Growing evidence of an emerging tick-borne disease that causes a Lyme-like illness for many Australia patients.

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Executive Summary:

Over the past three decades, thousands of Australian families have felt the impact of Lyme and other tick-borne diseases (TBDs), with an estimated10,000 individuals affected each year. Whether it is a laborer who cannot continue his work because of debilitating joint pain, or a child who misses school because of debilitating fatigue, pain and cognitive dysfunction, TBDs can have a significant effect on the day to day lives of Australians. Since Lyme disease was first identified in Australia in 1982, the disease has spread geographically, and in severity. It has been documented that there has been an increase in tickborne diseases in Australia, including early and late forms, as well as an increase in neurological cases.

The patient experience may be characterized by delays in diagnosis, confusion, frustration, ongoing illness, with, in many cases poor outcomes, disability and a significant financial burden. (Most recently, we have started to record deaths in Australia from tickborne diseases.)

Recognizing these facts, the Parliament of Australia has referred these matters to the Senate Community Affairs References committee for enquiry and report. The Senate acknowledged the

significant toll TBDs may exact on individuals, families, communities, and the state, noting that TBDs pose a serious threat to the health and quality of life of many residents and visitors to Australia.

The purpose of this inquiry should be to establish a Lyme and related tickborne diseases task force charged with exploring and identifying recommendations related to education and awareness, long-term effects of misdiagnosis, prevention, and surveillance. The intent of the recommendations are generally to improve Australia's response to the tickborne disease burden.

This submission reflects the history of TBDs in Australia, and includes specific recommendations as well as implementation strategies, case studies, and resource needs. While the Senate Inquiry will be the result of months of research and co-collaboration, it is clear that its report is merely the beginning of a much-needed dialogue and structured planning process across the country.

The primary recommendations in this submission focus on increased and improve surveillance, prevention of tick exposure strategies and tactics, as well as education and awareness for healthcare practitioners(HCPs), patients, the general public and other stakeholders.

In contemplating each recommendation, the author carefully considered each of the countries key stakeholders, including patients of all ages and their families, vulnerable populations, health care providers, domestic animals, researchers, Government agencies, policy makers, schools and community organisations, and the general public.

Key Themes:

1. Tickborne disease knowledge and research is evolving rapidly. It will be vital to encourage critical research, to understand the scope and scale of Lyme and other TBDs in Australia, and to develop options to improve the public health response and the community/ patient outcomes.
2. Different schools of thought exist among all stakeholders regarding Lyme. Ambiguities do exist so it is important to promote a strong and academically rigourous pursuit of better research to help clarify the best options for patients. We are encouraged to keep an open mind, and to continue to explore the nature of these diseases and their health impacts.
3. The most critical research gap is the lack of a gold standard test for Lyme and other tickborne infections; a test that can quickly and accurately diagnose the disease, and prove or disprove ongoing persistence. Research into bio- resonance for diagnosis and treatment of Lyme disease is producing encouraging results in Melbourne Australia.
4. Without more research and surveillance, it will be difficult to stay ahead of this rapidly evolving public health problem.
5. The cost to Australia of doing nothing is considerable.
6. Without targeted and significant funding, it is unlikely these recommendations can be deployed in an effective and impactful way.
7. Collaboration among the commonwealth's diverse stakeholders Will help ensure programs and strategies are innovative, effective, and measurable.

Too many Australians have suffered the consequences of Lyme and TBD's, and without action, thousands more remain at risk. This is important public health challenge affects all Australians -every state has reported ticks infected with bacteria. And yet our children, our elderly, and our

immunocompromised are most at risk and most vulnerable to their impact. Our actions now, will significantly impact Australian youth's risk and future potential.

The author respectfully requests Swift action on the enclose recommendations by all state leaders charged with ensuring the protection and well being of the Commonwealth's residents.

Growing evidence of an emerging tick-borne disease that causes a Lyme like illness for many Australian patients:
Senate Enquiry:
Summary Index:
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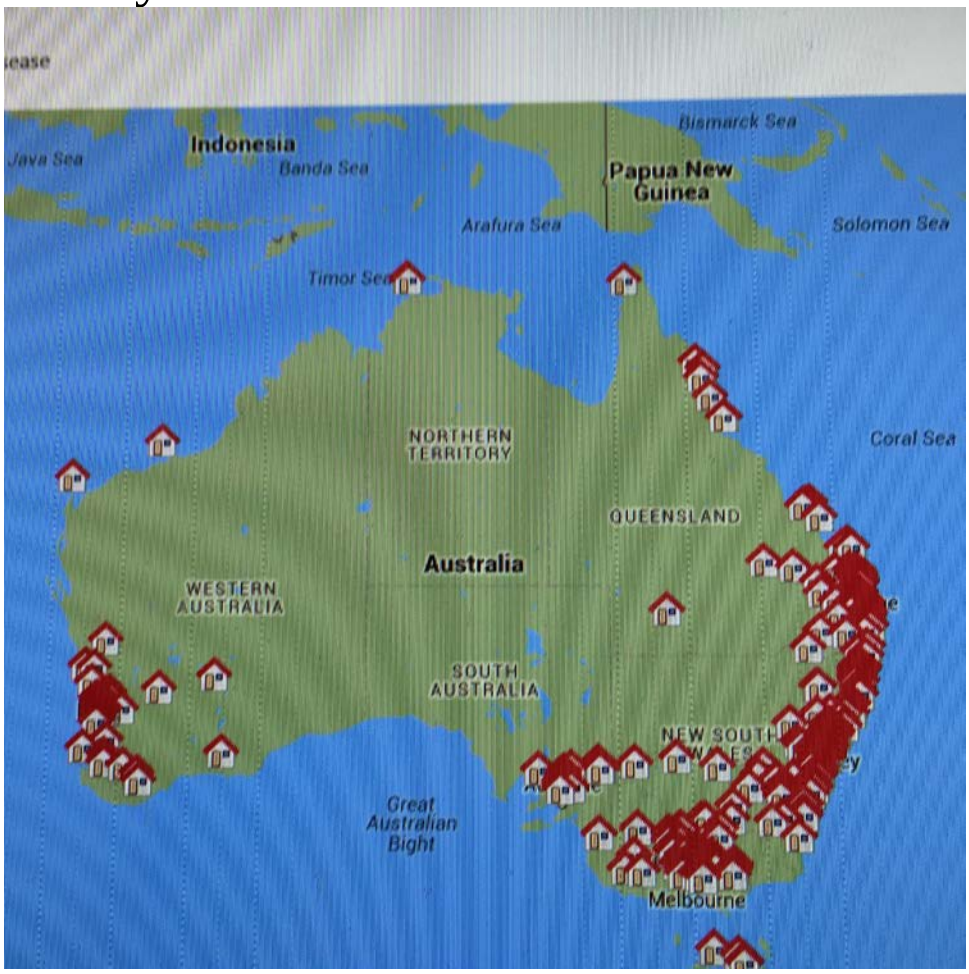
a. The prevalence and geographic distribution of Lyme-like illness in Australia:



Lyme Disease Association of Australia Map of Australia showing prevalence of proven cases of Lyme disease:

There are no formal figures on the illness, but the Lyme Disease Association estimates there are more than 10,000 diagnosed cases in Australia. The organisation estimates that many more cases, perhaps hundreds of thousands, are undisagnosed.

Significant Impact on the Quality of Life in Australia:

Lyme disease is a complex infection that has several manifestations and can affect different systems within the body including the skin, joints, heart, and nervous system. Lyme disease may carry significant and potentially lifechanging burdens, especially if the disease goes undiagnosed or untreated for a prolonged period. Unfortunately, it is common for Lyme disease to go undetected and untreated in absence of the hallmark bull's-eye (erythema migrans) rash, leading to

functional impairments and quality of life impacts, such as damage and infections of the joints, heart, and nervous system.

Many of the symptoms of TBDs are similar to those of other conditions, which further complicates diagnoses at any stage. Like syphilis, which is also caused by spirochete bacteria, some researchers also refer to Lyme disease as "the New Great Imitator." The fever, muscle aches, and fatigue associated with Lyme disease or other TBDS can be mistaken for viral infections, such as influenza or infectious mononucleosis. Joint pain can be mistaken for other types of arthritis, such as juvenile rheumatoid arthritis (JRA), and neurologic signs of Lyme disease can mimic those caused by other conditions, such as multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS).

As mentioned previously, Lyme disease and other TBDs can have significant consequences for patients, including – in the most severe cases – death. In 2013, a 52 year-old adult in New Jersey died from contracting the Powassan virus and in 2014, a three year-old child in Pennsylvania died from Rocky Mountain spotted fever. Recent research has identified co-infections with Lyme disease and one or more other TBD as a significant concern because of diagnostic challenges as well as the intensity and duration of symptoms.

Economic Costs of Tick-Borne Diseases:

Beyond the significant personal impacts these diseases may cause, TBDs also create a significant economic burden in Australia. Over $10 million in annual medical expenses in the Australia have been attributed to Lyme disease as well as up to $10,000 per patient annually in lost productivity. Lyme disease patients required 87 percent more visits to the doctor, and 71 percent more visits to the emergency room in comparison with those without Lyme disease.

Diverse Perspectives of the Medical Community:

The rapid expansion of TBDs in Australia is further complicated by a lack of consensus among researchers and healthcare practitioners (HCPs) in many critical areas. There are two organizations that have published guidelines for diagnosis and treatment of Lyme and other TBDs: the Infectious Disease Society of America (IDSA), and the International Lyme and Associated Diseases Society (ILADS).

The medical community varies in its approach to treating patients with Lyme disease, for example, the adherence to a specific timeframe for antibiotic treatment. Representation of the diversity of these views calls for a "broad spectrum of views to be represented and communicated to patients".

Areas of concern:

We require additional research to address unanswered questions about TBDs, such as:
1) What are the biologic processes in humans that cause ongoing symptoms following treatment?
2) How can we advance the development of innovative and more accurate diagnostic testing?
3) Are there additional, more effective treatments and multidisciplinary approaches? The recommendations within this report resulted from a multidisciplinary, collaborative group and will require significant, ongoing commitment from similarly diverse stakeholders, including healthcare

providers (HCPs), scientists, educators, policymakers, state agencies, local community groups, and patients.

Conclusion:

The human and economic costs of Lyme and other TBDs demand appropriate action and resources. The need for action is clear, and this report provides an important "first step" for moving the commonwealth in a healthier direction.

DEFINITIONS & TERMS

To effectively continue and advance a much-needed dialogue regarding Lyme and other TBDs in the Commonwealth of Australia, establishing a "common language" is critical.

Frequently Used Terms:

While Lyme disease is arguably the most commonly occurring and widely-recognized tick-borne disease, it is by no means the only one. As referenced in the definition below, different types of ticks can harbor a variety of microorganisms that can be harmful to humans, including Babesia, Anaplasma, Ehrlichia, Powassan Virus, Rocky Mountain Spotted fever, other Borrelia species, and possibly Bartonella – to name just a few.

To more accurately represent the full spectrum of infectious pathogens affecting Australians, this report will use the terms "tick-borne diseases" or "TBDs", unless otherwise specified.

Other common terms used in discussions related to TBDs include "post-treatment Lyme disease syndrome" and "Chronic Lyme Disease". Both are non-clarifying and as such, we have chosen to use "persistent symptoms" to describe long-term impact experienced by some patients who have been diagnosed with Lyme disease. 10

The following are definitions of terms that are found frequently in this report:11

Anaplasmosis is transmitted to humans by tick bites primarily from the blacklegged tick (Ixodes scapularis).

Antibiotic prophylaxis: Antimicrobial therapy following a known exposure to a bacterial pathogen that is given to prevent the development of disease, e.g. Lyme disease following short-term attachment and removal of a tick.
Babesiosis: Caused by microscopic parasites that infect red blood cells and are spread by certain ticks. In the U.S., tick-borne transmission is most common in particular regions and seasons: it usually peaks during the warm months. Although many people who are infected with Babesia do not have symptoms, effective treatment is available for those who do.

Bartonellosis: A disease or infection caused by bacteria of the genus Bartonella which primarily cause infection of nonhuman animals and are transmitted via insect vectors (fleas, lice, flies, etc.). The bacteria attack red blood cells and may cause severe anemia and high fever followed by skin eruption. Three species are well documented as human pathogens with as many as 7 others known

to be possible human pathogens based on the current evidence. A Bartonella infection in humans can manifest with little or no symptoms or as severe as ongoing febrile illness and more rarely, produce serious complications like endocarditis and encephalitis.

Borrelia burgdorferi: The causative agent (spirochete bacterium) in Lyme disease. The organism is transmitted to humans by tick vectors, primarily Ixodes scapularis (more commonly known as a deer tick).

Borrelia miyamotoi: A bacterial infection has recently been described as a cause of illness in the U.S. It is transmitted by the blacklegged tick (Ixodes scapularis). B. miyamotoi causes fever (that can be relapsing), chills, headache, and more rarely rash.

Clinically Diagnosed Lyme Disease Cases: Diagnoses based on medical history, symptoms, physical examination. May or may not be confirmed by lab tests.

Ehrlichiosis is transmitted to humans by the lone star tick (Ambylomma americanum), found primarily in the southcentral and eastern U.S. Epidemiology: Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems. 12

Erythema (chronicum) migrans: A rash due to the bite of a deer tick that spreads into a bull's-eye rash.

Hyperendemic: Exhibiting a high and continued incidence; used chiefly of human diseases.

Infectious Disease Society of America (IDSA): Professional organization that represents physicians, scientists and other health care professionals who specialize in infectious diseases. IDSA's purpose is to improve the health of individuals, communities, and society by promoting excellence in patient care, education, research, public health, and prevention relating to infectious diseases.

International Lyme and Associated Diseases Society (ILADS): A nonprofit, international, multidisciplinary medical society dedicated to the diagnosis and appropriate treatment of Lyme and its associated diseases.

Ixodes scapularis: The blacklegged tick or commonly known as a "deer tick", which can transmit the organisms responsible for anaplasmosis, babesiosis, and Lyme disease. This tick is widely distributed in the Northeastern and upper Midwestern United States. I. scapularis larvae and nymphs feed on small mammals and birds, while adults feed on larger mammals. Both can attach to humans.

Lyme disease is transmitted by the blacklegged tick (Ixodes scapularis) in the northeastern U.S. and upper Midwestern U.S. and the western blacklegged tick (Ixodes pacificus) along the Pacific coast.

Powassan disease is transmitted by the blacklegged tick (Ixodes scapularis) and the groundhog tick (Ixodes cookei). Cases have been reported primarily from northeastern states and the Great Lakes region.

Q-fever is usually spread when dust contaminated by dried placental material, birth fluids, urine, or feces from infected animals becomes airborne and is inhaled. Tick-borne transmission also has been documented and the bacterium that causes Q-fever has been identified in Dermacentor spp. ticks.

Rocky Mountain spotted fever (RMSF) is transmitted by the American dog tick (Dermacentor variabilis), Rocky Mountain wood tick (Dermacentor andersoni), and the brown dog tick (Rhipicephalus sanguineus) in the U.S. The brown dog tick and other tick species are associated with RMSF in Central and South America.

Seroconversion: The change of a serologic test from negative to positive, indicating the development of antibodies in response to infection or immunization.

Serology: Measurement of antibodies, and other immunological properties, in the blood serum.13

STARI (Southern tick-associated rash illness) is transmitted via bites from the lone star tick (Ambylomma americanum), found in the southeastern and eastern U.S.

Tick-borne rickettsial infections: Anaplasmosis, ehrlichiosis, and Rocky Mountain spotted fever (RMSF) are the most common tick-borne rickettsial infections in PA. These infections are caused by bacteria from the Rickettsiaceae family and spread by ticks. Each of these tick-borne rickettsial infections has distinct epidemiologic characteristics (type of tick(s) that spreads the bacteria, geographic distribution) and target different types of cells in the body during infection. Despite these differences, these illnesses cause similar symptoms—fever, myalgia, arthralgia, blood abnormalities, and rash (RMSF and ehrlichiosis)

Tularemia is transmitted to humans by the American dog tick (Dermacentor variabilis), the Rocky Mountain wood tick (Dermacentor andersoni), and the lone star tick (Amblyomma americanum). The bacteria that cause tularemia may also be transmitted after bare skin contacts infected animal tissues or contaminated soil or dust particles are inhaled. Tularemia occurs throughout the U.S.

Two-step testing for Lyme antibodies: CDC currently recommends a two-step process when testing blood for evidence of antibodies against the Lyme disease bacteria. Both steps use the same blood sample. The first step uses a testing procedure called “ELISA” (enzyme immunoassay) or, rarely, an “IFA” (indirect immunofluorescence assay). If this first step is negative, no further testing of the specimen is recommended. If the first step is positive or indeterminate (sometimes called “equivocal”), the second step should be performed. The second step uses a test called an immunoblot test, commonly, a “Western blot” test. Results are considered positive only if the ELISA/IFA and the immunoblot are both positive.

EDUCATION & AWARENESS of LYME Disease

This document has laid out specific trends regarding the increasing threat of Lyme and tick-borne diseases, as well as the increasing complexity of these diseases (complicated by multiple tick-borne infections). This clearly points to the need for prevention. Current state findings regarding physician practices and the patient experience point specifically to the need for education:

* Many common symptoms go unrecognized, even the mostly widely recognized bulls-eye rash16
* Physician practices vary significantly in both diagnosis and treatment17
* Patients experience significant delays in diagnosis and treatment18
* Patient outcomes are less than satisfactory19

Studies have found that some tick-borne disease patients experience delays in diagnosis and a portion remain sick for long periods of time. There is a critical need for healthcare education and reform to reduce such delays, and to improve the validity and effectiveness of diagnostic and treatment options available to patients. Thus access to medical care for Lyme disease is improved, and the burden of illness is reduced.

These recommendations should ensure that HCPs, insurers, patients, and governmental agencies are educated about the broad spectrum of scientific and treatment options regarding all stages of Lyme disease and related tick-borne illnesses.

Education would bring the healthcare community up to date with rapidly evolving science, the associated risks of exposure, what to do about bites and early stages of disease, and especially how to prevent the progression of disease to later stages with more incapacitating outcomes.
The goal is to catch disease earlier, and to provide a better understanding of disease processes and treatment options, to ultimately improve patient outcomes.

Most importantly, education would ensure that patients are properly informed, and positioned to evaluate the risks and benefits of different choices based on their response to treatment, with their HCPs.

Lastly, awareness and education of the general public, insurers, and governmental agencies would address prevention approaches, general awareness, and improve access to early and appropriate treatment.

SURVEILLANCE A comprehensive and thorough surveillance system for Lyme disease and other tick-borne infections, as with all vector-borne diseases, must incorporate both ecological surveillance (ticks and animal reservoirs/hosts) and disease surveillance (humans and domestic pets), which entails participation by multiple partners (e.g. PADOH, PADEP, universities, healthcare providers, veterinarians, etc.). Information collected through ecological and disease surveillance enables areas and risk groups with high rates of infection to be identified, which can help direct prevention efforts and prioritize how public health resources are distributed. It will also inform members of the medical and healthcare community on the specific types of tick-related infections that are being encountered most frequently in a particular state or geographical region of our commonwealth.

