study address a clearly focussed issue?	Yes - to determine the proportion of children diagnosed with Lyme disease who had a recognised tick bite and then compared this proportion between children with and without a diagnosis of Lyme disease.
Was the cohort recruited in an acceptable way?	Yes - study staff identified eligible patients and approached parents or legal guardians (patient age 1-17 years) and patients (age 18-21 years) to obtain written informed consent.
Was the exposure accurately measured to minimise bias?	Yes - for enrolled children a clinical phenotype was collected as well as a research biosample. Treating clinicians asked enrolled children and their parents or caregivers whether a tick bite had been recognised in the year prior to ED evaluation (time period selected to maximise recall). For children with a recognised tick bite, the time since the tick bite occurred (in weeks) was determined. Enrolled children were followed up by telephone one month from the time of enrolment.
Was the outcome accurately measured to minimise bias?	Yes - research samples were analysed in a single research laboratory. Children with positive or equivocal C6 EIA tests had an immunoblot performed and interpreted using standardised criteria. Any positive IgG or a positive IgM alone with <30 days of symptoms were classified as a positive immunoblot. A case of Lyme disease was defined as with a physician diagnosed erythema migrans lesion or a positive or equivocal C6 EIA followed by a positive immunoblot.
Have the authors identified all confounding factors?	Unclear. The authors noted several limitations of the study.
Have they taken account of the confounding factors in the design and/or analysis?	Unknown.

CASP Checklist for cohort studies	
Was the follow-up of subjects complete enough and long enough?	No - While the methodology stated enrolled children were followed up by telephone one month later, there was no information in the results or discussion about what the follow-up collected or how many children were actually followed up in the results or discussion.
Section B: What are the results of this study?	
What are the results of this study?	Of 1770 children undergoing emergency department evaluation for Lyme disease, 362 (20.5% per cent) of those with an available tick bite history, only a minority of those with Lyme disease had a recognised tick bite (60/325; 18.5 per cent, 95 per cent confidence interval 14.6-23.0 per cent).
How precise are the results?	The authors used Chi Square test to compare proportions. 95 per cent confidence intervals were provided.
Do you believe the results?	Yes. The authors noted several limitations. A limitation was that Lyme disease tests can be falsely negative early in disease and they did not routinely perform convalescent Lyme disease testing for children with initially negative test results.
Can the results be applied to the local population?	No - the authors noted each participating emergency department was located in a Lyme disease endemic area, and that their findings may not be applicable to regions with lower Lyme disease incidence or the primary care settings.
Do the results of this study fit with other available evidence?	Yes - that patients may not always recall a tick bite.
What are the implications of this study for practice?	This is one study in a Lyme disease endemic area in an emergency department setting. The authors acknowledge their findings may not be applicable to primary care settings.

COREQ Checklist for Qualitative studies

Qualitative studies were appraised for quality using the COREQ (Consolidated criteria for Reporting Qualitative research) Checklist available at http://cdn.elsevier.com/promis_misc/ISSM_COREQ_Checklist.pdf

Ali, A., Vitulano, L., Lee, R., Weiss, T. R., & Colson, E. R. (2014). Experiences of patients identifying with chronic Lyme disease in the healthcare system: A qualitative study. *BMC Family Practice*, *15*(1), 1–17. https://doi.org/10.1186/1471-2296-15-79

Topic	Item No.	Guide Questions/Description		Reported on Page No.
Domain 1: Research team and reflexivity				
Personal characteristics				
Interviewer/facilitator	1	Which author/s conducted the interview or focus group?	Lead author (A. Ali) and second author (L. Vitulano)	2
Credentials	2	What were the researcher's credentials?	Author details provided were: Lead author: Department of Pediatrics, Yale School of Medicine, USA. Second author: Child Study Center, Yale School of Medicine, USA.	8
Occupation	3	What was their occupation at the time of the study?	Department of Pediatrics, Yale School of Medicine	8
Gender	4	Was the researcher male or female?	Male	
Experience and training	5	What experience or training did the researcher have?	Lead author - CAM-trained research scientist. Second author (medical student)	2
Relationship with participants				
Relationship established	6	Was a relationship established prior to study commencement?	Participants were not known to the investigators prior the study.	2

Topic	Item No.	Guide Questions/Description		Reported on Page No.
Participant knowledge of the interviewer	7	What did the participants know about the researchers? E.g. personal goals, reasons for doing the research	None provided. Subjects were told that the aim of the study was to gain insights into the experiences of patients with CLD and that this information will be used to develop interventions to improve patient care and satisfaction	2
Interviewer characteristics	8	What characteristics were reported about the interviewer/facilitator? E.g. Bias, assumptions, reasons, and interests in the research topic	The interviewer was a Complementary and alternative medicines (CAM)-trained interviewer in the Department of Pediatrics. In the discussion, the authors commented that it is possible that patients were more open to discussing CAM in a study led by a CAM-trained investigator.	2, 7
Domain 2: Study design				
Theoretical framework				
Methodological orientation and Theory	9	What methodological orientation was stated to underpin the study? E.g. grounded theory, discourse analysis, ethnography, phenomenology, content analysis	The authors used a hermeneutic, phenomenological methodology; the focus of inquiry was placed on the patients' lived experience with chronic Lyme disease.	2
Participant selection				
Sampling	10	How were participants selected? E.g. purposive, convenience, consecutive, snowball	Purposive sampling. Enrollment continued until theoretical saturation was obtained; i.e., the point at which no new concepts emerged in a category, and in which categories were well characterised and differentiated.	2