Ecological surveillance provides essential information on the presence of: a) specific tick vectors in specified geographic areas, b) the pathogenic organisms that they carry, and c) animal disease

reservoirs and other animal hosts upon which the ticks feed. Ecological surveillance data along with an understanding of factors that impact transmission (e.g., timing of tick attachment for transmission, competency of tick species to transmit infection, host susceptibility to infection, etc.) are necessary to assess the potential for infection with Lyme and other tickborne diseases throughout the state where Australians live, work, and play.

Human disease surveillance for Lyme and other TBDs falls under a core public health activity that aims to provide information to help prevent and control disease, in order to promote the health and well-being of all Australian residents. Surveillance data along with observational epidemiologic studies can provide a better understanding of the burden and severity of specific tick-borne diseases among Australian residents. Serologic surveillance of domestic pets provides sentinel information and describes how specific tick-borne infections may impact residents' four-legged family members. Lastly, it is important to note that case classification definitions for disease surveillance purposes are not intended for clinical diagnosis, especially since case identification and investigation for surveillance are most often performed retrospectively after treatment decisions have been made.

OTHER RECOMMENDATIONS

The author recognizes that there are other areas of consideration that either go beyond (or that span) the areas of surveillance, education and awareness, and prevention. As such, this section includes several recommendations that are of an organizational nature as the Commonwealth moves forward on considering the recommendations herein for implementation. This section includes a recommendation related to estimating the implementation cost of our recommendations as well as establishing some structure for a continued advisory function. Alternatively, an organizational alternative is provided describing a body that would be both advisory and that would provide a coalition-oriented partnership approach among public, private, and non-profit stakeholders. This would help to leverage resources for implementing the Task Force recommendations.

Lyme Disease: Australian Government:
www.health.gov.au/lyme-disease
The Australian Government is monitoring Lyme disease, in consultation with the states and territories, through the Communicable Diseases Network Australia.

Page last updated: 02 February 2016

Lyme Disease

Even though the Chief Medical Officer's Clinical Advisory Committee on Lyme Disease (CACLD) has ceased, the Australian Government Department of Health will maintain an interest in an Australian Lyme disease-like syndrome. This website will provide updates on the department's work and relevant research findings on Australian Lyme disease-like syndrome.

- Senate Inquiry
- Department of Health Media Statement
- Recently Published Australian Research
- Preventing and Treating Tick Bites
- CMO Progress Report
- Australian guidelines for diagnosis of overseas acquired Lyme disease
- Previous Progress Report
- Scoping Study
- Department of Health's Response to the Scoping Study
- Consolidated List of Research Projects 2014
-
- Lyme Disease Treatment Round Table
- Research Funding
  - NHMRC
  - ARC
- Advice to Clinicians
- Clinical Advisory Committee on Lyme Disease (CACLD)

Senate Inquiry

The Australian Senate has established an inquiry into a Lyme-like illness in Australia. The URL link for making a submission is: (www.aph.gov.au/Parliamentary_Business/Committees/Senate/Community_Affairs/Lyme-like_Illness).

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Department of Health Media Statement

The Department of Health issued a media statement on Lyme Disease in Australia following several requests from the public and media outlets - a copy can be accessed below:

February 2016

- From a spokesperson for the Federal Department of Health (PDF)
- From a spokesperson for the Federal Department of Health (Word)

Recently Published Australian Research

- Inhibition of the endosymbiont "*Candidatus* Midichloria mitochondrii" during 16S rRNA gene profiling reveals potential pathogens in *Ixodes* ticks from Australia. Peter Irwin et al, 25 June 2015.
- Bacterial Profiling Reveals Novel "*Ca*.Neoehrlichia", *Ehrlichia*, and *Anaplasma* Species in Australian Human-Biting Ticks" Peter Irwin et al 28 December 2015

Preventing and Treating Tick Bites

- Preventing and Treating Tick Bites (PDF)
- Preventing and Treating Tick Bites(Word)

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Chief Medical Officer's progress report on Lyme disease in Australia

August 2015

- Chief Medical Officer's progress report on Lyme disease in Australia (PDF)
- Chief Medical Officer's progress report on Lyme disease in Australia (Word)

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Australian guideline on the diagnosis of overseas acquired Lyme Disease/Borreliosis

August 2015

- An Australian guideline on the diagnosis of overseas acquired Lyme Disease/Borreliosis (Word)
- An Australian guideline on the diagnosis of overseas acquired Lyme Disease/Borreliosis (PDF)

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Progress Report against the CACLD Terms of Reference

August 2014

The Progress Report from the Chief Medical Officer that details the activity achieved against each of the CACLD's terms of reference can be accessed below.

- Progress Report against the CACLD Terms of Reference (PDF)
- Progress Report against the CACLD Terms of Reference (Word)

Contents list

Scoping Study

The Department of Health contracted an expert in microbiology to conduct a Scoping Study to identify the research needs for an investigation into whether a causative tick borne microorganism for Lyme disease exists in Australia.

The Scoping Study can be accessed below.

November 2013

- Scoping Study (PDF)
- Scoping Study (Word)

Contents list

Department of Health's Response to the Scoping Study

In November 2013, the Department of Health sought public comment on the Scoping Study requesting that comments focus on the research programmes. Twenty-four submissions were received, along with eight expressions of support for the Lyme Disease Association of Australia's submission and four letters from people describing their personal situations (36 submissions in total). The department considered each submission and has prepared a response to the Scoping Study.

The Department of Health's response to the Scoping Study can be accessed below:

August 2014

- Department of Health's response to the Scoping Study (PDF)
- Department of Health's response to the Scoping Study (Word)

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Consolidated List of Research Projects

The Department of Health, in consultation with the CACLD and other clinical experts has identified a number of research projects that would assist in clarifying the Australian Lyme disease-like syndrome.

The consolidated list of research projects can be assessed below:

August 2014

- Consolidated list of research projects (PDF)
- Consolidated list of research projects (Word)

Contents list

Lyme Disease Treatment Round Table

The Department of Health hosted the Lyme Disease Treatment Round Table Meeting on Tuesday 27 May 2014. The outcomes can be accessed below:

August 2014

- Outcomes (PDF)
- Outcomes (Word)

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Research Funding

Advice provided by the CACLD, recommendations from the Scoping Study, and outcomes from the Lyme Disease Round Table Meeting have revealed potential research projects that would assist in clarifying the Australian Lyme disease-like syndrome.

The majority of Australian Government health and medical research funding is administered by the National Health and Medical Research Council (NHMRC). The Australian Research Council (ARC) has funded some Special Research Initiatives in the

health and medical areas however the ARC does not generally fund medical research. The ARC Medical Research Policy is available on the ARC web site. Researchers may also seek other avenues for funding including the higher education sector, business sector or the private non-profit sector. The Department of Health is not a research funding agency.

National Health and Medical Research Council

The Project Grant scheme is the NHMRC's main avenue of support for individuals and small teams of researchers undertaking biomedical, clinical, public health or health services research in Australian universities, medical schools, hospitals or other research institutions. The Project Grants scheme aims to fund research leading to improved health of all Australians. To achieve this aim the scheme provides support for projects with the following attributes:

- Investigator initiated research across all fields of research, from basic research through to research in clinical and community settings, relevant to health; and
- single investigators or small teams of researchers (up to 10 investigators) and early career researchers (new investigators).

All applications undergo a competitive peer-review process and project grant rounds usually start in December each year. Further information is available on the NHMRC website.

Australian Research Council

The ARC funds research and researchers under the National Competitive Grants Program (NCGP). As part of its commitment to nurturing the creative abilities and skills of Australia's most promising researchers, the NCGP provides:

- support for the highest-quality research leading to the discovery of new ideas and the advancement of knowledge
- financial assistance towards facilities and equipment that researchers need to be internationally competitive
- support for the training and skills development of the next generation of researchers
- incentives for Australia's most talented researchers to work in partnership with leading researchers throughout the national innovation system and internationally, and to form alliances with Australian industry

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Advice to Clinicians

August 2013

- CMO Advice to Clinicians on CACLD (PDF)
- CMO Advice to Clinicians on CACLD (Word)

In this section

- Communicable diseases information
- Poliomyelitis Outbreak Response Plan for Australia
- Antimicrobial resistance (AMR)
- Clinical Advisory Committee on Lyme Disease (CACLD)
- Factsheet for Patients and Consumers - Hepatitis A and frozen berry products recall.
- Foodborne illness in Australia: Annual incidence circa 2010
- Hepatitis A and frozen berry products recall
- Human Papillomavirus (HPV)
- Information for healthcare practitioners on Hepatitis A and frozen berry products recall
- **Lyme Disease**

- Middle East Respiratory Syndrome (MERS)
- Poliomyelitis
- Rabies, Australian bat lyssavirus and other lyssaviruses
- The First National Hepatitis B Strategy
- Vaccine Preventable Diseases
- Zika Virus

The Royal College of Pathologists of Australasia:
https://www.rcpa.edu.au/Library/College-Policies/Position-Statements/Diagnostic-Laboratory-testing-for-Borreliosis-Lyme
http://www.cdc.gov/lyme/resources/TickborneDiseases.pdf
Australian research into bio resonance testing and treatment of Lyme disease is proceeding in Australia.

LYME DISEASE IN AUSTRALIA

Stephen L. Doggett, Richard C. Russell, Richard Lawrence and David Dickeson

Originally appeared in: *Inoculum*, **4**: 1-4. Modified and updated; November, 1997.

History - Australia

The first Australian cases of a syndrome consistent with Lyme disease were reported from the Hunter Valley region of New South Wales in 1982. Serology was initially negative on one of the 6 patients, but later reported as positive in low titre. Cases of EM with febrile illness were reported in 1986 from the south and central coasts of New South Wales. All had negative serology. In Queensland, from 1986 to 1989, the State Health Laboratories tested 1,247 patients for *B.burgdorferi* antibody using an IFAT and reported 186 (15%) positive (titre 64) titres. In none of these cases was confirmatory serology (WB) undertaken.

In 1988 at Westmead Hospital, a multidisciplinary investigation of putative LD in coastal New South Wales began, encompassing clinical, serological, vector and reservoir host studies.

Clinical investigations - Australia

Over the past 6 years, due principally to local publicity, there has been an increase in serological testing for LD. This is often initiated by patients, who believe that LD may be an explanation for an undiagnosed health problem. Thus, most patients seen by infectious diseases specialists are self selected and referred for assessment on the basis of tick exposure and reported positive screening serology.

Patients frequently have long-standing symptoms for which no other diagnosis has been established including myalgia, arthralgia without objective evidence of joint disease, neurological symptoms such as frequent headaches, inability to concentrate and impairment of memory, and syndromes resembling chronic fatigue syndrome. The late LD dermatological manifestation, ACA, has not been reported in Australia.

A few cases of EM have been reported from South-Eastern Australia. However, diagnosis can be confounded by a spectacular erythematous hypersensitivity reaction to the bite of *I. holocyclus*, the most common tick biting humans in New South Wales. Of eight skin biopsies submitted to Westmead Hospital for spirochaete isolation, one, from a patient returning from a LD endemic area in Europe, was culture positive for *B.burgdorferi*. There has been no isolation from local patients.

Serological Investigations - Australia

No significant difference was found in seroprevalence rates for *B.burgdorferi* infection in humans between high (rural residents) and low (urban residents) tick exposure groups, using an IgG ELISA. The overall seropositive rate was 2.2% (9/400). The seroprevalence in New South Wales is comparable with that in non-LD areas, where 1-3% of human sera are seropositive due to cross reacting antibodies and contrasts with reports from known endemic areas, outside Australia, where rural populations have considerably higher seropositive rates. A serosurvey of dogs in New South Wales showed a similar result with 2.5% (6/239) seropositive and another from Brisbane also showed no evidence of *B.burgdorferi* infection. These suggest that southeastern Australia is a non-endemic area.

From 1988 to 1994 at Westmead Hospital, 78 (1.8%) of 4,372 from local patients with suspected LD were positive for IgG by ELISA and IFAT. All 78 were tested by WB, using North American and European strains of *Borrelia*; 46 sera showed one or more bands. None, including those with putative late stage disease, showed more than 4 specific bands and thus were all negative by international criteria. Twenty-four patients with various bacterial, viral or autoimmune syndromes unrelated to LD were tested in parallel and 11/24 showed one or 2 indicative bands. Thus a high degree of cross reactivity was demonstrated with non-LD patients.

Recently, there have been reports from eastern Australia of LD-like illness associated with WB serology yielding bands at 31kDa (OspA) and the highly-cross reactive 41kDa band. None of these results conforms with internationally accepted criteria for a positive WB. Concomitant with this are results of WB analysis of sera from patients with syndromes

unrelated to LD, >30% of which reacted with a 41kDa band and >10% with the OspA band.

The sensitivity of serological testing for LD sometimes depends on the strain of *Borrelia* used and could confound interpretation of results in Australia, where no local spirochaete has been isolated for use as a reference antigen.

Vector and Reservoir Host Investigations - Australia

To detect a possible causative agent, ticks were collected from areas associated with putative infections and examined for spirochaetes by dark field microscopy, culture of gut contents, and direct testing of ticks with PCR for the *Borrelia*-specific flagellin gene.

In total, over 12,000 ticks were tested including >1,000 by PCR. Spirochaete-like objects (SLOs), were observed in 92 cultures from bloodfed ticks but were not typical of *Borrelia* spp. They were found only found in cultures with bacterial contaminants, presumably from the bloodmeal. Electron micrographs were similar to those of SLOs recovered from contaminated cultures from ticks in Missouri, USA and were composed of aggregations of bacterial flagella, thought to originate from the contaminants. Molecular characterisation indicated that the SLOs shared some antigens with *B.burgdorferi*, but were not genetically related. Similar objects found in cultures from dissected bloodfed ticks taken from animals on the mid-north coast of NSW were purported to be related to *B.burgdorferi* and the probable cause of LD in Australia.

A small number (17) of native vertebrate animals were sampled by ear punch biopsy for culture and PCR investigation but there was no evidence of borreliae.

It is possible that the PCR primers used were unable to identify Australian spirochaetes. However, the tick gut contents were also negative by culturing and dark field microscopy.

Conclusions - Australia

There are some major differences between Australia and the endemic areas of the northern hemisphere with respect to the natural history of LD:

No ticks of the *I. persulcatus* complex, the principal vectors to humans in the northern hemisphere, occur in Australia. In eastern Australia, the logical candidate vector would be *I. holocyclus* which has a wide host range and is the most common tick biting humans. It was unable to transmit a North American strain of *B.burgdorferi* but an association with a so far undiscovered Australian spirochaete can not be excluded.

None of the mammal species identified as reservoir hosts in the northern hemisphere are present in Australia. There are reports of spirochaetes in Australian native animals, and a

local mammal could be a reservoir host for an indigenous spirochaete that occasionally infects humans through a tick vector and produces a clinical syndrome similar to LD; however, no spirochaete was detected in the 12,000 ticks or animals processed.

Summary

The diagnosis of LD outside known endemic areas cannot be based solely on serological tests especially if they fail to conform with internationally accepted criteria, because of the high incidence of false positive results.

A clinical diagnosis in a non-endemic disease area (especially of Stage II or III disease), is difficult to support without isolation of the causative agent from the patient, from other patients with similar illness or from a known vector in the region.

The existence of LD in Australia will remain controversial until an organism is isolated from a local patient and fully characterised, or until a tick-borne organism can be shown to be responsible for the human infection. If it exists it shares few of the epidemiological or clinical characteristics of US or European patterns of LD.

Further Reading

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Related Links

<http://www.uri.edu/artsci/zool/ticklab/HomePg.html> (Information from America for ticks, Lyme disease, Ehrlichiosis, Human Babesiosis).

<http://www.dis.strath.ac.uk/vie/LymeEU/index.htm> (Lyme disease from Europe, tick biology, control and images).

<http://www.lymenet.org/> (Lyme disease network from America).

B. Methods of reducing the stigma associated with Lyme-like illness, with a specific focus on the laboratory testing procedures and associated quality assurance processes, including recognition of accredited international testing:

'Our World is changing, our parasites are changing, too: small intracellular bacteria, such as the lyme agent, have learned how to persist in our bodies and to trigger more and more crippling pathologies.' Luc Montagnier, Nobel prize in Medicine for the discovery of HIV.

This document has laid out specific trends regarding the increasing threat of lyme and tickborne diseases, as well as the increasing complexity of these diseases(complicated by multiple tickborne infections). This is clearly points to the need for prevention.

Current findings regarding physician practices, and the patient experience, points specifically to the need for education:

1. many common symptoms go unrecognized, even the most widely recognized Bullseye rash
2. physician practices vary significantly in both diagnosis and treatment
3. patients experience significant delays in diagnosis and treatment
4. patient outcomes are less than satisfactory

Studies have found that's some tickborne disease patients experience delays in diagnosis and a portion remain sick for long periods of time. There is a critical need for healthcare education reform to reduce such delays, and to improve the validity and the effectiveness of diagnostic and treatment options available to patients. Thus access to medical care for Lyme disease is improved, and the burden of illness is reduced.

These recommendations should ensure that hates CPS, insurers, patients, and governmental agencies are educated about the broad-spectrum of scientific and treatment options regarding all stages of Lyme disease and related tickbourne illnesses.

Education would bring the healthcare community up to date with a rapidly evolving science, the associated risks of exposure, what to do about bites and early stages of disease, and especially how to prevent the progression of disease to late stages with more incapacitating outcomes. The goal is to catch disease earlier, and provide a better understanding of disease processes and treatment options, to ultimately improve patient outcomes.

Most importantly, education would insure the Patients are properly informed, and position to evaluate the risks and benefits of different choices based on the response to treatment, with their HCPs.

Lastly, awareness and education of the general public, insurers, and government agencies would address prevention approaches, general awareness, and improve access to early and appropriate treatment.

High-Risk Populations While anyone can be affected by a tick-borne disease, certain populations are more at-risk, including: • Individuals living in areas with high concentrations of Lyme and other TBDs; • School-age children (ages 5-14); • Older adults (ages 45-54); • Outdoor occupations (construction, landscaping, forestry, land surveying, farming, railroad work, utility line work, park or wildlife management, etc.); and • Outdoor recreationists (hikers, hunters, fishers, campers, etc.); • Immunocompromised.

Testimonial for Dr Horowitz:
More than 22 years ago [redacted] struggled with debilitating pain and unending insomnia, symptoms that left me barely able to work or to the deal confidently with daily life. I saw many specialists but still had no definitive diagnosis for my frightening symptoms. My neurologist said that he had heard of new internist it was gaining attention is it brilliant diagnostician. I made an appointment for a consultation. What did I have to lose?

I vividly remember my first visit with this physician. Dr Richard Horowitz sat next to me in the exam room. He read the copious laboratory test results I had brought with me, but then he gently asked me tell him the story of my illness. He listened intently, taking detailed notes, and urged me to slow down, so that I wouldn't feel rushed get everything out all at once. When I finished he placed his hand on my shoulder and said, everything will be okay. That's simple act of compassion in response to my narrative was so powerful! For the first time in more than a year I felt that I might regain my former Joy and energy; hi felt hopeful that I might find a way through the pain and recover my health.

Hope is a central human emotion, misunderstood and often mistaken for optimism. Having true hope is not about holding a rosy and unrealistic prospect for the future. It is not just think positive. Rather, hope is clear-eyed. True hope is seeing a viable route out of the darkness while acknowledging the obstacles that might impede your journey.
[redacted] PhD, New York.

Like many other physicians, Horowitz's initial understanding was that lyme disease is caused by tick-borne spirochetal bacteria, Borrelia burgdorferi, which can progress from characteristic expanding skin rash, erythema migrans (EM), to a wide variety of non-specific systemic symptoms that can affect any part of the body.

Appropriate and early antibiotic treatment can successfully treat many with the infection. However, in some people a tick bite can lead to disseminated infection and to disabling physical, cognitive,

and psychological manifestations. And some people manifest with puzzling multiple systemic symptoms that can occur throughout the body, which lead to complex lab results.

Because of this, lyme disease has been ignored or trivialized by the medical profession more than a quarter of a century. Many patients have seen 15 to 20 physicians before they walked through the doors of a qualified lyme doctor they're suffering is made worse as they repeatedly faced difficulty in obtaining appropriate care from often skeptical medical and insurance communities, as they unknowingly into one of the most virulent wars between two medical camps.

One camp, the Infectious Diseases Society of America (IDSA), believes that lyme disease is an easily diagnosable and treatable condition.

The other camp, the International Lyme and Associated Diseases Society (ILADS) believes that the blood tests to diagnose lyme disease are highly unreliable and that 30 days of antibiotics is often insufficient.

Chronically ill patients therefore go from doctor to doctor searching for answers.
A patient's journey typically begins with family doctor. A maximum of 30 days of antibiotics is the accepted standard of care for lyme disease. If patients report back that they not getting better, they are likely diagnosed as having post-lyme syndrome, chronic fatigue syndrome or fibromyalgia.

They are then given an antidepressant and the phone number of a local psychiatrist, or told to live with the symptoms.

When a child contracts lyme disease and can't concentrate in school and/or shows a decline in their grades and attention span, then the child is often diagnosed with attention deficit disorder (ADD), or perhaps there are problems at home. They are typically given Ritalin, and sent for behavioural therapy. This may help some of the symptoms yet fail to address the root problem.

Desperate patients go from their family doctors to specialists complaining of chronic fatigue, fevers, sweats and chills, stiff neck and headaches, light and sound sensitivities, dizziness, memory and concentration problems, joint aches and muscle aches that migrate around the body, tingling, burning sensations, chest pain, palpitations and shortness of breath, gastrointestinal problems, resistant neurological problems, sleep disorders, and/or a whole host of psychiatric symptoms, including depression, anxiety, and irritability. Or they get saddled with diagnoses of untreatable conditions.

Yes the answer might be that they are experiencing multiple systemic problems from lyme disease and its associated infections. Lyme and other tick-borne disorders have been identified across the world and are causing untold suffering and disability in millions of people every day. It is no wonder that Lyme and associated tick-borne diseases (TBDs) our called the great imitators.

Modern medicine is excellent in providing care for acute diseases. The medical system, however, lacks an understanding of and treatments for a myriad of chronic diseases.

Horowitz has successfully treated more than 12,000 Lyme patients over the past 26 years, many of whom have been misdiagnosed and given simplified medical labels. He believes that these patients are best described, as having multiple systemic infectious disease syndrome, Or (MSIDS).

Louis Pasteur's postulate 'one germ, one disease, which has been one of the hallmarks of medical diagnosis for the last century, is no longer applicable, at least in patients with multiple chronic diseases. Horowitz believes that we need a new paradigm to diagnose and treat chronic illness.

Chronically ill, complex patients, no matter which diagnosis we may give them, often have simultaneous multiple bacterial, parasitic, viral, and fungal causes for their illnesses. They also often have associated immune dysfunction with auto immune markers, large environmental toxin loads, hormonal dysfunctions, mitochondrial dysfunction, allergies with functional metabolic abnormalities, sleep disorders, and underlying psychological dysfunction.

Horowitz developed he's 16 point differential diagnostic map which is presented in his book ' Solving the mystery of lyme and disease'.

Today LYME disease is the number one vector borne infectious disease in the world. This refers to an infection transferred from an arthropod, or tick, to an individual person or animal. Horowitz believes that the identification of MSIDS, multiple systemic infectious disease syndrome, may prove to be the missing link in the diagnosis and treatment of LYME disease.

Conclusion:

The stigma associated with lyme-like illness can only be reduced through more accurate diagnosis and treatments for the diseases. This will be the result of worldwide research into LYME disease and Australian scientists are certainly capable of contributing to this.

C. The process for diagnosis of patients with a LYME like illness, with a specific focus on the laboratory testing procedures and associated quality assurance processes, including recognition of accredited international laboratory testing.

Remember, Lyme disease is a clinical diagnosis and the blood tests only help to confirm your clinical suspicion.

The Horowitz LYME-MSIDS questionnaire:

Answer the following questions as honestly as possible stop think about how you have been feeling over the previous month and how often you have been bothered by any of the following problems. Score the occurrence of each symptom on the following scale
none, mild, moderate, severe.

Lyme Disease Symptoms:

Section 1: Symptom frequency score: circle your score number:

0 None 1 Mild 2 Moderate 3 Severe

1. unexplained ***fevers***, sweats, chills, or flushing 0 1 2 3
2. unexplained ***weight change*** ; loss or gain 0 1 2 3
3. ***fatigue , tiredness*** 0 1 2 3
4. unexplained ***hair loss*** 0 1 2 3
5. ***swollen glands*** 0 1 2 3
6. ***sore throat*** 0 1 2 3
7. ***testicular or pelvic pain*** 0 1 2 3

8. unexplained ***menstrual irregularity*** 0 1 2 3

9. unexplained ***breastmilk production ; breast pain*** 0 1 2 3

10. ***irritable bladder*** or bladder dysfunction 0 1 2 3

11. ***sexual dysfunction*** Or loss of libido 0 1 2 3

12. ***upset stomach*** 0 1 2 3

13. change in ***bowel function***. .(constipation.or diarrhea) 0 1 2 3

14. ***chest pain*** or rib soreness 0 1 2 3

15. ***shortness of breath*** or cough 0 1 2 3

16. ***Heart palpitations*** , pulse skips, Heart block 0 1 2 3

17. history of a ***heart murmur*** or valve prolapse 0 1 2 3

18. ***joint pain*** or swelling 0 1 2 3

19. ***stiffness of the neck*** or back 0 1 2 3

20. ***muscle pain*** or cramps 0 1 2 3

21. ***twitching of the face*** or other muscles 0 1 2 3

22. ***headaches*** 0 1 2 3

23. neck cracks or ***neck stiffness*** 0 1 2 3

24. ***tingling, numbness, burning*** for stabbing sensations 0 1 2 3

25. ***facial paralysis*** Bell's palsy 0 1 2 3

26. eyes *vision double blurry* 0 1 2 3

27. ears Hearing *buzzing ringing ear pain* 0 1 2 3

28. Increased *motion sickness vertigo* 0 1 2 3

29. light-headedness *poor balance difficulty walking* 0 1 2 3

30. *tremors* 0 1 2 3

31. confusion *Difficulty thinking* 0 1 2 3

32. difficulty with *concentration or reading* 0 1 2 3

33. forgetfulness *poor short term memory* 0 1 2 3

34. *disorientation* getting lost going to the wrong places 0 1 2 3

35. difficulty with *speech or writing* 0 1 2 3

36. *mood swings irritability depression* 0 1 2 3

37. *disturbed sleep* too much too little early awakening 0 1 2 3

38. exaggerated symptoms or *worse hangover from alcohol* 0 1 2 3

Add up your totals from each of the four columns . This is your first score.

Section 2 : Most common Lyme symptoms score:

If you rated a three for each of the following in section 1, give yourself **five additional points :**

Fatigue

Forgetfulness poor short-term memory

joint pain or swelling

tingling numbness burning or stabbing sensations

disturbed sleep too much too little early awakening

Score:

Section 3 lyme incidence score

Now please circle the points for each of the following **statements you can agree with**:

1. You have ***had a tick bite with no rash*** or fluke symptoms 3 points

2. You have ***had a tick bite, an erythema migrans***, or an undefined rash, followed by flu like symptoms 5 points

3. You live in what is considered a ***Lyme-endemic area*** 2 points

4. You have a ***family member who has been diagnosed with Lyme*** and/or other tick borne diseases 1 point

5. You experience ***migratory muscle pain*** 4 points

6. You experience ***migratory joint pain*** 4 points

7. You experience ***tingling/burning/ numbness that migrates*** and/or comes and goes 4 points

8. You have received a ***prior diagnosis of chronic fatigue syndrome or fibromyalgia*** 3 points

9. You have received a prior diagnosis of a specific autoimmune disorder ***(lupus, MS, or rheumatoid arthritis***), or of a ***nonspecific autoimmune disorder*** 3 points

10. You have had a ***positive Lyme test*** (IFA, ELISA, Western blot, PCR, and/or borrelia culture) 5 points

Score for Section 3 ( )

Section 4: Overall health score:

1. Thinking about your overall physical health, **for how many of the past thirty days was your physical health not good?** _____Days

Award yourself the following points based on the total number of days:

0-5 days = 1 point

6-12 days = 2 points

13-20 days = 3 points

21-30 days = 4 points

2. Thinking about your *overall mental health*, for **how many days during the past thirty days was your mental health not good?** _____days

Award yourself the following points based on the total number of days:

0-5 days=1 point

6-12 days=2 points

13-20 days=3 points

21-30 days=4 points

Score for Section 4.2

Scoring:

Record your total scores for each section below and **add them together to achieve your final score:**

Section 1 Total:_____

Section 2 Total:_____

Section 3 Total:_____

Section 4 Total:_____

Final Score:_____

If you scored 46 or more, you have a high probability of a tick bourne disorder and should see a health care provider for further evaluation.
If you scored between 21 and 45, you possibly have a tickborne disorder then should see a health care provider for further evaluation.
If you scored under 21, you are not likely to have a tickborne disorder.

Understanding laboratory testing to diagnose Lyme disease

Various guidelines and case definitions are used to diagnose LYME disease. The IDSA guidelines are based on the CDCs strict surveillance definition, which was developed for national reporting of lyme disease and is primarily used by health departments for epidemiological purposes. The CDC surveillance case definition is narrow, meaning that only a minority of actual cases we'll meet it strict criteria. The definition can be satisfied in the following two ways:

A. A case with an EM rash 5 cm or larger
B. A case with at least one late, objective manifestation, such as meningitis, (inflammation occurring to the membranes covering the brain and spinal cord), cranial neuropathy (nerve damage of the cranial nerves), brief attacks of arthritis, aw AV Block(and electrical conduction defect in the heart) that is laboratory confirmed.

   Health care providers following the IDSA guidelines use these criteria.

   According to the CDC surveillance case definition, late manifestations require laboratory confirmation. This may involve obtaining a positive culture for Borrelia burgdorferi from blood, skin, a joint, or cerebral spinal fluid(CSF), all by identifying antibodies to the bacterium in the CSS; the most common method, however, known as the two tier testing algorithm, uses a specific sequence of blood tests.

   The first is an ELISA, the second is a Western blot. These are indirect tests of infection, because instead of identifying the organism itself, they look for antibodies to Borrelia bergdorferi that were made by the immune system. ELISA tests Measure the total amount of anti Borrelia burgdorferi antibodies present, while western blots identify individual

antibodies and look for specific protein patterns that are unique to Borrelia. If enough of these Borrelia proteins are present, the test is considered to be positive.

Although the surveillance case definition does firmly establish a Lyme disease diagnosis, many patients unfortunately do not meet with these criteria because these tests are often inaccurate. That is why the CDC has publicly stated on their website:" this surveillance case definition was developed for national reporting of LYME disease, it is not intended to be used in clinical diagnosis."

No one test or combinations of tests are perfect in establishing a laboratory diagnosis, and there are extensive scientific references in the medical literature documenting false negative blood tests. The CDC points out that there are problems with testing, and that a patient with LYME disease may not be diagnosed using these criteria.

The New York State apartment of health Study conducted in 1996, was reported to the CDC. Among the 1535 patients study, 81% of non-the M cases were not confirmed with the present two tiered testing algorithms. In other words, the presently used two tiered testing mr 81% of the LYME cases, especially when the patient did not have the bull's eye rash.

One of the most comprehensive reviews of the standard LYME tests comes from a 2005 study at John's Hopkins University, confirming the poor sensitivity of the ELISA. Working with early diagnosed LYME disease patients they studied state-of-the-art blood and DNA tests(PCR testing) for LYME and found serious flaws: when the standard two-step method recommended by the CDC was used on patients with other laboratory evidence of LYME disease, it was positive between 45% and 77% of the time.

As for the DNA tests, they reported that these rarely pick up otherwise confirmed LYME disease at all. Marangoni A. 2005 showed discrepant results. The sensitivity for the same blood tested varied between 36.8% and 70.5%.Ang, 2011 confirmed that not only was the ELISA and insensitive test, but that various Western blot kits often gave discordant results on the same specimen.

He concluded that the likelihood of a patient being diagnosed with LYME disease was highly influenced by which Elisa-Western blot combination was used. This confirmed the results of Bakken L published years before. Stricker R, 2007, found that the overall sensitivity of the combined ELIS-Western Blot was only 56%. These findings mirror what Dr Horowitz found in his own practice.

Another set of guidelines, develop by ILADS, States that lime disease is a clinical diagnosis. Cases meeting the CDC definition qualified under the ILADS definition, as do patients who have symptoms consistent with lyme disease and a history of potential exposure to the ticks that transmit the illness, particulate if symptoms cannot be rightfully attributed to other illnesses.

Positive two-tier testing is not required, because they hold the view that the present available testing is unreliable. In clinical practice, patients with persistent LYME disease

symptoms rarely meet CDC criteria.

These opposing views have confused many health care providers as to the best way to diagnose and treat LYME disease. Horowitz has found nine basic standards that help him establish a clear diagnosis.

1. LYME disease is a clinical diagnosis and lab results served to support the clinical diagnosis.

C.

2. An EM rash is definitive evidence of LYME disease and does not require Laboratory confirmation to make a diagnosis.

3. patients are often sero negative (have negative blood tests) if tested too early, or if antibiotics have been used early in the course of the disease, since this may prevent antibodies from being produced.

5. the two tiered protocol of using a lyme ELISA followed by a Western blot Will miss the majority of lyme patients, due to the insensitivity of the tests.

6.

5. the Western blot may provide us with more useful information, but it also has its limitations. There are multiple strains of Borrelia present in the United States (approximately 100), and there are over 300 strains worldwide. Blood tests often do not cross react between these strains, and consequently can lead to false negative results.

The utility of the Western blot is therefore based on the expertise of the laboratory performing the test, which strains of Borrelia the patient was exposed to, and identifying the specific bands on the Western blot that reflect exposure to Borrelia burgdorferi.

Borrelia specific bands reflect out the surface proteins (OSP) on the surface of the organism that is seeing more often in lyme disease than in other infections. If any of these bands are present what a Western blot, there is a higher likelihood that the patient has been exposed to lyme disease, especially with the right symptomatology. If two or more of these specific bands are present, the likelihood increases even further. Horowitz has found that is specialty lab, such as IGeneX, has a better chance of finding more Borrelia specific bands on the Western blot, because it uses different strains of Borrelia burgdorferi for its testing (both the B31 and 297 strains).

Use of this laboratory often has been considered controversial among some IDSA physicians, many ILADS physicians find it to be a reliable resource. IGeneX has passed the strict testing guidelines of New York state and California, any is certified by the US government via the centres for Medicare and Medicaid services (CMS).

6. **Other tests:**
polymerase chain reaction (PCR), a DNA test, is an important diagnostic tool for patients who have negative blood tests: but many require od ball samples overtime, using specimens from different body compartments such as serum, aspirated joint fluids, synovial tissue, urine, cord blood, placenta, and or spinal fluid, and it must be performed at a reliable

laboratory.

The PCR has an overall sensitivity of around 30% on any individual specimen, with a specificity of over 99% (it is highly specific for the disease, with few false positive results). Recent advances in more sensitive PCR tests were reported by research in 2012 used direct molecular detection and genotyping of Borrelia (identifying the specific species) increased the sensitivity to 62% in early lyme disease. Horowitz says he may need to send off several sets of PCR's on blood all your before getting back a positive result.

Other tests are occasionally used to help confirm a clinical diagnosis: the lymphocyte trans formation test (LTT, Borrelia ELISpot) and a commercial culture of Borrelia (Advanced Laboratories).

The Borrelia culture became commercially available in 2012 through Advanced Laboratories. Culture of Borrelia is the gold standard for testing, and is universally accepted by IDSA, ILADS, and the CDC.

7. in the north-east of the United States, 10% to 20% of Borrelia presently in ticks are not Borrelia burgdorferi, the cause of lyme disease, but are genetically related to Borrelia miyamotoi, the agent of relapsing fever in Japan. These organisms will not test positive for lyme disease so that a patient with lyme like illness may actually have been exposed to other strains of Borrelia, explaining sero negative test results for lyme disease.

8. other tickborne diseases such as Babesia (a malaria like parasite) and Bartonella (cat scratch disease) can be transmitted with the same tick bite that transmits lyme disease. These diseases complicate the clinical presentation, often making the symptoms of lyme disease much worse.

They are similar if you're to diagnose reliably using standard screening procedures e.g. Babesia smear (Giemsa stain) and Bartonella titer (Immunofluorescent assay, IFA) then testing must not only include antibody titers, but may need to include PCR assays as well.

9. any positive titer for one tick borne disease (TBD) suggests that other TBDs maybe present, since ticks are multiple co-infected. This is especially true for patients failing treatment regiments for any one specific disease process.

Horowitz differential diagnostic system was developed as a roadmap for identifying the multiple components of multiple systemic infectious disease syndrome (MSIDS). He reviews the potential 16 differential diagnoses against the results of the questionnaire and patient history. By doing so he can gather the most complete assessment of his patients current health and what needs to be done regarding testing and treatment going forward.

d. Evidence of investments in contemporary research into Australian pathogens specifically acquired through the bite of a tick and including other potential vectors:

Hope Australia's preliminary testing and treatment of patients with Lyme disease has demonstrated that they can detect Borrelia burgdorferi and co infections and depress their Measured Voltage response [MV second column, Table 1 below] using bio-resonance.

This patient tested positive for Lyme disease with Pathology testing in USA.
The patient travelled to various Lyme clinics around the world with limited improvement. She received further treatment in Melbourne with

significant improvement in her symptoms due to infection with Borrelia and co infections.