Topic	Item No.	Guide Questions/Description		Reported on Page No.
Method of approach	11	How were the participants approached? E.g. face-to-face, telephone, mail, email	Purposive sampling was used to recruit participants from Connecticut-based patient-oriented Lyme disease email lists and the website, craigslist.org. Recruitment announcements solicited participants with CLD (diagnosed by a clinician or by self-diagnosis) and were willing to complete an in-person interview.	2
Sample size	12	How many participants were in the study?	Twelve adults.	1
Non-participation	13	How many people refused to participate or dropped out? Reasons?	The authors did not say. Sampling was purposive and continued until theoretical saturation. Prior to each interview written informed consent was obtained.	2
Setting				
Setting of data collection	14	Where was the data collected? E.g. home, clinic, workplace	Interviews took place either at Yale University (New Haven, CT) or at participants' place of residence.	2
Presence of non-participants	15	Was anyone else present besides the participants and researchers?	The authors did not comment on this.	
Description of sample	16	What are the important characteristics of the sample?	All participants were Caucasian with a mean age of 41 years (range 21–69). Seven participants were college graduates, and eleven participants had health insurance. Of the eleven insured, CLD treatments were partially or fully covered for eight participants.	3
Data collection				

Topic	Item No.	Guide Questions/Description		Reported on Page No.
Interview guide	17	Were questions, prompts, guides provided by the authors? Was it pilot tested?	An interview guide was created based on the Health Belief Model dimensions of perceived susceptibility, severity, benefits, and barriers, as well as mediating factors such as cues to action and self-efficacy. The interview guide was provided as Table 1.	2,3
Repeat interviews	18	Were repeat interviews carried out? If yes, how many?	Interviews were conducted with twelve participants; one additional interview was conducted to corroborate findings and assess saturation.	2
Audio/visual recording	19	Did the research use audio or visual recording to collect the data?	Interviews were recorded digitally and transcribed by a HIPAA-compliant service (Transcription Plus LLC, Bristol, CT).	2
Field notes	20	Were field notes made during and/or after the interview or focus group?	Not addressed by the authors.	
Duration	21	What was the duration of the interviews or focus groups?	Interviews lasted between 60-90 minutes.	2
Data saturation	22	Was data saturation discussed?	Yes. Enrollment continued until theoretical saturation was obtained; i.e., the point at which no new concepts emerged in a category, and in which categories were well characterised and differentiated.	2
Transcripts returned	23	Were transcripts returned to participants for comment and/or correction?	Participants were not involved in the data analysis and interpretation.	3
Domain 3: analysis and findings				
Data analysis				

Topic	Item No.	Guide Questions/Description		Reported on Page No.
Number of data coders	24	How many data coders coded the data?	One investigator (AA).	3
Description of the coding tree	25	Did authors provide a description of the coding tree?	Codes were assigned to specific statements in each transcript, in five categories: 1) beliefs/understanding, 2) personal history/narrative, 3) consequences/limitations, 4) management, and 5) influences on care.	3
Derivation of themes	26	Were themes identified in advance or derived from the data?	Transcripts were analysed using standard methods of content analysis. After completing the first three interviews, transcripts were read by the multidisciplinary investigative team for an overall understanding to identify emergent themes. After subsequent interviews, and during the iterative process of reviewing each transcript as it is collected, the list of themes was revised multiple times. Codes were assigned to specific statements in each transcript, in five categories: 1) beliefs/understanding, 2) personal history/narrative, 3) consequences/limitations, 4) management, and 5) influences on care. Themes were then condensed from these categories and coded.	3
Software	27	What software, if applicable, was used to manage the data?	Data from all of the transcripts was coded using ATLAS.ti 6.1 (ATLAS.ti Scientific Software Development GmbH, Berlin).	3
Participant checking	28	Did participants provide feedback on the findings?	No.	

Topic	Item No.	Guide Questions/Description		Reported on Page No.
Reporting				
Quotations presented	29	Were participant quotations presented to illustrate the themes/findings? Was each quotation identified? E.g. participant number	Yes. Quotations were identified by participant number.	4-6
Data and findings consistent	30	Was there consistency between the data presented and the findings?	Yes.	3-7
Clarity of major themes	31	Were major themes clearly presented in the findings?	Yes. Four major themes were identified and then discussed.	3-6

APPENDIX C: AGREE II

The clinical guidelines included in this literature review were assessed using AGREE II tool and user's manual, where appropriate. The guidance on AGREE II is available at https://www.agreetrust.org/wp-content/uploads/2017/12/AGREE-II-Users-Manual-and-23-item-Instrument-2009-Update-2017.pdf.

AGREE II Domains	IDSA/AAN/ACR* (2019 draft)	NICE (2018)	ILADS (2014)	Olde Hartman et al. (2013)	DBG (2010)	IDSA^ (2006)
Scope and Purpose	100%	100%	83%	100%	33%	83%
Stakeholder involvement	83%	100%	53%	69%	61%	47%
Rigor of development	95%	100%	46%	46%	33%	50%
Clarity of presentation	94%	100%	28%	89%	22%	86%
Applicability	23%	100%	21%	46%	42%	23%
Editorial independence	79%	100%	58%	100%	25%	79%
Overall quality	6	7	3.5	6	2	5

*The IDSA/AAN/ACR draft Lyme disease clinical practice guidelines (Lantos et al., 2019) are draft. The draft guideline noted that external peer-review was pending.

^The IDSA (2006) Lyme disease guidelines (Wormser et al., 2006) have been archived by IDSA while the 2019 IDSA/AAN/ACR Lyme disease guidelines are being finalised.

We did not appraise the quality of three Australian guidelines included in this literature review (Communicable Diseases Network Australia, 2018; Lum et al., 2015; Therapeutic Guidelines, n.d.). All three guidelines are in current use within medical and diagnostic practice in Australia.

The CDNA guidelines are endorsed by CDNA, the Australian Health Protection Principal Committee (AHPPC) and released by the Department of Health. Their quality for inclusion in the DSCATT Clinical Pathway is not questioned in this literature review.

Therapeutic Guidelines advise the following regarding their guidelines and the use of their guidelines in Australia (https://www.tg.org.au/the-organisation/):

- Therapeutic Guidelines Limited (TGL) is an independent not-for-profit organisation. Its reputation is staked not only on its publications but also on its independence of government and commercial interests.
- They [the guidelines] are based on the latest international literature, interpreted by some of Australia's most eminent and respected experts, with input from an extensive network of GPs and other users.
- Therapeutic Guidelines are widely respected and are an accepted part of the Australian medical culture. They are used in all Australian medical and pharmacy schools, and are used extensively in public teaching hospitals and in community medical and pharmacy practices.
- Therapeutic Guidelines are endorsed by NPS MedicineWise, The Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, The Society of Hospital Pharmacists of Australia, and the International Society of Drug Bulletins. Individual titles are also endorsed by relevant specialty societies, colleges and peak bodies.

The Australian guideline on the diagnosis of overseas acquired Lyme disease/borreliosis (Lum et al. (2015)) was not appraised using AGREE II as it is not a clinical guideline and is the current Australian Department of Health guidance on diagnosis of Lyme disease in Australia.

APPENDIX D: AACODS GREY LITERATURE APPRAISALS

The grey literature included in this literature review was appraised using the AACODS checklist.
In preparing this checklist, Flinders Academic Commons' AACODS checklist was used. It can be accessed via https://dspace.flinders.edu.au/xmlui/bitstream/handle/2328/3326/AACODS_Checklist.pdf;jsessionid=F3E682274BB2C27D28BDA5BE08FBDFB8?sequence=4.

Name of document	Authority	Accuracy	Coverage	Objectivity	Date	Significance	*Allen + Clarke's* overall quality rating
Senate Community Affairs References Committee. (2016a). *Growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australian patients. Interim report* [Interim Report]. Commonwealth of Australia. https://www.aph.gov.au/Parliamentary_Business/Committees/Senate/Community_Affairs/Lyme-like_Illness/Interim_Report	YES Reputable organisation: the Senate. Report prepared by a Secretariat. Report published by Commonwealth of Australia. Not an authority in the field, but this is an inquiry into an emerging illness. No reference list or bibliography – submissions where highlighted are referenced as footnotes. All submissions were published on the Government website.	YES Clearly stated Terms of Reference. Supported by submissions from patients (the majority), advocacy groups, medical professionals, medical professional bodies, government authorities and links to websites. Also supported by expert opinion to Committee Hansard. Patient submissions are of self-reported experience. Advocacy groups had provided templates for	YES Content coverage set in Terms of Reference. Not all submissions, particularly from individual patients, were highlighted in report. All submissions were published.	YES Objective reporting of submissions expressing different points of view. The Committee provided a 'Committee View'.	YES Clearly dated.	YES Very relevant. In 2015 the Senate referred the matter to the Committee. This is the first public submission process and inquiry to gather information on the growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australians. The Committee made three recommendations, including to continue the inquiry in the 45th Parliament.	HIGH An important and relevant report from an official inquiry by the Senate. The reliability of the evidence about DSCATT is low, as most of the submissions were from patients with the information being self-reported.

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		patients to use. Medical professional body submissions are expert opinion with/without supporting scientific evidence. Government submission supported by evidence.					
Senate Community Affairs References Committee. (2016b). *Growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australian patients. Final report* [Final Report]. Commonwealth of Australia. https://www.aph.gov.au/Parliamentary_Business/Committees/Senate/Community_Affairs/Lymelikeillness45/Final_Report	YES Reputable organisation: theSenate. Report prepared by a Secretariat. Report published by Commonwealth of Australia. Not an authority in the field, but this is an inquiry into an emerging illness and this report summarises the evidence presented to the Committee.	YES Clearly stated Terms of Reference. Supported by submissions from patients (the majority), advocacy groups, medical professionals, medical professional bodies, government authorities and links to websites. Also supported by expert opinion to Committee	YES Content coverage set in Terms of Reference.	YES Objective reporting of submissions and the recommendations of the Committee are based on a synthesis of the evidence submitted during the Inquiry.	YES Clearly dated.	YES Very relevant and identifies gaps in current knowledge and areas where further investigation and research is required.	HIGH An important and relevant report from an official inquiry by the Senate.

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		Hansard.					
TMS Consulting Pty Ltd. (2018a). *Forum to consider the outcomes of the Australian Government's response to the Senate Community Affairs References Committee final report: Inquiry into the growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australians.* Department of Health. https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/DSCATT-Forum-Melbourne-18April%202018-Final-Report.pdf	YES Reputable Organisation recording the discussion/ consultation of DSCATT with patients.	YES The document provides a record a forum for State and territory health authorities, representatives from medical colleges and patient groups. It includes copies of government policy statements, and clinical perspectives.	YES The report records the discussion of current clinical and policy matters relating to DSCATT.	YES A useful statement of government, health professional and advocacy group positions.	YES Clearly dated.	YES Very useful resource.	HIGH An important and relevant report to inform the DSCATT Clinical Pathway.
TMS Consulting Pty Ltd. (2018b). *Patient group forum: Debilitating Symptom Complexes Attributed to Ticks*	YES Reputable organisation recording the discussion/	YES The document provides a record of a forum to hear patient groups. It	YEES Not intended to cover all aspects of DSCATT as this report focuses on	YES A valuable resource intended to record patient views being sought by	YES Clearly dated.	YES Very useful resource that reports lived experience of	HIGH An important and relevant report.