IGeneX Inc 9/10/13
IGENEX IGM Result+ve
CDC/NYS result +ve
31kDa IND
34 kDa IND
83-93 kDa IND

Fry Laboratories
Special stains (Stained blood Film Test)
Moderate (11-20 organisms per total fields observed) coccobacilli adherent to erythrocytes. This is suggestive of epierythrocytic bacteria or an unspecified infections and/or parasitic disease.

Australia:
She tested +ve to bio- resonance testing as shown in Table 1 below . The MV=4 when Borrelia was first tested, is now 2,(baseline) following treatment.

Bioresonance testing of patient with Lyme disease signs and symptoms and +ve USA pathology tests for Borrelia:

Lyme infections	Resonant	MV	MV

		frequency	Tested	treated
Borrelia burgdorferi	Main infection	380,000	4	2
Mycoplasma	Co-infection	346,000	3	2
Herpes simplex	Co-infection	345,500	3	2
Herpes zoster	Co-infection	418,000	5	2
Epstein barre virus (EBV)	Co-infection	372,500	3	2
Cytomegalovirus (CMV)	Co-infection	409000	5	2
+ Patient's PAP smear shows cervical hyperplasia				

Table 1.

Notes: The bio-resonance machine generates frequencies from 80,000 to 560,000 cycles per second in 1000Hz steps and measures the response from the patient as MV after the frequency is transmitted to the patient. There is usually a baseline level of response, such as the 2 shown in the MV treated column following several weeks of treatment. Infections that have previously been experienced by the patient but which are no longer significant will have a baseline measurement after treatment. The recognition of the likely infection according to the registered frequencies are derived from Dr Clark's research. There is no linkage between the frequencies.

[http://www.huldaclarkzappers.com/frequency.pdf]

MV = millivolt, is the resonance response value to the frequency currently applied,[the voltage returned from the patient], following the application of a resonant frequency voltage, graphically it is the height of the peak seen during the bio-resonance scan.

Treatment:

If treatment immediately follows testing by feeding back the stored significant test frequencies, for 3 minutes each, then the MV response of the infections can be reduced close to a base line level and the symptoms of the patient improve.

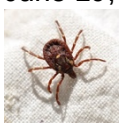
Immediate treatment, after testing, overcomes the problems of the infections changing their phenotypes before treatment.

Microbial toxins and other waste materials have to be cleared through the normal detoxification channels of the body (colon, liver and kidneys. These channels must be clear and functioning well in order to avoid autointoxication during treatment.

The preparation of the patient involves washing & drying their hands and being relaxed. They should be tested in a quiet room sitting in a comfortable chair or lying on an examination couch.

Researchers closer to ending debate around Lyme disease and ticks in Australia

June 29, 2015



As debate surrounding whether Lyme disease is associated with tick bites in Australia continues to rage, a team of Murdoch University researchers, together with colleagues at the **University of Sydney** **and** **Curtin University**, have made a discovery that helps solve part of the puzzle.
The research has provided new information about the bacteria associated with the Australian paralysis tick (*Ixodes holocyclus*) and their potential to cause disease in people.
Lead researcher, **Professor Peter Irwin** **of Murdoch University**, has been collecting ticks from around Australia to study whether they carry disease-causing bacteria.
"We did not find any evidence of the Lyme disease-causing bacterium *Borrelia burgdorferi*, but instead discovered a single isolate of a relapsing fever *Borrelia*, and other potential pathogens, including a new type of *Neoehrlichia* bacterium," Professor Irwin said.
The relapsing fever Borrelia and other bacteria found could potentially cause symptoms consistent with Lyme-like disease including extreme fatigue and nausea, but more research is needed to confirm this.
The research was complicated by the fact that bacteria in ticks are masked by large amounts of a single endosymbiont (an organism which lives within other organisms).
"We developed a new method of blocking amplification of the endosymbiont, or abundant bacteria, to reveal potential pathogens," Professor Irwin said.
"This research represents a new approach to what will be a challenging investigation to answer the most controversial and difficult of questions about which, if any, microorganisms transmitted by ticks cause illness in people in Australia."
The research provides a clearer picture but will not put an end to debate regarding a link between Lyme disease and ticks in Australia.
"We are still a long-way from knowing what, if any, disease is transmitted by ticks in Australia," Professor Irwin said.
"We need to test many more ticks yet."
This research has received funding from the **Australian Research Council** and from Bayer Animal Health and Bayer Australia. The research results have been published in the journal *Parasites and Vectors*.
- The Experts Box - - End the Experts Box -
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Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease

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Abstract

Ticks transmit more pathogens to humans and animals than any other arthropod. We describe the 2.1 Gbp nuclear genome of the tick, *Ixodes scapularis* (Say), which vectors pathogens that cause Lyme disease, human granulocytic anaplasmosis, babesiosis and other diseases. The large genome reflects accumulation of repetitive DNA, new lineages of retro-transposons, and gene architecture patterns resembling ancient metazoans rather than pancrustaceans. Annotation of scaffolds representing ~57% of the genome, reveals 20,486 protein-coding genes and expansions of gene families associated with tick–host interactions.

We report insights from genome analyses into parasitic processes unique to ticks, including host ‘questing', prolonged feeding, cuticle synthesis, blood meal concentration, novel methods of haemoglobin digestion, haem detoxification, vitellogenesis and prolonged off-host survival.

We identify proteins associated with the agent of human granulocytic anaplasmosis, an emerging disease, and the encephalitis-causing Langat virus, and a population structure correlated to life-history traits and transmission of the Lyme disease agent.

Ticks (subphylum Chelicerata: suborder Ixodida) are notorious ectoparasites and vectors of human and animal pathogens, transmitting a greater diversity of infectious agents than any other group of blood-feeding arthropods. Ticks are responsible for serious physical damage to the host, including blood loss and toxicosis.

Tick-borne diseases result in significant morbidity and thousands of human and animal deaths annually. The genus *Ixodes* includes multiple species of medical and veterinary importance, most notably serving as vectors of Lyme borreliosis in North America, Europe and Asia.

Lyme disease is the most prevalent vector-borne disease in the northern

hemisphere[1]. In the USA, 22,014 confirmed human cases were reported in 2012 (ref. [2]), with ~10-fold more infections suspected[3].

In Europe, ~65,500 Lyme borreliosis patients are documented annually[4]. In the USA, *Ixodes scapularis* also vectors the infectious agents that cause human babesiosis, human granulocytic anaplasmosis, tick-borne relapsing fever and Powassan encephalitis. The increased incidence and distribution of Lyme disease and other tick-borne diseases[5] necessitates new approaches for vector control.

Subphyla Chelicerata (includes ticks and mites) and Mandibulata (includes insects) shared a common ancestor 543–526 million years ago (Myr ago)[6].

Tick life cycles differ in many aspects from those of insects (Fig. 1) and include long periods of host attachment and blood feeding, as well as months living off-host without feeding. ‘Three-host' ticks such as *I. scapularis* require a host blood meal at each life stage. Feeding occurs over several days and involves a period of slow feeding followed, after mating and insemination, by rapid consumption of a large blood meal. The synthesis of flexible new cuticle is a unique feature that permits the engorgement of ixodid ticks during feeding[7]. Moulting occurs off-host, and the subsequent developmental stage will ‘quest' for a new host from vegetation. *I. scapularis* exhibits a wide host range including small, ground-dwelling vertebrates, birds, white-tailed deer and humans.

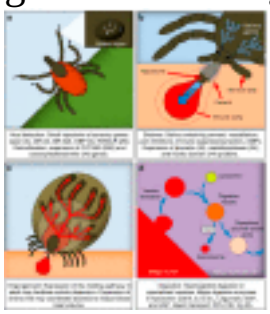

Figure 1

Genes associated with the unique parasitic lifestyle of *Ixodes scapularis*.

The *I. scapularis* genome assembly is the first for a medically important acarine species. It affords opportunities for comparative evolutionary analyses between disease vectors from diverse arthropod lineages and serves as a resource for the exploration of how ticks parasitize and transmit pathogens to their vertebrate hosts.

Results

The first genome assembly for a tick vector of disease

The assembly, IscaW1, comprises 570,640 contigs in 369,495 scaffolds (N50=51,551 bp) representing 1.8 Gbp, including gaps (Table 1, Supplementary Table 2). The *ab initio* annotation of 18,385 scaffolds >10 Kbp in length and representing 1.2 Gbp (57% of the genome) predicted 20,486 protein-coding genes, and 4,439 non-coding RNA genes (Supplementary Figs 1–6 and Supplementary Table 3). Ixodid ticks typically have haploid genomes that exceed 1 Gbp (ref. 8). In contrast, the 90 Mbp genome of the two-spotted spider mite, *Tetranychus urticae*, a horticultural pest, is the smallest of any known arthropod, and contains <10% transposable elements9. Repetitive DNA is estimated to comprise ~70% of the *I. scapularis* genome10, reflecting an extreme case of tandem repeat and transposable element accumulation.

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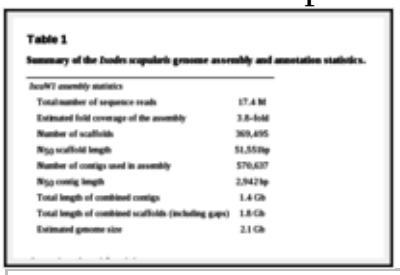

Table 1

caption a4

caption a8**Summary of the *Ixodes scapularis* genome assembly and annotation statistics.**

The *I. scapularis* genome possesses 26 acrocentric autosomes and two sex chromosomes (XX:XY)11,12. Fluorescent *in situ* hybridization (FISH)-based physical mapping was used to develop a karyotype and physical map12 (Fig. 2; Supplementary Tables 12 and 15). Mapping revealed that

tandem repeat accumulation in centromeric or peri-centromeric regions, also noted in some other arthropods13, is high in *I. scapularis* and comprises ~40% of genomic DNA10. The low complexity tandem repeat families, ISR-1, ISR-2 and ISR-3, account for ~8% of the genome12 (Supplementary Text). The most abundant ISR-2 (95–99 bp; ~7% of the genome) is localized at the near-terminal heterochromatic regions of the chromosomes (Fig. 2).

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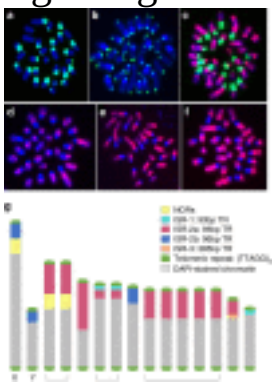

caption a8

Organization of DNA on the *Ixodes scapularis* chromosomes.The moderately repetitive fraction of the genome (~30% of genomic DNA10) contains numerous copies of Class I and Class II transposable elements (Supplementary Tables 13 and 14 and Supplementary Text). For example, 41 well-represented elements (that is, comprising a full-length canonical and/or consensus sequence (Supplementary Figs 7 and 8)) of the long-terminal repeat (LTR) retro-transposon family, estimated to make up <1% of the genome, were identified. Thirty-seven members of the Ty3/gypsy group were identified, with the remainder being Pao/Bel-like. Two (Mag and CsRn1) of the six well-known insect Ty3/gypsy lineages were confirmed in the tick and two new clades, Squirrel and Toxo, are likely specific to the subphylum Chelicerata (Supplementary Fig. 8). Structural characterization of elements belonging to these lineages revealed shared

features that include the CCHC gag and GPY/F integrase domains, and two ORFs matching gag and pol. The LTRs possess the TG..CA pattern[14] and their integration generates a duplication of 4 bp.

Non-LTR retro-transposons comprise about 6.5% of the genome. Sequence conservation and transposable element copy number suggest recent activity in the *I. scapularis* CR1, I and L2 clades; these elements are also abundant in birds, mammals and lizards, and the possibility of horizontal transposable element transmission warrants further investigation. The R2, RTE and LOA non-LTR retro-transposon clades found in mosquitoes and *Drosophila* were not identified in the tick.

Seemingly intact *mariner* and *piggyBac* transposable elements were identified, indicating possible recent or active transposition, and 234 miniature inverted-repeat transposable elements (MITEs) were annotated. These MITEs range in copy number from 50 to 14,500 and occupy ~5% of the genome. Collectively, these findings suggest a genome permissive to high repeat accumulation.

Approximately 60% of tick genes have recognizable orthologs in other arthropods, about half of which are maintained across representative species of the major arthropod lineages (Supplementary Fig. 9). Approximately 50% of the remaining genes have homologs and ~1/5th of tick genes appear unique (*T. urticae* has a similar proportion of unique genes); these provide an important resource to understand tick-specific processes and develop highly selective interventions.

Analysis of gene models and 20,901 tentative consensus sequences (the Gene Index Project; compbio.dfci.harvard.edu/tgi) compiled from 192,461 expressed sequence tags (ESTs) identified ~22% of *I. scapularis* genes as paralogs (Supplementary Note 1 and Supplementary Table 11). This is in line with estimates for *Homo sapiens* (15%)[15] and the nematode, *Caenorhabditis elegans* (20%)[16].

Complementary analyses of paralogs[17] suggest two duplication events in

I. scapularis, involving hundreds of genes that took place within the last 40 million years, consistent with the radiation of ticks through Europe, America and Africa. The tick mitochondrial genome retains the inferred ancestral arthropod organization as predicted by its phylogenetic position[18] (Supplementary Fig. 10).

The genome-scale quantitative molecular species phylogeny (Supplementary Text) inferred from single-copy orthologs from OrthoDB[19], confirms the expected position of Chelicerata as basal to crustaceans and insects (Fig. 3a). The rate of molecular evolution of *I. scapularis* genes is slightly slower than that of other representative arthropods, and considerably slower than the rapidly evolving dipterans.

Quantification of shared intron positions (Fig. 3b) and lengths (Fig. 3c) among orthologs reveals that *I. scapularis* shares greater than 10 times more intron positions exclusively with the non-arthropod species compared with the crustacean *Daphnia pulex* (Supplementary Figs 11–14 and Supplementary Tables 7–10).

The species tree topology is reconstructed using only intron presence/absence data, but its branch lengths reveal that *I. scapularis* intron positions are more similar to those of the outgroup species, than to the other arthropods.

This distinction is underscored by the contrasting length distributions of shared introns; *I. scapularis* lengths are most similar to those of mouse and other vertebrates, and an order of magnitude greater than in *D. pulex* and the representative insect species analysed. Ancestral eukaryotic genes likely possessed high intron densities similar to those of modern mammals[20].

The tick genome, therefore, supports an intron-rich gene architecture at the base of the arthropod radiation and more similar to that of ancestral metazoans than extant pancrustaceans.

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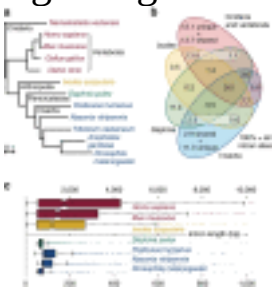

caption a8**Molecular and intron evolution of *Ixodes scapularis* orthologs.**

Ticks as parasites

Tick mouthparts (chelicerae and barbed hypostome) attach to and create a feeding lesion in the dermis of the host (Fig. 1b). Tick saliva consists of a complex mixture of peptides and other compounds that facilitate attachment and disarm host haemostasis, inflammation and immunity, thereby enabling prolonged blood feeding.

Antimicrobials in the saliva21 presumably prevent bacterial overgrowth within the ingested blood and/or feeding lesion. Transcriptome analyses indicate that tick saliva is exceptionally diverse compared with that of haematophagous insects22. Also, genes encoding salivary gland products are evolving rapidly in comparison with other gene families, possibly due to the immune pressure imposed by the host.

Notably, the genome reveals an expanded repertoire (74, 0.4% of the predicted proteome) of proteins containing a Kunitz domain (Supplementary Table 16), implicated in protease inhibition and channel-blocking activity, with roles in inhibiting coagulation, angiogenesis and vasodilation.

The tick genome is the richest source of this gene family identified to date. In contrast, only 0.05% of human and 0.1% of bovine proteins have this signature domain23, while the mosquito vectors *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles gambiae* have only five, eight and four

proteins with this domain, respectively.

Other tick gene expansions of note include the lipocalins (40 genes), linked to anti-inflammatory activity in other systems[24], and the metalloproteases (34 genes), which are involved in fibrin degradation and inhibition of angiogenesis[25]

Ticks have evolved a novel mechanism for haemoglobin digestion. Haemolysis of host erythrocytes occurs in the midgut but the digestion of blood meal proteins takes place within specialized vesicles of midgut epithelial cells following internalization by pinocytosis (Fig. 1d). Haemoglobin digestion occurs via a cascade of proteolytic enzymes resulting in dipeptides and free amino acids that are transcytosed into the haemolymph (Supplementary Text and Supplementary Table 21).

Orthologs of *Ixodes ricinus* haemoglobinolytic enzymes[26] were identified in the *I. scapularis* genome that contains multiple genes for *cathepsin D* (three genes), *cathepsin L* (three genes), and *serine carboxypeptidase* (four genes), suggesting the relative importance of these enzymes in haemoglobin digestion.

Haemoglobinolytic enzymes have also been identified in other tick species[27,28], suggesting that this mode of haemoglobin digestion is widespread throughout the Ixodida. Liberated haem is transported from the digestive vesicles by transport proteins to haemosomes, unique storage vesicles where haem is detoxified by formation of haematin-like aggregates[29].

Thus, haemoglobinolysis in ticks is similar to that in endoparasitic flatworms and nematodes. However, tick-specific intracellular digestion in midgut epithelial vesicles and haem detoxification in specialized haemosomes could offer novel acaricide targets (Supplementary Text and Supplementary Table 21).

Haem is associated with multiple essential functions as it complexes with proteins that perform oxygen transport and sensing, enzyme catalysis and electron transfer30. However, ticks are incapable of *de novo* haem synthesis, and it has been proposed that they rely on haem recovery from the diet31.

The identification of orthologous genes in *I. scapularis* for the enzymes hemF, hemG and hemH associated with the production of protohaem (Supplementary Fig. 15 and Supplementary Table 20) suggests these may be remnants of a once functional haem synthesis pathway that became redundant following adaptation to a blood diet.

In the absence of *de novo* synthesis, haem storage in ticks is likely essential, especially during the extended periods that occur between blood feeding and during egg development. In ticks, two families of storage proteins ensure haem availability and protect against the toxicity of a haem-rich diet: haemlipoglyco-carrier proteins (CPs) and the yolk proteins, vitellogenins (Vgs)32 (Fig. 1d).

CPs are predominant in all tick developmental stages except the embryo. In contrast, Vg is produced in the fat body and midgut of adult females during vitellogenesis (Fig. 4), and is transported via the haemolymph to the developing oocyctes where it is stored as vitellin. Vitellin is the main protein in the egg and the likely source of haem for developing embryos33. Ten putative *CP* genes, the most described from a tick to date, and two *Vg* genes were identified in the *I. scapularis* genome (Supplementary Fig. 16 and Supplementary Table 22).