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(DSCATT). Department of Health. https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/DSCATT-Syd-Forum-report-27July2018.pdf	consultation of DSCATT with patients.	includes government policy statements, qualitative patient narratives, and summary of workshop comments.	lived experience and personal stories of living with Lyme disease.	government agencies. Patient views are self-reported.		people with DSCATT.	The qualitative evidence about DSCATT is useful as it describes patient concerns however, the evidence is self-reported.
Department of Health. (2018a). *Position statement: Debilitating Symptom Complexes Attributed to Ticks*. Department of Health. http://www.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/Posit-State-Debilitating-Symptom-Complexes-Attributed-Ticks-June18.pdf	YES Reputable organisation.	N/A Position Statement is succinct.	N/A Position Statement is succinct.	N/A Position Statement states government actions.	YES Dated 2018.	YES Statement adds context and clearly states actions the government is taking to raise awareness of DSCATT.	HIGH An important and relevant statement from the Australian Government Department of Health.
Department of Health. (2018b). *Position statement: Lyme disease in Australia*. Department of Health. http://www.health.gov.au/int	YES Reputable organisation.	N/A Position Statement is succinct.	N/A Position Statement is succinct.	N/A Position Statement states government actions.	YES Dated 2018.	YES Statement adds context and clearly states actions the government is taking to fund	HIGH An important and relevant statement from the Australian Government

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ernet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/Posit-State-Lyme-June18.pdf						research on Lyme disease in Australia.	Department of Health.
Department of Health. (2018c). *Release of the National Serology Reference laboratory report: Investigation of the performance of assays for Lyme disease in Australia. Questions and answers*. Department of Health. https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/NRL-QA-2018.pdf	YES Reputable organisation.	N/A Q&A document is succinct.	N/A Q&A document is succinct.	N/A Q&A document states why NRL was asked to undertake the investigation.	? Not dated. The file name is dated as 2018.	YES Document adds context to the Department-commissioned investigation.	HIGH Report adds context to the investigation into how Australian laboratories would perform when diagnosing Lyme disease.
Allen + Clarke. (2019). *DSCATT Think Tank summary report* (p. 45). Department of Health. https://www1.health.gov.au/internet/main/publishing.nsf/Content/4594AB5B9B2A90D4CA257BF0001A8D43/$File/DSCATT-Think-Tank-2019.pdf	YES Reputable organisation. Commissioned by Department of Health. A reference list/bibliography was not required for this report.	YES Clearly stated purpose of the Think Tank. This report records the key discussion points and outcomes on DSCATT at the Think Tank. The	YES The participants at the Think Tank included patient groups, State and Territory government officials and medical professional	YES The findings of the Think Tank were reported objectively but were based on self-reported and anecdotal information from patient groups and medical opinion.	YES Clearly dated.	YES Useful resource that included stakeholder input on key areas of the DSCATT Draft Clinical Pathway.	HIGH Think Tank Report acknowledges stakeholder input into key discussion areas of relevance to the development of the DSCATT Clinical Pathway. Informed the

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		information was provided by patients, patient advocacy groups and medical professionals who attended the Think Tank.	groups. Not all who were invited attended.				development of the Draft DSCATT Clinical Pathway.
National Serology Reference Laboratory Australia. (2017). *Final report: Investigation into the performance of assays for Lyme disease in Australia.* National Serology Reference Laboratory. https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/NRL-2017.pdf	YES Highly reputable, globally recognised expert and commissioned by Department of Health.	YES Clearly stated purpose and method of the investigation.	YES Eight institutions provided serum samples; four in Australia and four overseas.	YES NRL is recognised as an independent laboratory in Australia and its independence was a key consideration in its selection as the laboratory to undertake the project. Objective report.	YES Clearly dated.	YES A highly significant report that concluded there was nothing to suggest that testing performed by NATA/RCPA accredited medical testing laboratories in Australia is not of good quality. This report addressed concerns raised by some submitters to the Senate Inquiry.	HIGH Report adds context and assurance of the performance of assays for diagnosing Lyme disease in Australian NATA/RCPA accredited laboratories. Provided the follow up service.
Mackenzie, J. S. (2013). *Scoping study to develop a research project(s) to investigate the presence or*	YES Reputable individual author with recognised	YES Clearly stated Terms of Reference supported by	YES This report identified research needs for an	YES The report provided an objective review	YES Clearly dated.	YES Valuable resource that was consistently	HIGH Report adds context. It was the result of extensive

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absence of Lyme disease in Australia [Final Report]. Department of Health. https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/scoping-study-2013.pdf	expertise and published by a reputable organisation. Detailed list of references.	consultation with the Chief Medical Officer's Clinical Advisory Committee on Lyme disease, Lyme disease and Borrelia experts, and researchers conducting or considering research projects that examine tick-borne disease in Australia at the time of this report.	investigation into whether a causative tick-borne microorganism (Borrelia) for Lyme disease exists in Australia.	of the evidence and research projects.		referenced/ mentioned by key stakeholders. Relatively recent scoping paper that identified research needs for whether Lyme disease exists in Australia.	research backing and relevant stakeholder consultation.
Brunton, G., Sutcliffe, K., Hinds, K., Khatwa, M., Burchett, H., Dickson, K., Rees, R., Rojas-Garcia, A., Stokes, G., Harden, M., Stansfield, C., Sowden, A., & Thomas, J. (2017). *Stakeholder experiences of the diagnosis of Lyme disease: A systematic review* (p. 75). Department of Health Reviews Facility. https://researchonline.lshtm.ac.uk/id/eprint/4656944/1/Ly	YES The authors are from universities in the UK with lead authors from University College, London. Report was commissioned by the Policy Research Programme in the Department of Health UK.	YES This report is a systematic review. There are clear aims and review questions, a detailed methodology including inclusion criteria, data extraction, quality appraisal and synthesis. A	YES There were specific inclusion criteria. Relevant studies were sought from within a systematic evidence map covering the whole range of research evidence on Lyme disease in humans published in or since 2002,	YES Strengths and limitations of the literature were discussed.	YES Clearly dated. December 2017.	YES The authors noted the review is the first to systematically identify and assess the evidence of patients' and clinicians' experiences of diagnosis of Lyme disease. The aim of the work was to	HIGH An important systematic review produced for the Department of Health UK that found even in Lyme disease endemic areas clinicians find it challenging to diagnose Lyme disease accurately.

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me%20disease%20stakeholder%20experiences%202017%20Brunton.pdf	Detailed reference list provided.	scientific advisory group of academics and clinicians with expertise in Lyme disease provided advice as needed on technical issues.	produced in the first phase of the project.			help understand the issues that may help or hinder the diagnosis of Lyme disease in real-world clinical settings.	
General Practice Mental Health Standards Collaboration. (2019). *Working with the stepped care model: Mental health services through general practice*. Royal Australian College of General Practitioners. https://gpmhsc.org.au/getmedia/a3c419ef-68e9-4c32-b78f-f97b16d06541/Working-with-the-stepped-care-model.pdf.aspx	YES Reputable organisations and collaborations.	YES Clearly stated aims for the guide and intended audience in the Executive Summary.	YES Expressed limitations on when stepped care services may not be appropriate.	YES Balanced guide through a collection of peak bodies.	YES Clearly dated.	YES Valuable resource that adds context on how peak bodies encourage communication between GPs and PHNs, and promotion of shared-care decision making for carers and consumers.	HIGH Guide adds context. Illustrates how peak health bodies envisage shared-care and a Stepped Care Model.
Australian Commission on Safety and Quality in Health Care. (n.d.). *FAQs about partnering with consumers in the NSQHS Standards (second edition)*.	YES Reputable organisation.	N/A FAQ webpage is succinct.	N/A FAQ webpage is succinct.	N/A FAQ webpage explained the Partnering with Consumers Standard and the	NO Not dated.	YES Adds context on how to partner with consumers in NSQHS Standards.	HIGH FAQ webpage adds context. Government body providing reputable information around

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https://www.safetyandquality.gov.au/faqs-about-partnering-consumers-nsqhs-standards-second-edition#what-is-person-centred-care?				changes made to the Partnering with Consumers Standard since the first edition of the NSQHS Standards.			Partnering with Consumers Standard and changes accompanying the second edition of the NSQHS Standards.
Australian Commission on Safety and Quality in Health Care. (2018). *Fact sheet 1: Person-centred organisations: Achieving great person-centred care*. Australian Commission on Safety and Quality in Health Care. https://www.safetyandquality.gov.au/sites/default/files/2019-06/fact-sheet-1-achieving-great-person-centred-care.pdf	YES Reputable organisation.	N/A Fact sheet is succinct.	N/A Fact sheet is succinct.	N/A Fact sheet provided a summary of person-centred care information.	? Fact sheet is not dated. The webpage that the fact sheet is located on has the published date of 2018.	HIGH Adds context on what a NSQHS Standards' person-centred care means and looks like.	HIGH Fact sheet adds context. Government body providing reputable information around person-centred care.
Australian Rickettsial Reference Laboratory. (n.d.). *Tests performed at the ARRL*. Australian Rickettsial Reference Laboratory. https://www.rickettsialab.org.au/tests-performed	YES Reputable organisation.	N/A Information on tests performed at the ARRL is succinct.	N/A Information on tests performed at the ARRL is succinct.	N/A Webpage provided information on tests performed at the ARRL.	NO Not dated.	YES Adds context of the tests that are currently being performed in an Australian laboratory.	HIGH Webpage adds context on the types of tests that are being offered in the Australian Rickettsial

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							Reference Laboratory.
Cancer Council Australia. (2015). *National Cancer Council Control Policy: Position statement—Complementary and alternative therapies*. https://wiki.cancer.org.au/policy/Position_statement_-_Complementary_and_alternative_therapies	YES Reputable organisation. Detailed reference list.	N/A Position Statement is succinct.	N/A Position Statement is succinct.	N/A Position Statement states organisation's stance on complementary and alternative therapies.	? Not dated. The webpage's date was located in the 'History' tab.	YES Adds context on a reputable organisation's stance on complementary and alternative therapies.	HIGH Position statement adds context around complementary and alternative therapies.
Centers for Disease Control and Prevention. (n.d.). *Lyme Disease | 2017 Case Definition*. Centers for Disease Control and Prevention. https://wwwn.cdc.gov/nndss/conditions/lyme-disease/case-definition/2017/#:~:text=A%20systemic%2C%20tick%2Dborne%20disease,60%25%2D80%25%20of%20patients.	YES Reputable organisation.	N/A Case definition is succinct.	N/A Case definition is succinct.	N/A 2017 case definition states its purpose and intended use.	? 2017 case definition but the publication date is not present on the webpage.	YES Webpage provides a recent definition of Lyme disease by a reputable government body.	HIGH Adds context by providing a clear case definition of Lyme disease by a reputable government body.
Centers for Disease Control and Prevention. (2018, December 21). *Laboratory tests not recommended for*	YES Reputable organisation.	N/A List of laboratory tests that are not	N/A List of laboratory tests that are not	N/A List of laboratory tests lists examples of unvalidated	YES Clearly dated.	YES Webpage provides a recent list of examples of	HIGH Adds context of the laboratory test for Lyme disease not