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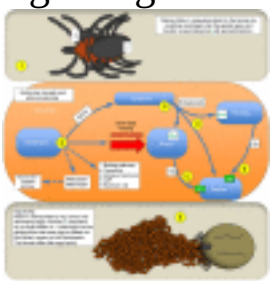

caption a8**Model of neuroendocrine processes controlling mating and egg production in *Ixodes scapularis*.**

The genome contains orthologs for at least 39 invertebrate neuropeptide genes (Supplementary Tables 25–28), including peptides that regulate ecdysis, cuticle synthesis, hardening and tanning. Orthologs involved in insect moulting34, that is, corazonin, eclosion hormone, cardioactive peptide and buriscon α and β, were identified (Fig. 4).

Additional novel putative neuropeptide genes were identified based on the presence of tandem repeats in conserved C-terminal sequences, including the canonical sequences for amidation and dibasic (or monobasic) cleavage signals (Supplementary Table 25).

ESTs matching corazonin, eclosion hormone and bursicon α and β were found in the synganglion transcriptome of adult *Dermacentor variabilis*35, which do not moult, suggesting previously unrecognized roles for these neuropeptide hormones. Companion analyses36 identified major differences in gene expression between *I. scapularis* and the soft tick, *Ornithodoros turicata* (Argasidae) in response to feeding that may explain how synganglion neuropetides regulate different life styles of the two tick families.

The identification of orthologs of neuropeptides known to regulate insect moulting provides a much needed starting point to understand the regulation of development in ticks and in the modification of cuticle to accommodate the approximately 100-fold increase in size that occurs during blood feeding (Fig. 4).

In ticks, over-hydration from large blood meals is counterbalanced by hormonally controlled salivary secretion into the host, presumably regulated by neuropeptides and their G-protein-coupled receptors (GPCRs) (Fig. 1c). The homologs of many insect neuropeptides, protein hormones, biogenic amines and associated GPCRs37 (Supplementary

Tables 25–28) that steer processes such as diuresis, behaviour, reproduction and development38, were identified in *I. scapularis*.

Some of the neuropeptide genes identified encode multiple neuropeptides. Of note is the extreme number of copies (19) of the kinin gene, which ranges from one to eight in other arthropods38 (Supplementary Table 28), suggesting that high peptide copy number is also needed for effective diuresis.

In accordance, four kinin GPCRs are present (Supplementary Table 28). The tick has 20 GPCRs for five biogenic amines, a number similar to that for all other sequenced arthropods37, suggesting an early evolutionary origin of these molecules and a core set of highly conserved arthropod signalling molecules. Typically in insects, each neuropeptide interacts with one, or at most two, GPCRs37.

Remarkably, the numbers of some neuropeptide GPCRs have expanded significantly (up to 10-fold) in *I. scapularis* (Supplementary Tables 26 and 28). This includes the GPCRs for AKH/corazonin-related peptide, allatostatin-A, diuretic hormones (calcitonin- and CRF-like), inotocin, kinin, pigment-dispersing-factor, sulfakinin, and tachykinin (Supplementary Table 28)37.
In insects, these GPCRs are involved in regulating meal size (kinin), satiety (sulfakinin) and diuresis (kinin, tachykinin and calcitonin-like diuretic hormone)38. In ticks, the increased efficacy and fine regulation of diuresis may be accomplished through an increased repertoire of diuretic GPCRs rather than via corresponding neuropeptides, emphasizing their potential as targets for tick control.

Blood feeding is essential for reproduction in adult female ticks (Fig. 4). In lower insects, reproduction is largely regulated by juvenile hormone III. Biochemical evidence suggests that ticks do not synthesize juvenile hormone III and instead employ ecdysteroids to initiate vitellogenesis (Fig. 4, reviewed in33). In insects, the final hydroxylations for the synthesis of ecdysteroids are performed sequentially by cytochrome

P450s (CYP450s) encoded by the *Halloween* genes (Supplementary Fig. 17 and Supplementary Table 19).

Genes for all steroidogenic CYP450s except for *phantom* were identified in the *I. scapularis* genome and putative gene duplications were identified for *disembodied* and the *spook/spookier* clades, suggesting conservation of ecdysteroid regulated processes between ticks and insects. Genes for seven of the nine enzymes in the insect mevalonate pathway that produces the juvenile hormone precursor, farnesyl-pyrophosphate (farnesyl-PP), were identified in the tick genome (Supplementary Fig. 18 and Supplementary Table 18).

There are five insect enzymes involved in the conversion of farnesyl-PP to juvenile hormone III. Only the gene for farnesol oxidase in the juvenile hormone branch was found in the *I. scapularis* genome (Supplementary Table 18) and is transcribed in the synganglion of *I. scapularis* and *D. variabilis*. The tick genome reveals a striking expansion of the methyl transferase family (44 genes) and EST data indicate that at least 26 of these are transcribed (Supplementary Fig. 19).

However, the *I. scapularis* methyl transferases studied so far lack the juvenile hormone binding motif. An ortholog of the insect cytochrome P450 (CYP15A1) that adds the epoxide to methyl farnesoate to produce juvenile hormone III was not found in either the tick genome (Supplementary Table 18) or synganglion transcriptomes.

The neuropeptides, allatostatin and allatotropin, which perform a variety of functions in insects, including the regulation of juvenile hormone biosynthesis, were also identified in the tick (Fig. 4).

Important questions remain as to the role of the mevalonate-farnesal pathway in tick reproduction and development. In a complementary study, transcripts for genes in the mevalonate-farnesal pathway were identified from the synganglion of two hard and one soft tick species[39].
The *I. scapularis* genome reflects a parasitic lifestyle requiring

detoxification of multiple xenobiotic factors (Fig. 1a).

We identified a record 206 CYP450 (Supplementary Table 23) and 75 carboxylesterase/cholinesterase-like genes, including five putative acetylcholinesterase genes (Supplementary Table 24). CYPs are haem-containing enzymes that catalyse biological oxidation reactions, many of which detoxify xenobiotics, including acaricides.

In contrast, the body louse, *Pediculus humanus*, also an obligate blood-feeding ectoparasite, has 36 CYPs, the fewest known in an animal[40], while the plant feeding mite, *T. urticae* has 81 (ref. [9]). Carboxylesterases are also associated with metabolic detoxification in animals.

While the function of these enzymes is not known, the abundance of these genes in *I. scapularis* may reflect the need to detoxify large blood meals from diverse hosts and toxicants encountered during off-host stages. As a parasite that lives largely off-host, *I. scapularis* has developed unique mechanisms for host detection that are reflected in the genome (Fig. 1a).

The sensory system in ticks includes setiform sensilla for chemo-, mechano-, thermo- and hygroreception, non-setal sensilla and dorsal light-sensing cells. Chemoreception occurs presumably through the unique Haller's organ located on the tarsi that are presented when ticks ‘quest' for a host. In insects, smell and taste are mediated by families of membrane receptors and extracellular ligand-binding proteins[41].

The chemoreceptor genes identified in the tick genome belong to the gustatory receptor and ionotropic glutamate receptor (iGluR)-related ionotropic receptor families. Sixty-two gustatory receptors were identified that fall into three major clades (Supplementary Fig. 20, Supplementary Table 29 and Supplementary Note 1).

The largest of the clades (43 genes) is exclusive to *I. scapularis* and the relatively short branch lengths compared with those for other

representative species, suggest a recent lineage-specific expansion. Although phylogenetically distant, this clade is related to the Dipteran sugar receptors and a set of three distinctive *D. pulex* gustatory receptors[42].

The second clade includes 16 tick gustatory receptors, also more closely related to the sugar receptors than to other representative gustatory receptors, with branch lengths suggesting an early diversification. The remaining clade (three genes) clusters with the largest *D. pulex* expansion. Of the 29 *IR/iGluR* genes identified, 15 are likely of the chemosensory type (ionotropic receptor) and 14 are canonical iGluRs (Supplementary Fig. 21 and Supplementary Tables 30 and 31).

Members of the insect odorant receptor, odorant-binding protein (OBP) and chemosensory protein B families[43] were not identified in the tick and only one member of the chemosensory protein (CSP) family was found. Our analysis supports the hypothesis that the origin of insect odorant receptors and OBPs occurred after the split of the lineages Hexapoda and Crustacea (~470 Myr ago)[42,44]; the CSPs, however, are predicted to appear before the split of the Chelicerata and Pancrustacea lineages.

Phylogenetic analyses indicate that odorant receptors belong to a divergent lineage originated from gustatory receptors, while OBPs could have derived from a CSP-like ancestor[44]. Both events may have occurred concomitantly as an adaptation of ancestral hexapods to the terrestrial environment (380–450 Myr ago).

Chelicerate olfaction may, therefore, rely exclusively on ionotropic receptors, which are expressed in olfactory organs across Protostomia[45], although it is also possible that some gustatory receptors have been recruited to this sensory function, as in *Drosophila melanogaster*[46].

Comparative transcriptomics has identified putative GPCRs, ionotropic receptors, odorant turnover enzymes and other transcripts specific to the

Haller's organ in ticks[47]. Evidence suggests the potential involvement of female specific cuticular lipids and a non-volatile mounting pheromone in *I. scapularis* during mating[48]. These data and morphological studies provide an emerging model for research on tick chemical communication and new control methods.
The tick possesses a small repertoire of photon-sensitive receptors compared with most insects. Genes for three opsin GPCRs were identified (Fig. 1a, Supplementary Table 26) and include orthologs of the insect putative long-wavelength sensitive ‘visual' opsins, the honey bee ‘non-visual' pteropsin likely involved in extraocular light detection and regulation of circadian rhythm[49], as well as the *D. melanogaster* Rh7 opsin[50].

Orthologs of the insect UV and short wavelength receptors were not identified. This indicates a reduced visual system as compared with other blood-feeding arthropods (Supplementary Text) that rely heavily on visual processes during flight for location of mates, hosts and oviposition sites.

During host detection, olfactory, mechano- and thermoreception may offset limited visual acuity and wavelength detection in the tick.

Ticks as vectors of pathogens and parasites

Ticks are biological vectors of viruses, bacteria and protozoa that are typically acquired via the blood meal and transmitted through saliva during feeding (Fig. 5). The tick immune system has several mechanisms to fend off pathogen invasion. Most components of the Toll, IMD (Immunodeficiency), JAK-STAT (Janus Kinase/Signal Transducers and Activators of Transcription) immune pathways and the RNA interference-antiviral signalling pathways were identified in the tick genome (Supplementary Figs 22 and 23 and Supplementary Table 17).

The repertoire of immunity-related genes also includes akirins, antimicrobial peptides, caspases, defensins, oxidases, the fibrinogen-

related protein family of ixoderins, lysozymes, thio-ester containing proteins and peptidoglycan-recognition proteins (Supplementary Table 17).
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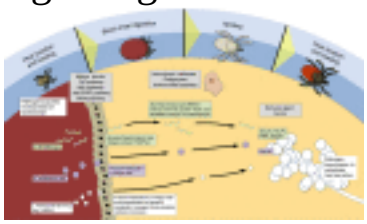

caption a8**Key features of pathogen transmission by *Ixodes scapularis*.**

Multiple infection factors facilitate transmission of the Lyme disease pathogen, *Borrelia burgdorferi* (Fig. 5). These include the tick salivary gland proteins Salp15, Salp20, Salp25D, tick salivary lectin pathway inhibitor and tick histamine-release factor, as well as the tick receptor for OspA and tick protein tre31, and the *Borrelia* lipoprotein BBE31 (ref. 51). Increasingly, research is focused on interactions with *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), the causative agent of human granulocytic anaplasmosis prevalent in the USA and Europe52. The *I. scapularis* proteins P11, SALP16, α1, 3-fucosyltransferases and the X-linked inhibitor of apoptosis E3 ubiquitin ligase are required for *A. phagocytophilum* infection and transmission, and modification of the tick cytoskeleton by *A. phagocytophilum* increases infection53,54,55. To establish infection, *A. phagocytophilum* inhibits apoptosis in midgut and salivary gland cells through the JAK/STAT and intrinsic pathways56. In response, the extrinsic apoptosis pathway is induced in tick salivary glands.

All known components of these pathways were identified in the tick with the exception of the Perforin ortholog (Supplementary Table 17). Systems biology analyses56 revealed that the generalized responses of tick cells to *A. phagocytophilum* infection include changes in protein processing in the endoplasmic reticulum and glucose metabolism. Protein misfolding is increased in infected tick cells, a possible strategy by which *A.*

phagocytophilum evades the cellular response to infection.

The subsequent activation of protein targeting and degradation, reduces endoplasmic reticulum stress and prevents cell apoptosis, and may also benefit the pathogen through provision of raw materials critical for an obligatory intracellular parasite with reduced biosynthetic and metabolic capacity[57].

In addition, *A. phagocytophilum* can induce an increase in expression of antifreeze glycoproteins, enhancing *I. scapularis* survival in cold temperatures[58], and downregulate Porin expression to inhibit apoptosis, increasing tick colonization[55,56].

Tick cells respond to pathogen infection by decreasing glucose metabolism and increasing Subolesin and Heat Shock Protein expression, and limiting rickettsial infection[59,60].

We used quantitative proteomics to further characterize tick–*Anaplasma* interactions, and identify differential protein expression in an *I. scapularis* ISE6 cell line in response to infection; 735 unique peptides assigned to 424 different *I. scapularis* proteins, were identified (Supplementary Tables 32–35).

In total, 83 proteins were differentially represented (50 under- and 33 over-represented; Supplementary Fig. 24 and Supplementary Table 32). Under-represented (13) and over-represented (8) proteins were identified during early infection (11–17% infected cells at 3 days post-inoculation). Most were also represented as infection advanced when the number of under- and over-represented proteins increased to 50 and 31, respectively (56–61% infected cells; 10 days post-inoculation).

Analysis of protein ontology demonstrated differences between under- and over-represented proteins in both early and late infections for cell growth (adducin, spectrin and β-tubulin) and transport (Na+/K+ ATPase, voltage-dependent anion-selective channel or mitochondrial porin and

fatty acid-binding protein; Supplementary Tables 32–34).

The genome of a *Rickettsia* (Alphaproteobacteria: Rickettsiales) species, *Rickettsia* endosymbiont of *Ixodes scapularis* (REIS), was assembled from both bacterial artificial chromosome clones and recruited whole-genome shotgun reads (available at GenBank, NZ_ACLC00000000).

Phylogenomics analysis of the REIS genome, which comprises a single 1.82 Mbp chromosome and four plasmids, indicates a novel non-pathogenic species that is ancestral to all Spotted Fever Group *Rickettsia* species, providing a valuable resource for understanding the evolution of symbiosis versus pathogenicity61.

Much less is known about the molecular mechanisms involved with viral interactions in ticks. Research suggests the RNA interference pathway provides an important defense against virus infection in tick cells, with a significant expansion of *Ago* genes in comparison with insects62. In a companion proteomics study of the *I. scapularis* ISE6 cell line following infection with the Langat virus63, 266 differentially expressed tick proteins were identified.

Functional analyses suggest perturbations in transcription, translation and protein processing, carbohydrate and amino acid metabolism, transport and catabolism responses. The majority of differentially expressed proteins were downregulated, similar to the proteomics profile described above. Interestingly, 121 differentially expressed proteins lacked homology to known orthologs, suggesting these may be unique to *I. scapularis*.

Population structure of *Ixodes scapularis* in North America

The restriction-site-associated DNA sequencing (RADseq) technique was employed for genome-wide discovery of single-nucleotide polymorphisms (SNPs) and examination of genetic diversity within and among eight *I. scapularis* populations from the north-east, mid-west and south-east regions of the USA and the Wikel reference colony. F-statistics were used to assess genetic distance as evidence of selection. FIS values

(range 0.003–0.012; Supplementary Table 36) suggest random mating or low levels of inbreeding among members comprising each population.

Further supporting this hypothesis, among all populations, the average observed heterozygosity (Ho) per variable SNP was comparable (range 0.013–0.016) to expected heterozygosity (He) (range 0.013–0.018) and the nucleotide diversity over all SNP loci (π) (range 0.015–0.019) was comparable among samples. FST values (range 0.03–0.16; Supplementary Table 37), support a single species classification for *I. scapularis* across North America as previously reported64.

Low-moderate genetic variation (FST=0.03–0.06) was observed among northern tick populations from Indiana, Maine, Massachusetts, New Hampshire and Wisconsin, and moderate variation (FST=0.07–0.09) among southern populations from Florida, North Carolina and Virginia. FST analyses revealed signatures of north–south structure in *I. scapularis* populations. Moderate-to-high genetic variation was observed between northern versus southern populations (FST=0.10–0.15). Interestingly, low genetic variation (FST=0.03–0.06) was observed between populations from the mid-west (Indiana and Wisconsin) versus the north-east (Maine, Massachusetts and New Hampshire), two areas associated with a high prevalence of human Lyme disease cases. As expected, moderate-to-high genetic variation was observed between the reference Wikel colony and field populations (FST=0.07–0.16).
The population structure of *I. scapularis* was separately analysed using a subset of representative SNPs. Membership probabilities, interpreted as proximities of individuals belonging to each cluster, revealed five clades (Fig. 6), with clear separation of the Wikel colony from field populations. Clustering of Indiana and New Hampshire, and Massachusetts, Maine and Wisconsin populations, indicates significant shared alleles, while the Virginia, Florida and North Carolina populations may share a small number of alleles. Interestingly, the population structure suggests a genetic component associated with differences in the natural history of northern and southern *I. scapularis* and a correlation to the prevalence of human Lyme disease cases. The incidence of Lyme disease is greatest in

the upper mid-west and north-east where *I. scapularis* populations feed predominantly on deer as adults and complete the life cycle over 2 years. In contrast, southern populations exploit a wider range of vertebrate hosts and are not quiescent during winter64,65. These data provide important resources to determine the genetic basis of host preference and vector competence, and the correlation with Lyme disease transmission.
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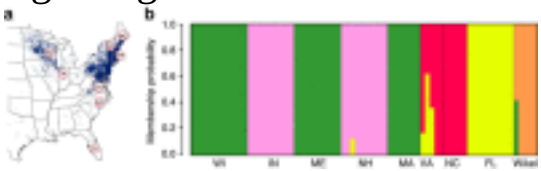

caption a8**Population structure of *Ixodes scapularis* across North America.** **Genome-based interventions to control tick-borne disease**
Prevailing methods of tick control rely heavily on the use of repellents and acaricides. Resistance to currently applied pesticides that disrupt neural signalling and tick development has prompted the search for novel targets. GPCRs represent a source of candidate targets for development of novel interventions. High-throughput target-based approaches have been employed to discover new mode-of-action chemistries that selectively inhibit the *I. scapularis* dopamine receptors66. The ligand-gated ion channels (LGICs) offer another rich source of targets. iGluRs play a major role in neurotransmission and chemosensory signalling within arthropods67. Twenty-nine putative *iGluR* genes and 32 putative cys-loop receptors were identified in the *I. scapularis* genome (Fig. 7, Supplementary Table 31). Among the *iGluR* genes, 14 encode members of the three principal subclasses of synaptic iGluRs (AMPA, Kainate and NMDA; Supplementary Fig. 21 and Supplementary Tables 30 and 31), while the remaining 15 more divergent sequences likely belong to the chemosensory ionotropic receptor subfamily (see above). The cys-loop LGIC family also contains six candidate glutamate-gated Cl− channels (GluCls), 12 nicotinic acetylcholine receptor subunits, and four GABA-gated chloride channels. One histamine-gated Cl− channel and one pH-gated Cl− channel gene were also identified. Both the iGluRs and cys-loop

LGIC families contain tick-specific genes with no apparent insect ortholog. This striking divergence may contribute to the apparent ineffectiveness of some insecticides on acaricidal targets[67]. Classifying LGIC candidates by functional expression is underway and an example is shown for a GluCl (Fig. 7; Supplementary Fig. 25). Selective targeting of tick LGICs and GPCRs may offer routes to new, safe and effective acaricides.