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Lyme disease. Centers for Disease Control and Prevention. https://www.cdc.gov/lyme/diagnosistesting/labtest/otherlab/index.html		recommended is succinct.	recommended is succinct.	laboratory tests for Lyme disease.		unvalidated laboratory tests for Lyme disease by a reputable government body.	recommended by a reputable government body.
Centers for Disease Control and Prevention. (2019a, November 8). *HHS federal research updates on Lyme disease diagnostics | Lyme Disease*. https://www.cdc.gov/lyme/diagnosistesting/HHS-research-updates.html	YES Reputable organisation.	N/A HHS federal research updates is extensive.	N/A HHS federal research updates is extensive.	N/A HHS federal research updates on Lyme disease diagnostics.	YES Clearly dated.	YES Video on webpage provides an extensive update on HHS federal research of Lyme disease diagnostics.	HIGH Adds context on the most recent update available by this reputable government body on HHS federal research for Lyme disease diagnostics.
Centers for Disease Control and Prevention. (2019b, November 8). *Post-Treatment Lyme Disease Syndrome*. Centers for Disease Control and Prevention. https://www.cdc.gov/lyme/postlds/index.html	YES Reputable organisation.	N/A Information page is succinct.	N/A Information page is succinct.	N/A Post-Treatment Lyme Disease Syndrome (PTLDS) described by a reputable organisation.	YES Clearly dated.	YES Webpage provides recent and reputable information on PTLDS.	HIGH Adds context on what PTLDS is by a reputable government body.
Centers for Disease Control and Prevention. (2019c, November 20). *Diagnosis and testing of Lyme disease*. Centers for Disease Control	YES Reputable organisation.	N/A Information page is succinct.	N/A Information page is succinct.	N/A Lyme disease diagnosis and testing information	YES Clearly dated.	YES Recent and reputable information of Lyme disease	HIGH Adds context on Lyme disease diagnosis and testing information

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and Prevention. https://www.cdc.gov/lyme/diagnosistesting/index.html				by a reputable organisation.		diagnosis and testing.	by a reputable government body.
Centers for Disease Control and Prevention. (2020, September 25). *Lyme disease treatment*. Centers for Disease Control and Prevention. https://www.cdc.gov/lyme/treatment/index.html	YES Reputable organisation.	N/A Information page is succinct.	N/A Information page is succinct.	N/A Lyme disease treatment information by age category, drug, dosage, maximum doses and duration of dosage.	YES Clearly dated.	YES Recent and reputable information on Lyme disease treatment.	HIGH Adds context on Lyme disease treatment, as provided by a reputable government body.
Chitnis, A., Dowrick, C., Byng, R., Turner, P., & Shiers, D. (2011). *Guidance for health professionals on medically unexplained symptoms (MUS)*. Forum for Mental Health in Primary Care. https://dxrevisionwatch.files.wordpress.com/2013/06/guidance-for-health-professionals-on-mus-jan-2011.pdf	YES Collaboration of reputable organisations. Detailed references list.	YES Document driven by clear key learning points.	YES Clear guidance document for health professionals on MUS.	YES Clear factual display of information.	YES Clearly dated.	YES Reputable and clear source of information for health professionals on MUS.	HIGH Adds context on MUS and how peak medical organisations have provided guidance to health professionals.
Choosing Wisely Australia. (n.d.). *Antibiotic resources for clinicians*. Choosing Wisely Australia. https://www.choosingwisely.org.au/resources/health-	YES Reputable body.	YES Webpage provided factual resources to clinicians and their patients.	YES Clear overview of antibiotic resources for clinicians and their patients.	YES Factual and objective language on the list of	NO Not dated.	YES Reputable body providing credible resources on antibiotics.	HIGH Adds context on guidelines, continuing professional

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professionals/antibiotic-resources-for-clinicians				antibiotic resources.			development (CPD) and tools, calculators and apps on antibiotics.
Department of Health. (2019). *PHN Primary Mental Health Care Flexible Funding Pool Implementation Guidance – Stepped Care*. Department of Health. https://www1.health.gov.au/internet/main/publishing.nsf/Content/2126B045A8DA90FDCA257F6500018260/$File/1.%20PHN%20Guidance%20-%20Stepped%20Care%20-%202019.pdf	YES Reputable organisation.	YES Clearly stated document purpose; provides a foundation to support other PHN mental health guidance documents relating to the Primary Mental Health Care Flexible Funding Pool.	YES Clearly stated document purpose and its parameters.	YES Factual language where opinion of authors are not clear from the document.	YES Clearly dated.	YES Recent and credible information on stepped care through the Australian Primary Mental Health Care Flexible Funding Pool.	HIGH Adds context on primary mental health care funding that is recent and in the Australian setting.
Department of Health. (2020, August 17). *Debilitating Symptom Complexes Attributed to Ticks (DSCATT)*. https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm	YES Reputable organisation.	YES DSCATT information webpage by a relevant government department.	YES DSCATT information webpage by a relevant government department.	YES DSCATT information webpage by a relevant government department.	YES Clearly dated.	YES Recent and credible information on DSCATT.	HIGH Adds context and supported by recent and credible information on DSCATT.
Graves, S. (n.d.). *Update on Australian Rickettsial*	YES	N/A	N/A	N/A	NO Not dated.	YES	HIGH