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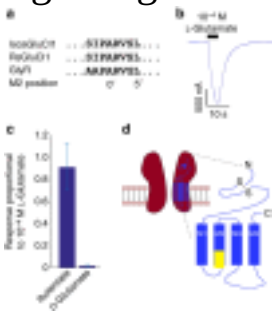

caption a8**De-orphanizing *Ixodes scapularis* receptors as candidate targets for the development of new acaricides.**Go to:

Discussion

The genome sequence of *I. scapularis*, the first for a medically important chelicerate, offers insights into the molecular processes that underpin the remarkable parasitic lifestyle of the tick and its success as a vector of multiple disease-causing organisms. Foundational studies of genome organization and population structure will advance research to determine the genetic basis of tick phenotypes, and efforts are ongoing to discover novel chemistries that selectively disrupt molecular targets mined from the genome. This study is a pioneering project for genome research on ticks and mites of public health and veterinary importance, with efforts proposed to expand genomic resources across this phyletic group. In 2011, the National Institutes of Health approved the sequencing of additional species of hard ticks, including European and Asian *Ixodes* species, the soft tick *Ornithodoros moubata* (Family Argasidae) and the *Leptotrombidium* mite vector of scrub typhus (Superorder Acariformes)[68]

(Supplementary Table 38). The *I. scapularis* genome offers a roadmap for research on tick–host–pathogen interactions to achieve the goals of the One Health Initiative69 and improve human, animal and ecosystem health on a global scale.

Go to:

Methods

Genome sequencing, assembly and annotation

The genome of *I. scapularis* Wikel strain was sequenced in a joint effort by the Broad Institute and the JCVI and funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health. The *I. scapularis* Wikel strain (Quinnipiac University, Hamden, CT) genome was sequenced to approximately 3.8-fold coverage using Sanger sequencing and assembled using the Celera Assembler configured to accommodate high repeat content within the genome and heterozygosity in the donor population (Supplementary Table 1). The assembly and raw reads are available at GenBank under the project accession ABJB010000000, consisting of contig accessions ABJB010000001-ABJB011141594 and VectorBase as IscaW1, 3 May 2012. The annotation of the *I. scapularis* genome was performed via a joint effort between the JCVI and VectorBase. The genome annotation release (IscaW1.4) is available at VectorBase (https://www.vectorbase.org/) and GenBank (accession ID: ABJB010000000). Forty-five bacterial artificial chromosome clones, ~183,834 ESTs and 45 microRNAs were also sequenced and annotated (Supplementary Figs 4–6 and Supplementary Tables 4–6).

Proteomics of *Ixodes-Anaplasma* interactions

The *I. scapularis* ISE6 cells were inoculated with *A. phagocytophilum* (human NY18 isolate) or left uninfected. Uninfected and infected cultures (n=5 independent cultures each) were sampled at early infection (11–17% infected cells (Avg±s.d., 13±2)) and late infection (56–61% infected cells (Avg±s.d., 58±2)) and used for proteomics. Protein extracts from the four experimental conditions, control uninfected early, infected early, control uninfected late and infected late (100 μg each) were gel-concentrated, digested overnight at 37 °C with 60 ng μl−1 trypsin (Promega, Madison, WI, USA) and the resulting tryptic peptides from each proteome were extracted and iTRAQ labelled for the analysis. The samples were

fractionated by isoelectric focusing and each fraction analysed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) using a Surveyor LC system coupled to a linear ion trap mass spectrometer model LTQ (Thermo Finnigan, San Jose, CA, USA) and protein identification was carried out using SEQUEST algorithm (Bioworks 3.2 package, Thermo Finnigan), allowing optional (Methionine oxidation) and fixed modifications (Cysteine carboxamidomethylation, Lysine and N-terminal modification of +144.1020 Da). The MS/MS raw files were searched against the alphaproteobacteria combined with the arachnida Swissprot database (Uniprot release 15.5, 7 July 2009) supplemented with porcine trypsin and human keratins. This joint database contains 638,408 protein sequences. False discovery rate of identification was controlled by searching the same collections of MS/MS spectra against inverted databases constructed from the same target databases. The alphaproteobacteria Swissprot database was used to identify *Anaplasma* and discard possible symbiotic bacterial sequences from further analyses.

Ixodes scapularis genetic diversity and population structure

74 RADseq libraries were produced from female *I. scapularis* representing nine ‘populations' from the states of Florida, Indiana, Maine, Massachusetts, North Carolina, New Hampshire, Virginia and Wisconsin and the Wikel reference colony. RADseq libraries were constructed using 1 μg genomic DNA from individual ticks, separately digested with the *Sbf*I restriction enzyme. Adaptor ligated libraries were pooled and sequenced at the Purdue Genomics Core Facility on the Illumina HiSeq 2500 in Rapid run mode. Further analysis was performed by the Bioinformatics Core at Purdue University. Illumina reads were corrected for restriction site, clustered and de-multiplexed (sorted by barcode) using the ‘process_radtags.pl' script of STACKS. For SNP identification, reads from each sample were separately aligned to the IscaW1 assembly using the end-to-end mode and default parameters of Bowtie2 v 2.1.0. Genetic diversity within and between *I. scapularis* populations was calculated using 745,760 SNPs across 35,460 polymorphic loci. F-statistics were used to assess genetic distance or differentiation as evidence of selection where FIS is the inbreeding coefficient of an individual (I) relative to the

subpopulation (S) and FST is the difference in allele frequency between subpopulations (S) compared with the total population (T). The population structure of *I. scapularis* across North America was separately analysed using a subset of 34,693 representative SNPs (1 SNP per polymorphic locus). The 'population' step from STACKS was used to analyse genetic diversity and fastStructure (beta release) was used to analyse population structure. Detailed methods are available in Supplementary Text. All variation data are available at NCBI SRA (SRP065406), VectorBase and via BioMart: http://biomart.vectorbase.org.

Functional expression of tick LGICs

Expression studies were performed on mature oocytes extracted from anaesthetised female *Xenopus laevis*. Briefly, complementary RNA encoding IscaGluCl1 was injected at 1 mg ml−1 using a Drummond Nanoject injector into oocytes that had been treated for 20–40 min in a 2 mg ml−1 solution of collagenase type 1A (Sigma UK) in calcium-free saline. Following 3–5 days incubation at 18 °C in saline supplemented with penicillin (100 units per ml), streptomycin (100 µg ml−1), gentamycin (50 µg ml−1) and 2.5 mM sodium pyruvate, oocytes were secured individually in a Perspex chamber (~90 µl) and perfused continually in saline at 5 ml min−1. They were impaled by two glass microelectrodes filled with 3 M KCl (resistance 1–5 MOhm in saline), with which the oocytes were voltage clamped at −100 mV using an Axoclamp 2A amplifier. Solutions were applied in the perfusing saline. The saline consisted of (in mM): NaCl 100, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, adjusted to pH 7.6 with 10 M NaOH.

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Additional information

Accession codes: The data reported in this paper are archived at GenBank under the project accession ABJB010000000, consisting of contig accessions ABJB010000001-ABJB011141594, and at VectorBase (IscaW1, 3 May 2012). The genome annotation release (IscaW1.4) is available at GenBank (accession ID: ABJB010000000) and VectorBase (https://www.vectorbase.org/) and RADseq data have been deposited in the NCBI Sequence Read Archive (SRA) under accession code SRP065406.

How to cite this article: Gulia-Nuss, M. *et al*. Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. *Nat. Commun.* 7:10507 doi: 10.1038/ncomms10507 (2016).

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Supplementary Material

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caption a9**Supplementary Information:**
Supplementary Figures 1-25, Supplementary Tables 1-38, Supplementary Note 1, Supplementary Methods and Supplementary References
Click here to view.(7.0M, pdf)

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Acknowledgments

This project has been funded in part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services (NIAID, NIH, DHHS) under contract numbers N01-AI30071, HHSN272200900007C, HHSN266200400001C and 5R01GM77117-5. Its contents are solely the responsibility of the authors and do not represent the official views of the NIH. Additional grants and contracts supporting work described in this manuscript were from the NIH-NIAID (HHSN266200400039C and HHSN272200900039C) to F.H.C., and a subcontract under HHSN272200900039C to C.A.H. and J.M.M., the **Australian Research Council** Discovery Project (DP120100240) to S.C.B. and R.S., the Ministerio de Ciencia e Innovación of Spain (BFU2007–6292; BFU2010–15484) to J.R., BIO2009–07990 and BIO2012–37926 to J.V. NIH-1R01AI090062 to Y.P., L.S., and J.K., NIH 1R21AI096268 and NSF IOS-0949194 to R.M.R., the Xunta de Galicia of Spain (10PXIB918057PR) to J.M.C.T. and M.T., BFU2011–23896 and EU FP7 ANTIGONE (278976) to J.F., the USDA-NRI/CREES (2008-35302-18820) and Texas AgriLife Research Vector Biology grant to P.V.P. and European Research Council Starting Independent Researcher Grant (205202) to R.B., J.M.R was supported by the intramural program of the NIAID, R.M.W. by a Marie Curie International Outgoing Fellowship PIOF-GA-2011–303312, E.M.Z. by Swiss National Science Foundation awards 31003A-125350 and 31003A-143936, J.M.G. by an NIH-NCATS award TL1 TR000162 and NSF Graduate Research Fellowship (DGE

1333468), V.C. by a Boehringer Ingelheim Ph.D. Fellowship, F.G.V. by a Fundação para a Ciência e a Tecnologia, Portugal fellowship (SFRH/BD/22360/2005), C.J.P.G. and F.H. by The Lundbeck Foundation (Denmark), and J.J.G. by NIH awards HHSN272200900040C, R01AI017828 and R01AI043006. Support from the Broad Genomics Platform is gratefully acknowledged.

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Footnotes

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Author contributions C.A.H., V.M.N. and S.K.W. wrote the genome sequencing proposal. C.A.H. and S.K.W. generated DNA and RNA for sequencing. E.C., D.L., V.M.N., C.M.F. B.B., K.N. and F.H.C. coordinated genome sequencing, assembly and automated annotation. C.A.H. coordinated genome analyses and J.F., M.G.-N., C.A.H., A.B.N, J.M.M., D.B.S., D.E.S., R.M.R., J.R. and R.M.W. coordinated manuscript preparation. All other authors are members of the *Ixodes scapularis* genome sequencing consortium and contributed annotation, analyses or data to the genome project.

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post-content

Int J Parasitol. 2016 Mar;46(3):213-20. doi: 10.1016/j.ijpara.2015.12.005. Epub 2016 Jan 23.

Amblyomma sculptum tick saliva: α-Gal identification, antibody response and possible association with red meat allergy in Brazil.

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Abstract

The anaphylaxis response is frequently associated with food allergies, representing a significant public health hazard. Recently, exposure to tick bites and production of specific IgE against α-galactosyl (α-Gal)-containing epitopes has been correlated to red meat allergy. However, this association and the source of terminal, non-reducing α-Gal-containing epitopes have not previously been

established in Brazil. Here, we employed the α-1,3-galactosyltransferase knockout mouse (α1,3-GalT-KO) model and bacteriophage Qβ-virus like particles (Qβ-VLPs) displaying Galα1,3Galβ1,4GlcNAc (Galα3LN) epitopes to investigate the presence of α-Gal-containing epitopes in the saliva of Amblyomma sculptum, a species of the Amblyomma cajennense complex, which represents the main tick that infests humans in Brazil. We confirmed that the α-1,3-galactosyltransferase knockout animals produce significant levels of anti-α-Gal antibodies against the Galα1,3Galβ1,4GlcNAc epitopes displayed on Qβ-virus like particles. The injection of A. sculptum saliva or exposure to feeding ticks was also found to induce both IgG and IgE anti-α-Gal antibodies in α-1,3-galactosyltransferase knockout mice, thus indicating the presence of α-Gal-containing epitopes in the tick saliva. The presence of α-Gal-containing epitopes was confirmed by ELISA and immunoblotting following removal of terminal α-Gal epitopes by α-galactosidase treatment. These results suggest for the first known time that bites from the A. sculptum tick may be associated with the unknown etiology of allergic reactions to red meat in Brazil.

KEYWORDS:
Alpha-Gal; Amblyomma sculptum; IgE; Red meat allergy; Tick saliva
PMID: 26812026 [PubMed - in process]

Sci Rep. 2016; 6: 18920.
Published online 2016 Jan 6. doi: 10.1038/srep18920
PMCID: PMC4702108

Mitochondrial genome evolution and tRNA truncation in Acariformes mites: new evidence from eriophyoid mites

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Funding: **Australian Research Council (DP120100240 to RS), and Australia-China Science & Research Fund (ACSRF00980 to RS).**
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Go to:

Abstract

article-meta
The subclass Acari (mites and ticks) comprises two super-orders: Acariformes and Parasitiformes. Most species of the Parasitiformes known retained the ancestral pattern of mitochondrial (mt) gene arrangement of arthropods, and their mt tRNAs have the typical cloverleaf structure. All of the species of the Acariformes known, however, have rearranged mt genomes and truncated mt tRNAs. We sequenced the mt genomes of two species of Eriophyoidea: *Phyllocoptes taishanensis* and *Epitrimerus sabinae*. The mt genomes of *P. taishanensis* and *E. sabinae* are 13,475 bp and 13,531 bp, respectively, are circular and contain the 37 genes typical of animals; most mt tRNAs are highly truncated in both mites. On the other hand, these two eriophyoid mites have the least rearranged mt genomes seen in the Acariformes. Comparison between eriophyoid mites and other Aacariformes mites showed that: 1) the most recent common ancestor of Acariformes mites retained the ancestral pattern of mt gene arrangement of arthropods with slight modifications; 2) truncation of tRNAs for cysteine, phenylalanine and histidine occurred once in the most recent common ancestor of Acariformes mites whereas

truncation of other tRNAs occurred multiple times; and 3) the placement of eriophyoid mites in the order Trombidiformes needs to be reviewed.
Eriophyoidea is the largest superfamily in the subclass Acari (mites and ticks), with more than 4,400 described species[1]. Eriophyoid mites have exceptional morphological and biological characters in comparison to other mites and ticks: two pairs of legs (instead of four pairs), small body size (~200 μm body length), and high host specificity[2], to name a few. Some species of eriophyoid mites are pests to crops[3] and forests[4], such as the wheat curl mite, *Aceria tosichella*[5,6]. Other than causing yield loss, some eriophyoid mites can transmit and spread viruses, which can cause further damages to plants[6,7].
The typical animal mt genome is circular, 15–20 kb in length with 37 genes[8], and was found in most mites and ticks investigated to date except for *Steganacarus magnus* (16 tRNA genes not identified, possibly due to tRNA truncation)[9], *Metaseiulus occidentalis* (*nad3* and *nad6* not identified, 18 genes duplicated)[10] and *Leptotrombidium pallidum* (2 genes duplicated)[11]. Of the 39 species from the superorder Parasitiformes investigated to date, 19 species retained the ancestral pattern of mt gene arrangement for arthropods; these species are from four families: Argasidae (soft ticks, 11 species)[12,13,14], Allothyridae (holothyroid mites, 1 species)[12], Nuttalliellidae (hard ticks, 1 species)[13] and Ixodidae (hard ticks, 6 species)[15,16,17]. Rearrangement of mt genes was found in the other 20 species from five families: Ixodidae (16 species)[18,19], Varroidae (1 species)[20], Phytoseiidae (2 species)[10,21] and Ologamasidae (1 species)[22]. In contrast to Parasitiformes, all of the 27 species from the other superorder, Acariformes, have rearranged or highly rearranged mt genomes (details below).
The mt tRNAs of animals usually possess a cloverleaf secondary structure composed of four arms: AA-arm, D-arm, AC-arm and T-arm[23]. The only exception is the tRNA for serine (anticodon GCT), which lost D-arm in nearly all animals; this is apparently an ancestral feature for animals[23]. Loss of D-arm or T-arm occurred in other tRNAs but was not common in animals. Large-scale tRNA truncation was first found in nematodes, in which 20 of the 22 tRNAs lack T-arm and the two tRNAs for serine (anticodons GCT and TGA) lack the D-arm[24]. Later, truncated tRNAs

were also found in Acariformes11, Araneae25,26,27, Pseudoscorpiones28, Scorpiones29,30, Thelyphonida30, Acanthocephala31, Insecta32 and Protura33. All of the 25 species of Acariformes mites known have many truncated tRNA genes whereas species from the other superorder, Parasitiformes, do not have truncated tRNAs except for the tRNA for serine (anticodon GCT), and the tRNA for cysteine, which lacks D-arm in *Varroa destructor*20, *Phytoseiulus persimilis*21, *M. occidentalis*21, *Haemaphysalis flava*14, *Rhipicephalus microplus*14 and *R. sanguineus*14. Prior to this study, no complete mt genomes have been investigated for eriophyoid mites. To understand the evolution of mt genomes in the Acariformes, we sequenced the mt genomes of two eriophyoid mites, *Phyllocoptes taishanensis* Xue & Hong, 2005 (Eriophyidae: Phyllocoptini) and *Epitrimerus sabinae* Xue & Hong, 2005 (Eriophyidae: Phyllocoptini). We found rearrangement of mt genes in both eriophyoid mites relative to the hypothetical ancestor of arthropods. Further, both species have highly truncated tRNAs. Here, we present the novel features of the mt genomes of *P. taishanensis* and *E. sabinae* and discuss the evolution of mt genomes in the Acariformes, tRNA truncation and the phylogeny of eriophyoid mites in the light of new evidence from these two mites.