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Infections. https://www.asid.net.au/documents/item/415	Expert and reputable author.	Presentation slides on an update of Australian Rickettsial infections.	Presentation slides on an update of Australian Rickettsial infections.	Presentation slides on an update of Australian Rickettsial infections by an expert in the field.		Overview on Australian Rickettsial infections.	Adds context on Australian Rickettsial infections by an expert in the area.
House of Representatives Standing Committee on Health. (2016). *Inquiry into Chronic Disease Prevention and Management in Primary Health Care*. Commonwealth of Australia. https://www.aph.gov.au/Parliamentary_Business/Committees/House/Health/Chronic_Disease/Report	YES Reputable organisation.	YES Clear Terms of Reference.	YES Clear Terms of Reference.	YES Clear Terms of Reference, overview of Committee Membership, and summary of the Inquiry's objective and scope.	YES Clearly dated.	YES Recent report on the findings of the Inquiry into chronic disease prevention and management in primary health care.	HIGH Adds context on the findings of the most recent Australian parliamentary inquiry into chronic disease prevention and management in primary health care.
Lyme Disease Association of Australia. (2012). *Lyme disease: Australian patient experience in 2012*. Lyme Disease Association of Australia. https://www.lymedisease.org.au/wp-content/uploads/2012/11/lda-lyme-disease-_australian-	NO Patient advocacy organisation. Reference list provided.	NO The report presents the findings of an online survey accessed through LDAA website. The findings are based on self-reported information from patients who	? The survey was promoted by LDAA on its News page and through its emailing lists and links posted on its Facebook page. It was limited to those who could access it online.	NO Biased language and author's standpoint is clear through the report.	YES Clearly dated. 2012.	? Overview of Australian patient experience with Lyme disease, especially on treatment for Lyme disease although findings are self-reported through a survey and from an	LOW While it provides findings about the patient experience of Lyme disease in Australia, the findings are self-reported and at high risk of bias.

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patient-experience-in-2012-22nov12.pdf		participated in the survey.				advocacy group, therefore not representative.	
Marzec, N. S., Nelson, C., Waldron, P. R., Blackburn, B. G., Hosain, S., Greenhow, T., Green, G. M., Lomen-Hoerth, C., Golden, M., & Mead, P. S. (2017). Serious bacterial infections acquired during treatment of patients given a diagnosis of chronic Lyme disease – United States (MMWR Morb Mortal Wkly Rep, pp. 607–609). Centres for Disease Control and Prevention. https://doi.org/10.15585/mmwr.mm6623a3	YES Reputable organisation. The report is a MMWR published by the CDC. Reference list provided.	YES The report describes five cases to illustrate complications resulting from unproven treatments for 'chronic Lyme disease'.	YES An important Morbidity and Mortality Report from the CDC on unproven treatments for Lyme disease. Cited on the CDC website.	YES Reports of serious bacterial infections acquired during treatment of patients given a diagnosis of 'chronic Lyme disease'. Report is published by the CDC.	YES Clearly dated.	YES An important report with implications for public health practice from the CDC. That is, clinicians, public health practitioners and patients should be aware that treatment for 'chronic Lyme disease' lack proof of effectiveness and can result in serious complications.	HIGH An important report with implications for public health practice from the CDC. That is, clinicians, public health practitioners and patients should be aware that treatment for 'chronic Lyme disease' lack proof of effectiveness and can result in serious complications.
Mead, P., Petersen, J., & Hinckley, A. (2019). *Updated CDC recommendation for Serologic Diagnosis of Lyme disease.* Morbidity and Mortality Weekly Report, 68(32), 703.	YES Reputable organisation. The report is a MMWR published by the CDC. Reference list provided.	YES An official updated CDC recommendation for serologic diagnosis of Lyme disease.	YES An important Morbidity and Mortality Report from the CDC that has public health implications.	YES Updated recommendations from CDC based on the FDAhaving cleared several Lyme disease serologic assays	YES Clearly dated.	YES Internationally significant update from the CDC about serologic diagnosis of Lyme disease with indications for	HIGH Internationally significant update from the CDC about serologic diagnosis of Lyme disease with indications for

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https://doi.org/10.15585/mmwr.mm6832a4				with new indications for us.		public health practice.	public health practice.
National Association of Testing Authority, Australia. (n.d.). *About accreditation*. https://www.nata.com.au/about-nata/about-accreditation	YES Highly reputable organisation.	N/A Information on accreditation.	N/A Information on accreditation.	N/A Information on accreditation by the relevant Australian authority.	LOW Not dated.	YES Clear information on accreditation from the relevant Australian authority.	HIGH Adds context around accreditation by the relevant authority.
National Health and Medical Research Council. (2014). *Talking with your patients about Complementary Medicine—A Resource for Clinicians*. National Health and Medical Research Council. https://www.nhmrc.gov.au/about-us/publications/talking-your-patients-about-complementary-medicine-resource-clinicians	YES Reputable organisation.	YES Government document to guide clinicians on their discussions with patients about complementary medicine (CM).	YES Clear intended use of this document; intended to help clinicians to have collaborative and patient-centred discussions about CM use.	YES Government document to guide clinicians on their discussions with patients about complementary medicine.	YES Clearly dated.	YES Guidance provided to clinicians on their discussions with patients about complementary medicine.	HIGH Adds context on Australian's recent government recommendations on how clinics could approach conversations on CM with their patients.
Public Health England. (2018, July 31). *Lyme disease: Differential diagnosis*. GOV.UK. https://www.gov.uk/guidance	YES Reputable organisation.	YES Clear government information on Lyme disease's	YES Webpage clearly states that its purpose is to provide	YES Government information on Lyme disease's	YES Clearly dated.	YES Clear information on Lyme disease diagnosis, including skin rashes,	HIGH Adds context from a reputable and recent government source on Lyme