Go to:

Results

General features of the mt genomes of the two eriophyoid mites, *P. taishanensis* and *E. sabinae*

The mt genomes of *P. taishanensis* and *E. sabinae* are 13, 475 bp and 13, 531 bp long respectively, are circular and have 37 genes: 13 protein-coding genes (PCG), two ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes (Fig. 1; Table 1). Genes are on both strands: one strand has 27 genes whereas the other strand has 10 genes. The start codons of the 13 PCGs were ATN in *E. sabinae*. In *P. taishanensis*, 11 of the 13 PCGs use ATN as start codons whereas *cox1* and *atp8* appear to start with CTG, which is a rare start codon for animal mitochondria (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi ?chapter=cgencodes). We noticed that in the water mites, *Unionicola parkeri* and *U. foili*, another rare start codon, TTG, was used34,35. The stop codons were TAA or TAG in both eriophyoid species; incomplete

stop codons, T, was found in protein-coding genes that precede a tRNA gene. In both species of eriophyoid mites, TAA was the most common stop codon and was used in nine of the 13 PCGs in *P. taishanensis* and 10 of the 13 PCGs in *E. sabinae* (Table 1). The putative control region (CR) of *P. taishanensis* is short, only 47 bp in size, lying between *rrnL* and *trnW*. The putative CR of *E. sabinae* is 94 bp and is in a different location between *trnK* and *atp6*. No conserved regions were found between the CRs of the two eriophyoid mites. No other non-coding regions longer than 16 bp were found in the mt genomes of these two mites.

fig ft0fig mode=article f1

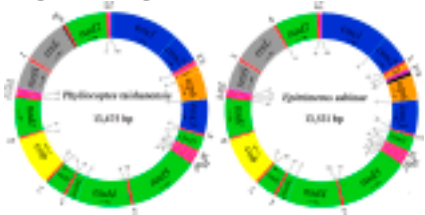


Figure 1

caption a4

caption a8**Map of the mitochondrial genomes of *Phyllocoptes taishanensis* (A) and *Epitrimerus sabinae* (B).**

table ft1table-wrap mode=article t1

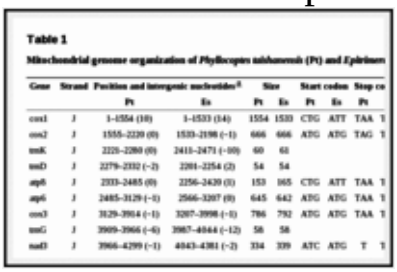


Table 1

Mitochondrial genome organization of *Phyllocoptes taishanensis* (Pt) and *Epitrimerus*

Gene	Strand	Position and intergenic nucleotides[a]		Size		Start codon		Stop co	
		Pt	Es	Pt	Es	Pt	Es	Pt	
cox1	J	1–1554 (10)	1–1533 (14)	1554	1533	CTG	ATT	TAA	T
cox2	J	1555–2220 (0)	1533–2198 (−1)	666	666	ATG	ATG	TAG	T
trnK	J	2221–2280 (0)	2413–2471 (−10)	60	61				
trnD	J	2279–2332 (−2)	2201–2254 (2)	54	54				
atp6	J	2333–2485 (0)	2256–2420 (1)	153	165	CTG	ATT	TAA	T
atp6	J	2485–3129 (−1)	2566–3207 (0)	645	642	ATG	ATG	TAA	T
cox3	J	3129–3914 (−1)	3207–3998 (−1)	786	792	ATG	ATG	TAA	T
trnG	J	3909–3966 (−6)	3987–4044 (−12)	58	58				
nad3	J	3966–4299 (−1)	4043–4381 (−2)	334	339	ATC	ATG	T	T

Table 1

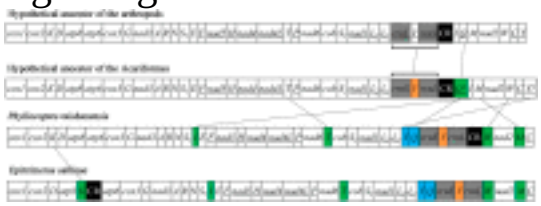
caption a4

caption a8**Mito genome organi *Phyllocoptes ta* and *Epitrimeru***

Eriophyoid mites have the least rearranged mt genomes among Acariformes mites

Like in other mites of the Acariformes, rearrangement of mt genes occurred in both of the eriophyoid mites. We calculated breakpoints with CREx as a measure of the extent of mt gene rearrangement from that of

the hypothetical ancestor of arthropods[36]. Among the 12 different patterns of mt gene arrangement observed in the 27 species of Acariformes mites known, *P. taishanensis* has the least rearranged mt genome with 13 breakpoints and *E. sabinae* has the third least rearranged mt genome with 16 breakpoints (Table 2). In *P. taishanensis*, two rRNA genes (*rrnS* and *rrnL*) and seven tRNA genes (*trnI*, *trnT*, *trnY*, *trnQ*, *trnV*, *trnW*, *trnM*) changed their locations relative to their counterpart genes in the hypothetical ancestor of arthropods. In *E. sabinae*, two rRNA genes (*rrnS* and *rrnL*) and eight tRNA genes (*trnK*, *trnI*, *trnT*, *trnY*, *trnQ*, *trnV*, *trnW*, *trnM*) changed locations (Fig. 2). Four derived gene arrangements, *trnS1-trnI-trnE*, *nad6-trnT-cob*, *trnY-trnQ-rrnS-trnV-rrnL*, and *trnW-nad2-trnM-trnC*, were found in both eriophyoid mites but not in any other mites; these novel gene arrangements were candidate synapomorphies (i.e. shared derived characters) for the eriophyoid mites.
fig ft0fig mode=article f1



caption a8**Mitochondrial gene arrangements in the hypothetical ancestor of Acariformes, *Phyllocoptes taishanensis* and *Epitrimerus sabinae*.**table ft1table-wrap mode=article t1

Table 2

Mitochondrial gene-arrangement breakpoints relative to the hypothetical ancesto

Superorder	Order	Taxon	Number of breakpoints
Parasitiformes	Ixodida	Argasidae (11 species)	0
Parasitiformes	Ixodida	Ixodidae (16 species)[1]	7
Parasitiformes	Mesostigmata	*Sylochyrus rarior*	7
Acariformes	Trombidiformes	*Phyllocoptes taishanensis*	13
Parasitiformes	Mesostigmata	*Varroa destructor*	14
Acariformes	Trombidiformes	Demodicidae (2 species)	15
Acariformes	Trombidiformes	*Epitrimerus sabinae*	16
Acariformes	Sarcoptiformes	Sarcoptiformes (6 species)	24
Acariformes	Trombidiformes	*Ascoschoengastia* sp.	25
Acariformes	Trombidiformes	*Walchia hayashi*	25

caption a8**Mitochondrial gene-arrangement breakpoints relative to the hypothetical ancestor of arthropods.**

Truncated tRNAs of the eriophyoid mites

Fifteen mt tRNA genes of *P. taishanensis* and 16 mt tRNA genes of *E.*

sabinae were identified with tRNAscan-SE37 or ARWEN38 programs. The other seven tRNA genes of *P. taishanensis* (*trnA*, *trnG*, *trnQ*, *trnR*, *trnS1*, *trnT*, *trnV*) and the other six tRNA genes of *E. sabinae* (*trnA*, *trnG*, *trnI*, *trnQ*, *trnR*, *trnS1*) were found manually based on conserved nucleotides and the anticodon sequences. The putative mt tRNAs were highly truncated in both *P. taishanensis* (47 to 61 bp) and *E. sabinae* (47 to 67 bp) (Figs 3 and
and4).
4). Sixteen of the 22 tRNAs have atypical secondary structures, missing either D-arm or T-arm in both mites. The majority of tRNAs also have mismatches on T-arm, D-arm, acceptor arm or anticodon arm.
fig ft0fig mode=article f1

caption a8**Inferred secondary structures of the 12 mt tRNAs of *P. taishanensis* (Pt) and *E. sabinae* (Es).**fig ft0fig mode=article f1

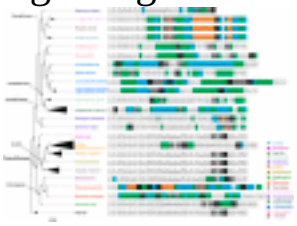
caption a8**Inferred secondary structures of the 10 mt tRNAs of *P. taishanensis* (Pt) and *E. sabinae* (Es).**

The phylogenetic position of Eriophyoidea

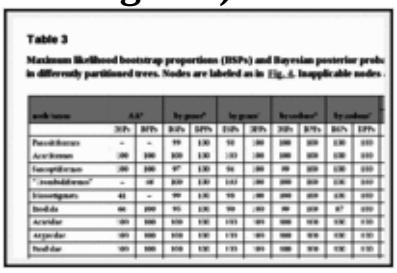
The super-family Eriophyoidea was traditionally in the order Trombidiformes. In our analysis, however, the two eriophyoid species

were not grouped with spider mites (Tetranychidae), follicle mites (Demodicidae), chigger mites (Trombiculidae) and water mites (Unionicolidae), which were in the Trombidiformes. Rather, these two eriophyoid mites were grouped with Sarcoptiformes mites in ML and BI trees with strong support (Figures S1A-S1B, S1F, BSPs =100; Figure S2A, BPPs =100), or at the base in the Acariformes (Fig. 5, Figures S1C-S1E, Figures S2B-S2H). Excluding the two eriophyoid mites, the other Trombidiformes mites formed a monophyletic group with strong support (Table 3; Figures S1A-S1D, BSPs =100; Figures S2A, S2C-S2H, BPPs =100; Figure S2B, BPPs = 58). In addition, our phylogenetic analyses support the monophyly of Acariformes in all ML and BI trees (Table 3; Fig. 5, Figures S1A-S1F, BSPs >97; Figures S2A-S2I, BPPs =100). Monophyly of the other superorder, Parasitiformes, was recovered in ML and BI trees constructed with nucleotide sequences (Table 3; Figure S1A-S1D, S1F, BSPs >98; Figure S2A, S2C-S2I, BPPs =100), but was rejected in ML and BI trees constructed with amino acid sequences (Table 3, Figure S1E, Figure S2B). Also, three orders (Ixodida, Mesostigmata and Sarcoptiformes) were recovered as monophyletic groups in most of our analyses (Table 3, Fig. 5). Nine families (Acaridae, Argasidae, Demodicidae, Eriophyidae, Ixodidae, Phytoseiidae, Tetranychidae, Trombiculidae and Unionicolidae) were recovered as monophyletic groups in all of our analyses (Table 3, BSPs =100, BPPs =100). Intriguingly, monophyly of Acari was rejected as Pseudoscorpiones was grouped with Acariformes with strong support (Figure S1F, BSPs =98; Figure S2I, BPPs = 100). Our result thus conflicts with previous analyses based on rDNA in which Solifugae was grouped with Acariformes[39,40,41], indicating the weaknesses of current hypotheses on the ordinal relationships in the Arachnida.

fig ft0fig mode=article f1



caption a8**Maximum likelihood trees inferred with nucleotide sequences by 16 partitions (13 PCGs, 2 rRNA genes and concatenated tRNA genes).**table ft1table-wrap mode=article t1



caption a8**Maximum likelihood bootstrap proportions (BSPs) and Bayesian posterior probabilities (BPPs) for nodes in differently partitioned trees. Nodes are labeled as in Fig. 4. Inapplicable nodes are indicated with.**Go to:

Discussion

The ancestral pattern of mt gene arrangement of Acariformes mites

Mitochondrial gene arrangement is relatively stable within major animal lineages8. This is, however, not the case for the Acari (ticks and mites). For the superorder Parasitiformes, the ancestral pattern of mt gene arrangement to arthropods was found in 11 species of Argasidae, one species of Allothyridae and six *Ixodes* species of Ixodidae (Fig. 5). Four Mesostigmata species and 16 Ixodidae species have rearrangement of mt genes (Fig. 5, Table 2). In the other superorder Acariformes, however, rearrangement of mt genes was found in all of the 27 species whose mt genomes have been sequenced. Even the species in the same genus differ from one other in mt gene arrangement, such as the two water mites in the genus *Unionicola*34,35 and three chigger mites in the genus *Leptotrombidium*11,42 (Fig. 5). The two eriophyoid mites investigated in the current study were from the same tribe (Phyllocoptini) of the family Eriophyidae but differ from one another in mt gene arrangement.

What was the ancestral pattern of mt gene arrangement for Acariformes mites ? Establishment of the ancestral pattern will lay the ground for understanding how mt genome organization evolved in Acariformes

mites. Intriguingly, in comparison to other species of the Acariformes, the two eriophyoid mites have the least rearranged mt genomes and retained most of the ancestral pattern of mt gene arrangement of arthropods (Figs 2 and
and5).
5). The conserved mt gene-arrangement characters present in the two eriophyoid mites and other Acariformes mites allowed us to infer that the ancestral pattern of mt gene arrangement of the Acariformes retained likely the ancestral pattern of mt gene arrangement of arthropods with slight modifications (Fig. 2). The modifications include: 1) translocations of *trnQ* and *trnY*; these two tRNA genes are rearranged in all of the 27 species of Acariformes mites investigated to date and furthermore, their locations vary among different Acariformes lineages (Figs 2 and
and5)
5) inversion of *rrnS-trnV-rrnL* as a cluster, which is seen in the two eriophyoid mites and five Sarcoptiformes mites (Fig. 5); however, in seven of the 27 Acariformes species, *rrnS* and *rrnL* are translocated but not inverted, so there are alternative interpretations for the location and transcription orientation of *rrnS-trnV-rrnL* in the ancestor of Acariformes mites.

tRNA truncation in Acariformes mites

Truncated mt tRNAs have been found in several orders of the class Arachnida including Araneae25,26,27, Acariformes11, Pseudoscorpiones28, Scorpiones29,30, and Thelyphonida30. In the superorder Acariformes, truncated tRNAs are common and have been observed in all of the 27 species whose mt genomes have been sequenced. Sixteen of the 22 tRNAs in the two eriophyoid mites investigated in the current study lack D-arm or T-arm (Figs 3 and
and4).
4). Nineteen tRNAs lack D-arm or T-arm in spider mites (Tetranychidae); in some species, the tRNAs for phenylalanine and glutamine lack both D-arm and T-arm (Table S1)43,44. Fifteen tRNAs lost D-arm or T-arm in Demodicidae, including five tRNAs losing both D-arm and T-arm45. Twenty tRNAs in Pyroglyphidae46,47, 17 to 18 tRNAs in Trombiculidae11,34,42, 21 tRNAs in Acaridae and Psoroptidae48,49,50, 15 tRNAs in Unionicolidae34,35, lack D-arm or T-arm (Table S1).

Large-scale tRNA truncation was first observed, and best studied in nematodes[24]. Twenty of the 22 tRNAs lack T-arm and the two tRNAs for serine lack D-arm in all nematodes except for several species in the class Enoplea. In *Trichinella spiralis*, eight tRNAs have the cloverleaf secondary structure with both D- and T-arms whereas several others lack both D- and T-arms[51]. tRNA truncation in the Acariformes mites has some similarity to that in nematodes but has their distinctive features. First, *trnK* has the typical cloverleaf structure in all Acariformes mites except for *Steganacarus magnus*[9] (but K was not identified in this mite, see Table S1), whereas the other 21 tRNAs lack either D-arm or T-arm or both arms in one or more species of Acariformes mites. Three tRNAs, for cysteine, phenylalanine and histidine respectively, lack T-arm in all known Acariformes mites (Table S1), indicating that T-arm loss in these three tRNAs is likely ancestral to Acariformes mites. The other 18 tRNAs, however, vary in their secondary structures among the Acariformes mites: either having a cloverleaf structure, or lacking D-arm, T-arm or both arms (Table S1). The pattern of D-arm or T-arm loss appears to be consistent within a family but differs between families (Table S1). Thus, the loss of D-arm or T-arm in these tRNAs may have occurred multiple times independently in different lineages of the Acariformes mites.

Limited evidence from nematodes indicates that truncation does not seem to disable mt tRNAs from function. Okimoto *et al.* hybridized mt tRNA gene-specific probes to the RNAs of two nematodes, *Caenorhabditis elegans* and *Ascaris suum*, and obtained evidence for the transcription of at least nine *C. elegans* and three *A. suum* mt tRNA genes[52]. Each tRNA transcript has the same size of its corresponding tRNA gene, to which three nucleotides (CCA) were added after transcription. Okimoto *et al.* concluded that the mt tRNAs without D-arm or T-arm were functional. Further, Suematsu *et al.* and Ohtsuki and Watanabe showed that the evolution of truncated mt tRNAs in nematodes was linked to EF-Tu protein: the paralogs of this protein acquired differential binding abilities to tRNAs with deleted domains[53,54]. Recently, Juhling *et al.* showed computational evidence that tRNAs of the nematodes of the class Enoplea that lack both D-arm and T-arm were functional[51]. The Acariformes mites provided another system for further experimental and computational

investigation into the evolution and function of truncated mt tRNAs.

Should Eriophyoidea be placed in the order Trombidiformes?

The subclass Acari comprises two superorders, Acariformes and Parasitiformes[55]. The former includes two orders (Trombidiformes and Sarcoptiformes), while the later include four orders (Opilioacarida, Holothyrida, Ixodida, Mesostigmata)[55]. The monophyly of Acari, however, is not without controversy, when species of other major lineages of Arachnida were included into analyses[28,40,41,56]. Pseudoscorpiones was always grouped with Acariformes in our analyses, which is consistent with Ovchinnikov and Masta[28]. Several phylogenetic studies of Acariformes[39,40,41] and Parasitiformes[57] have been conducted previously using molecular data; however, eriophyoid mites were not included. The current taxonomic assignment of Eriophyoidea to the order Trombidiformes[55] is controversial, due to the distinctive morphology of eriophyoid mites such as having only two pairs of legs, lack of ontogenetic diversity and absence of respirator system[58]. Indeed, André placed Eriophyoidea outside Trombidiformes[59] Our analyses based on different partitions and inference methods showed consistently that Acariformes, Parasitiformes and several families (Acaridae, Argasidae, Demodicidae, Ixodidae, Phytoseiidae, Tetranychidae, Trombiculidae and Unionicolidae) were monophyletic, which was consistent with previous studies using mt genes[9,18,27,39,44,45,46,50] or nuclear genes[18,39,57]. The monophyly of Trombidiformes, however, was rejected in our analyses (Figure S1, Figure S2). The monophyly of Trombidifromes was also rejected in a recent study by Gu *et al.* based on the mt genome sequences of 16 species of Acariformes mites due to spider mites (Tetranychidae) grouped with sarcoptiform mites[50]. In our analyses, when the two species of eriophyoid mites were excluded, the rest of the Trombidiformes species were always together in a monophyletic group. Our results thus raised the need for further phylogenetics studies on Acariformes mites with more taxa included especially from Tydeoidea and Eupodoidea, which were thought to be the sister groups of Eriophyoidea[60]. Further morphological studies are also necessary to elucidate the position of eriophyoid mites in Acariformes.

In conclusion, we sequenced the mt genomes of two species of eriophyoid mites, *P. taishanensis* and *E. sabinae*. These two mites have the least

rearranged mt genomes seen in the Acariformes and have highly truncated mt tRNAs. Our comparison between the eriophyoid mites and other mites and ticks showed that the most recent common ancestor of Acariformes mites retained the ancestral pattern of mt gene arrangement of arthropods with slight modifications. The truncation of three tRNAs (for cysteine, phenylalanine and histidine, respectively) likely occurred once in the common ancestor of Acariformes mites. Truncation of other tRNAs, however, occurred multiple times independently in different lineages of Acariformes mites. Our phylogenetic analyses of mt genome sequences rejected the monophyly of the order Trombidiformes when eriophyoid mites were included. Further phylogenetics studies on Acariformes mites including more taxa from different lineages is needed to clarify the position of eriophyoid mites.