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/lyme-disease-differential-diagnosis		differential diagnosis.	"information to assist with differentiating Lyme disease from other causes of rash, neurological or non-specific symptoms".	differential diagnosis.		neurological symptoms, persistent non-specific systemic symptoms and other infections associated with tick bites.	disease differential diagnosis.
Public Health Laboratory Network. (2017). *Q Fever laboratory case definition (LCD)*. Department of Health. https://www1.health.gov.au/internet/main/publishing.nsf/Content/D731BDA5ED9E3038CA257BF0001D3C83/$File/Q-Fever-LCD-27-Nov-2017.pdf	YES Reputable body. Detailed references list.	YES Clear laboratory case definition (LCD) for Q Fever.	YES Clear LCD for Q Fever.	YES Clear and factual LCD for Q Fever.	YES Clearly dated.	YES Clear LCD for Q Fever by a reputable body.	HIGH Adds context with a recent, clear and factual LCD for Q Fever.
Royal Australasian College of Physicians. (2002). *Chronic fatigue syndrome: Clinical practice guidelines—2002* (p. 40). Royal Australasian College of Physicians.	YES Reputable organisation. Detailed references list.	YES Clear guideline development summary.	YES Clear guideline development summary.	YES Clear guideline development outline, and literature review and evidence ratings.	YES Clearly dated.	YES Clinical guidelines on chronic fatigue syndrome by an Australian peak health body.	HIGH Clinical guidelines on chronic fatigue syndrome by an Australian peak health body, but dated in 2002.
Royal Australian College of General Practitioners. (2016). *IM16 Integrative medicine contextual unit*.	YES Reputable organisation.	YES	YES	YES Clear and factual information and	YES Located document	HIGH Information on incorporating the	YES Adds context on how peak health

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https://www.racgp.org.au/download/Documents/Curriculum/2016/IM16-Integrative-medicine.pdf	Detailed references list.	Clear information on integrative medicine.	Clear information on integrative medicine.	language used to discuss integrative medicine.	date from the organisation's website.	use of evidence-based, safe and ethical integrative therapies with conventional medicine.	body recently recommended integrative medicine practice.
Royal College of General Practitioners. (2020). *Lyme Disease Toolkit*. https://www.rcgp.org.uk/clinical-and-research/resources/toolkits/lyme-disease-toolkit.aspx#:~:text=Clinicians%20should%20be%20aware%20of,and%20other%20health%20care%20professionals	YES Reputable organisation.	YES Clear overview of Lyme disease.	YES Succinct toolkit for Lyme disease.	YES Clear and factual information on Lyme disease.	? Date is not clearly stated.	YES Factual information on Lyme disease by a reputable peak health body.	HIGH Adds context on Lyme disease with comprehensive information on its key facts, diagnosing, testing, treating, persistent symptoms and misdiagnosis, resources and support.
Royal College of Pathologists of Australasia. (n.d.). *Lab accreditation*. Royal College of Pathologists of Australasia. https://www.rcpa.edu.au/Patients/Lab-Accreditation#:%E2%89%88:text=All%20pathology%20laboratories%20in%20Australia,Ac	YES Reputable organisation.	N/A Clear information on laboratory accreditation.	N/A Clear information on laboratory accreditation.	N/A Clear information on laboratory accreditation in Australia and New Zealand.	LOW Not dated.	YES Information on lab accreditation.	HIGH Adds context on Australian and New Zealand laboratory accreditation, with further relevant sources.

Name of document	Authority	Accuracy	Coverage	Objectivity	Date	Significance	*Allen + Clarke's* overall quality rating
creditation%20Advisory%20Council%20(NPAAC)							
Royal College of Pathologists of Australasia. (2019). *Position statement: Diagnostic laboratory testing for Lyme disease (or similar syndromes) in Australia and New Zealand*. Royal College of Pathologists of Australasia. https://www.rcpa.edu.au/Library/College-Policies/Position-Statements/Diagnostic-Laboratory-testing-for-Borreliosis-Lyme	YES Reputable organisation.	N/A Position Statement is succinct.	N/A Position Statement is succinct.	N/A Position Statement by a peak health body.	YES Clearly dated.	YES Clear information and flow-diagram to guide laboratory testing of patients with suspected Lyme disease in Australia.	HIGH Adds context on diagnostic laboratory testing for Lyme disease or similar syndromes in Australia and New Zealand.
Royal College of Psychiatrists. (2017). *Medically unexplained symptoms*. Royal College of Psychiatrists. https://www.rcpsych.ac.uk/mental-health/problems-disorders/medically-unexplained-symptoms	YES Reputable organisation. Detailed and credible references list.	YES Factual information on MUS that are without an obvious physical cause.	YES Explicit, intended audience stated; anyone with physical symptoms without an obvious physical cause.	YES Clear purpose and disclaimer.	YES Clearly dated on the downloadable version.	YES Succinct information on MUS, and further information.	HIGH Adds context with peak mental health body information on medically unexplained symptoms.
US Department of Veterans Affairs. (2009). *VHA Directive 2009-053 Pain management*. US Department of Veterans Affairs.	YES Reputable organisation.	YES Pain management standards of care and procedures	YES Pain management standards of care and procedures	YES Government directive on pain management.	YES Clearly dated. Expired on 31 October 2014.	YES Directive on various pain management, assessment and	MED Pain management directive adds context.

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https://www.va.gov/painmanagement/docs/vha09paindirective.pdf		information is succinct.	information is succinct.			treatment procedures.	
Williamson, M., Tudball, J., Toms, M., Garden, F., & Grunseit, A. (2008). *Information use and needs of complementary medicines users*. National Prescribing Service. https://www.westernsydney.edu.au/__data/assets/pdf_file/0007/537406/Information_Use_and_Needs_of_Complementary_Medicines_Users.pdf	YES Reputable organisation.	YES Stated methods, codes were repeatedly reviewed and refined on complementary medicines (CMs).	YES Clearly stated strengths and limitations of the study.	YES Balanced and clear study with clear research questions and design.	YES Clearly dated.	YES Provides comprehensive overview of CMs, Australian's thoughts on them, how they're used, how information is gained and preferred source of CMs information.	HIGH Adds context around Australia's view on CMs.