Go to:

Methods

Collection of mites

E. sabinae and *P. taishanensis* were collected in May 2013 in Nanjing, China. *E. sabinae* was collected from *Juniperus chinensis* (Cupressaceae) (China savin), while *P. taishanensis* from *Cedrus deodara* (Pinaceae) (Deodar cedar). Mite samples were either used immediately for DNA extraction or were preserved in 100% ethanol at −20 °C prior to DNA extraction. Samples of each eriophyoid species were also mounted to slides as voucher, using modified Berlese medium[61] for morphological check with Zeiss A2 (microphoto camera AxioCam MRc) microscope. All of the specimens and vouchers were deposited at the Arthropod Collection, Department of Entomology, Nanjing Agricultural University, China.

DNA extraction, mt genome amplification and sequencing

Genomic DNA was extracted from both individual and pooled specimens for each species, using a DNeasy Blood and Tissue Kit (QIAGEN), following the modified protocol[62]. For *E. sabinae*, a 658-bp fragment of *cox1* and a 413-bp fragment of *rrnL* were initially amplified by PCR with the primer pairs LCO1490–HCO2198[63] and LRJ12287–LRN13398[64] (see Additional file Table S2). PCR products were purified and sequenced directly using Sanger method at Majorbio (Shanghai, China). Specific primers for *E. sabinae*, ECOISR3 and E16SSR2, were designed from the

sequences of the *cox1* and *rrnL* fragments, respectively. PCR with these two primers produced a 1.7-kb amplicon, which was sequenced using Sanger method at Majorbio. Another pair of primers, PF2–PR2, were designed from the sequence of the 1.7-kb amplicon. An 11.2-kb amplicon was produced with PF2–PR2 primer pair and was sequenced with Illumina Hiseq 2000 platform at the Beijing Genome Institute, Hong Kong (BGI-HK).

For *P. taishanensis*, a 658-bp fragment of *cox1* and a 407-bp fragment of *rrnL* were initially amplified by PCR with the primer pairs LCO1490-HCO2198[63] and LRJ12287–LRN13398[64] (see Additional file Table S2). The PCR products were purified and sequenced directly using Sanger method at Majorbio. Two pairs of specific primers, TYCB3R2-TY16sR1 and RTYCB3F5-RTY16sF4, were designed from the sequences of the *cox1* and *rrnL* fragments. The PCR with TYCB3R2-TY16sR1 produced a 1.7-kb amplicon, which was sequenced using Sanger method at Majorbio. The PCR with RTYCB3F5-RTY16sF4 produced an 11.2-kb amplicon, which was sequenced with Illumina Hiseq 2000 platform at the BGI-HK. The initial PCRs contained 12.5 µL of PCR SuperMix (Transgen Biotech Co., Ltd., Beijing, China), 2 µl of template DNA, and 1.25 µM of each primer, in a total volume of 25 µL. The PCR cycling conditions were: 3-min denaturation at 96 °C; 35 cycles of 10-sec denaturation at 95 °C, 30-sec annealing at 46 °C and 1.5-min extension at 72 °C; 5-min final extension at 72 °C; and then held at 4 °C. PCR products were checked on 1% agarose gel. PrimeSTAR GXL DNA polymerase (TAKARA) was used in the long PCRs with the cycling conditions: 98 °C for 10 sec, 68 °C for 2 to 10 min (depends on the length of regions between *rrnL* and *cox1*). The reaction mixture contained 0.5 µl GXL DNA Polymerase, 5 µl buffer, 2 µl dNTP mixture, 0.75 µl of each primer, 1 µl of template DNA and Milli-Q water added to total volume of 25 µl. Positive and negative controls were executed with each PCR. PCR products were checked on 1% agarose gel. PCR products were purified with QIAquick Spin PCR Purification Kit (QIAGEN).

Assembly of Illumina sequence-reads, gene identification and gene

rearrangement analysis

Illumina sequence-reads obtained from the mt genome amplicons of *P. taishanensis* and *E. sabinae* were assembled into contigs with Geneious 6.1.6 (Biomatters Ltd.). The transfer RNA (tRNA) genes were identified using tRNAscan-SE[37] and ARWEN[38] or identified manually based on anticodons and secondary structures. tRNA genes of the two eriophyoid mites were verified by comparison of secondary structures and conserved nucleotide sequences with those of the Acari species reported in published literature. PCGs were identified by open reading frame search in Geneious and BLAST searches of GenBank[65]. The two rRNA genes, *rrnL* and *rrnS*, were also identified by BLAST searches of GenBank based on sequence similarity and conserved sequence motifs. The start and stop nucleotides of *rrnL* and *rrnS* cannot be determined exactly and were assumed to be immediately after their upstream genes and before their downstream genes. Breakpoints were calculated with CREx[36] web server (http://pacosy.informatik.uni-leipzig.de/crex) as a measure of the extent of mt gene rearrangement; the two eriophyoid mites were compared with the hypothetical ancestor of arthropods (see Table 3 for details). The nucleotide sequences of mt genomes of *P. taishanensis* and *E. sabinae* have been deposited in GenBank under accession numbers KR604967 and KR604966.

Phylogenetic analyses

Sequences of the mt genomes of 64 Acari species, including 39 Parasitiformes mites[10,11,12,13,14,15,16,17,18,19,20,21,22,66,67] and 25 Acariformes mites[9,11,34,35,42,43,44,45,46,47,48,49,50,68], and two outgroup species were retrieved from GenBank (see Additional file Table S3). We used two horseshoe crabs, *Limulus polyphemus*[69] and *Carcinoscorpius rotundicauda*, as outgroups. Horseshoe crabs have ancestral gene arrangement of arthropod and are in the same subphylum, Cheilcerata, as mites and ticks. To investigate the phylogenetic position of Acari in Arachnida, 18 more mt genomes were retrieved from GenBank, including two species of Amblypygi[30], four species of Araneae[26,30], two species of Opiliones[30,70], three species of Scorpiones[29,30], two species of Solifugae[30,71], one species of Thelyphonida[30], two species of Pseudoscorpiones[28] and two species of Ricinulei[71]. Amino acid sequences of the PCGs were aligned individually using MAFFT v7.205[72]

web server (http://mafft.cbrc.jp/alignment/server/) with G-INS-i strategy for global homology and manually inspected before concatenation. Nucleotide sequences of PCGs were aligned using TranslatorX[73] web server (http://translatorx.co.uk/) using MAFFT to compute the protein alignments. rRNA and tRNA genes were aligned individually using Muscle algorithm implemented in MEGA 6.06[74]; large gaps and ambiguous sites were deleted manually.

We analyzed the datasets of mt genome sequences as two types of matrix: amino acid sequences of PCGs and nucleotide sequences of all genes (13 PCGs, 2 rRNA genes and 22 tRNA genes). The datasets were partitioned by genes, by codon positions and optimal partitioning as determined by PartitionFinder (Table S4). Amino acid sequences were partitioned by 13 genes. Nucleotide sequences partitioned by genes resulted in 16 datasets: 13 PCGs, 2 rRNA genes and concatenated tRNA genes. Partitioning by codon positions resulted in six datasets: one for each base of codons, two for each rRNA gene and one for the combined tRNAs. Both types of partitioned nucleotide sequences were run twice independently, once with the third codon positions of PCGs included and once without the third codon positions. To avoid redundant sampling of each genus, which may potentially affect a complex lower-level phylogeny, two representative species from each genus (*Argas*, *Ornithodoros*, *Amblyomma*, *Haemaphysalis*, *Ixodes*, *Rhipicephalus*, *Tetranychus*, *Leptotrombidium*) were included in the analyses. The reduced datasets included 43 taxa and only Baysian inference was run based on the best models found by PartitionFinder. To check if RNA genes could affect the topology, we also ran Baysian inference with the nucleotide sequences of the 13 PCGs based on the best models found by PartitionFinder. The datasets with 18 additional Arachnid mt genomes were ran in ML and Baysian analyses with nucleotide sequences of the 13 PCGs by 13 partitions.

The best models of the datasets were predicted by jModelTest 2.1.1[75] and PartitionFinder v1.1.1[76], using the Bayesian Information Criterion (BIC). PartitionFinder was set using unlinked branch lengths, greedy search for nucleotide sequences and amino acid sequences. MtRev + I + G + F was chosen as the best amino acid substitution model for most PCGs, except for *nad4*, for which JTT + I + G + F was chosen as the best model.

Nucleotide substitution model GTR + I + G was chosen as the best of 16 partitions. The ML analyses were performed using the GTRGAMMA model for nucleotide partitions and MtRev + I + G + F (JTT + I + G + F for *nad4*) for amino acid partitions in the program raxmlGUI1.377,78. For nodal support evaluation, a nonparametric bootstrap with 1,000 replicates was used. Mixed-model Bayesian analyses were performed with MrBayes 3.2.279 using separate data partitions. For BI, one cold chain and three heated chains were run with the combined dataset for 2 million generations. The average standard deviation of split frequencies fell down quickly. After 0.2 million generations, the average standard deviation number was below 0.01 in most of the BI trees. The convergence of the parameter estimates was performed with Tracer v1.6. The consensus three was edited with FigTree1.4.0.

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Additional Information

How to cite this article: Xue, X.-F. *et al.* Mitochondrial genome evolution and tRNA truncation in Acariformes mites: new evidence from eriophyoid mites. *Sci. Rep.* **6**, 18920; doi: 10.1038/srep18920 (2016).

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Supplementary Material

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caption a9**Supplementary Information:**
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Acknowledgments

This research was funded by the National Natural Science Foundation of China (31172132), the Fundamental Research Funds for the Central Universities (KYZ201405), the State Scholarship Fund of China, the ****Australian Research Council (DP120100240 to RS), and Australia-China Science & Research Fund (ACSRF00980 to RS).**

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Footnotes

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Author Contributions X.-F.X., X.-Y.H. and R.S. designed the research. X.-F.X., J.-F.G. and Y.D. performed the research. X.-F.X. and R.S. analyzed the data. X.-F.X., X.-Y.H. and R.S. wrote the manuscript. All authors have read and approved

the final manuscript.

to:

Bacterial Profiling Reveals Novel “Ca. Neoehrlichia”, Ehrlichia, and Anaplasma Species in Australian Human-Biting Ticks

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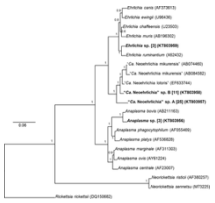
- Published: December 28, 2015
- DOI: 10.1371/journal.pone.0145449

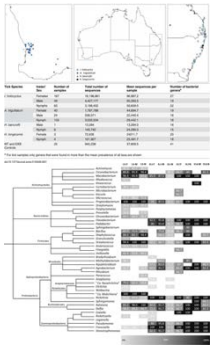
Article

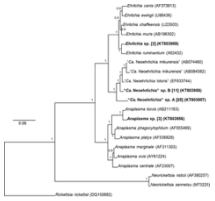
Abstract

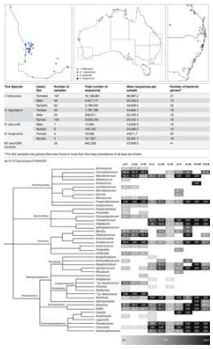
In Australia, a conclusive aetiology of Lyme disease-like illness in human patients remains elusive, despite growing numbers of people presenting with symptoms attributed to tick bites. In the present study, we surveyed the microbial communities harboured by human-biting ticks from across Australia to identify bacteria that may contribute to this syndrome. Universal PCR primers were used to amplify the V1-2 hyper-variable region of bacterial 16S rRNA genes in DNA samples from individual *Ixodes holocyclus* (*n* = 279), *Amblyomma triguttatum* (*n* = 167), *Haemaphysalis bancrofti* (*n* = 7), and *H*. *longicornis* (*n* = 7) ticks. The 16S amplicons were sequenced on the Illumina MiSeq platform and analysed in USEARCH, QIIME, and BLAST to assign genus and species-level taxonomies. Nested PCR and Sanger sequencing were used to confirm the NGS data and further analyse novel findings. All 460 ticks were negative for *Borrelia* spp. by both NGS and nested PCR analysis. Two novel "*Candidatus* Neoehrlichia" spp. were identified in 12.9% of *I*. *holocyclus* ticks. A novel *Anaplasma* sp. was identified in 1.8% of *A*. *triguttatum* ticks, and a novel *Ehrlichia* sp. was identified in both *A*. *triguttatum* (1.2%) ticks and a single *I*. *holocyclus* (0.6%) tick. Further phylogenetic analysis of novel "*Ca*. Neoehrlichia", *Anaplasma* and *Ehrlichia* based on 1,265 bp 16S rRNA gene sequences suggests that these are new species. Determining whether these newly discovered organisms cause disease in humans and animals, like closely related bacteria do abroad, is of public health importance and requires further investigation.

Figures









Citation: Gofton AW, Doggett S, Ratchford A, Oskam CL, Paparini A, Ryan U, et al. (2015) Bacterial Profiling Reveals Novel "*Ca*. Neoehrlichia", *Ehrlichia*, and *Anaplasma* Species in Australian Human-Biting Ticks. PLoS ONE 10(12): e0145449. doi:10.1371/journal.pone.0145449
Editor: Bradley S. Schneider, Metabiota, UNITED STATES
Received: October 12, 2015; **Accepted:** December 3, 2015; **Published:** December 28, 2015

Data Availability: All NGS 16S sequences are available from NCBI Bioproject database (PRJNA298108). Sanger sequencing results for Anaplasmataceae 16S sequences are available from the GenBank accessions cited in text.
Funding: This study was part-funded by the Australian Research Council (LP13010050), www.arc.gov.au, Bayer Healthcare, www.healthcare.bayer.com, and Bayer Australia Ltd., www.bayer.com.au. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Competing interests: The authors have read the journal's policy and have the following competing interests: This research was part funded by Bayer Healthcare and Bayer Australia Ltd. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Introduction

Over the last 30 years in Australia there have been reports of an illness in humans, the onset of which has been putatively associated with parasitism by ticks, most

frequently the Australian paralysis tick (*Ixodes holocyclus*) [1]. This undetermined disease usually presents as acute flu-like symptoms including headache, fever, and fatigue that can persist for weeks to months, and may develop into a severe chronic illness that can include, but is not limited to, myalgia, arthralgia, chronic migraine, and a systemic inflammatory syndrome [1, 2]. Similarities between these symptoms and those of Lyme disease have led to the controversial diagnosis by some physicians of Lyme disease in Australian patients [3, 4].

In the northern hemisphere, Lyme disease is caused by the bacteria *Borrelia burgdorferi* sensu lato and is transmitted by several species of *Ixodes* ticks, including *I*. *ricinus* and *I*. *persulcatus* in Europe and Asia, and *I*. *scapularis* and *I*. *pacificus* in North America, none of which occur in Australia [5, 6]. *Borrelia burgdorferi* sensu lato is not considered by many physicians to occur in Australia, and over 20 years of scientific effort has failed to find sufficient evidence of *B*. *burgdorferi* sensu lato in Australian ticks, wildlife, or humans that did not acquire *Borrelia* infection overseas [1, 2, 7]. Consequently, there is significant public concern and medical uncertainty over the diagnosis and treatment of a Lyme disease-like illness in Australia, and there is a need for robust scientific inquiry to clarify the aetiology of this illness.

Ixodes holocyclus is the most significant Australian tick species from both a medical and veterinary perspective [8]. It is the tick most commonly found parasitising humans and domestic animals in its enzootic range, which spans coastal areas along almost the entire east coast of Australia and includes many of Australia's most densely populated regions [9]. Its natural wildlife hosts include a variety of small marsupials such as bandicoots (*Isoodon* spp. and *Perameles* spp.) and possums (*Trichosurus vulpecula* and *Pseudocheirus peregrinus*) [8]. *Ixodes holocyclus* causes life-threatening paralysis in domestic animals through envenomation, and in humans it can cause weakness, paralysis, and dermatological and allergic reactions, including mammalian meat allergies [10]. It is also a vector of the human pathogens *Rickettsia australis* and *R*. *honei*, agents of Queensland tick typhus and Flinders Island spotted fever, respectively [11, 12]. On the west coast of Australia, the most common human-biting tick is the ornate kangaroo tick, *Amblyomma triguttatum* [8], which is a putative host of *Coxiella burnetii*, the aetiological agent of Q fever, and the spotted fever pathogen *R*. *gravesii* [8, 13, 14].

Recently, a survey of bacteria harboured by *I*. *holocyclus* ticks from New South Wales (NSW), Australia, using bacterial 16S rRNA gene (16S) profiling, identified four novel candidate pathogens, including a relapsing fever group *Borrelia* sp., an *Anaplasma* sp., and two novel "*Candidatus* Neoehrlichia" species [7]. Phylogenetic analysis of 300 bp 16S rRNA gene sequences from these bacteria revealed that the *Borrelia* and "*Ca*. Neoehrlichia" were closely related to the known human tick-borne pathogens *B*. *duttonii* and "*Ca*. N. mikurensis", respectively [7], which share some clinical similarities to those described by patients suffering Lyme disease-like illness in Australia [15, 16]. The novel *Anaplasma* sp. was closely related to the tick-borne pathogen of cattle, *A*. *bovis* [7]. None of these candidate pathogens had

been described previously in Australia.
The present study was designed in order to better understand the range and genetic diversity of microorganisms potentially transmitted to humans by ticks in Australia. As previously described [7], next-generation sequencing (NGS) and bioinformatics tools were used to profile bacterial populations within ticks removed from people around Australia. Additionally, species-specific PCR assays, Sanger sequencing, and Bayesian phylogenetic reconstructions were implemented to further analyse and confirm results obtained by NGS.

Methods

Ethics statement

This research complies with the *Australian Code for the ResponsibleConduct of Research*, 2007, and was approved by the Murdoch UniversityHuman Research Ethics Committee (Permit No. 2011–005). All tickcollections were opportunistic and were volunteered by people who hadeither removed the ticks from themselves, or had them removed by a medicalprofessional during outpatient treatment. Participants provided writtendocumented consent to participate in this study, and the consent procedurewas approved by the Murdoch University Human Research Ethics Committee(Permit No. 2011–005).

Tick collection and identification

A total of 460 individual ticks were collected from patients attending the outpatient clinic at the Mona Vale Hospital (Mona Vale, NSW, n = 63), or solicited through media coverage and word-of-mouth (n = 397) from people experiencing tick-bite within Australia between 2013 and 2015. Information about the geographical location (Fig 1) and the date of the tick bite was obtained, and all ticks were confirmed (by medical history or questionnaire) to be actively blood feeding on humans at the time of removal. Ticks were preserved in 70% ethanol immediately after removal and morphologically identified into species, instar, and sex, at the Department of Medical Entomology, Westmead Hospital, or at Murdoch University, using standard keys [8, 17]. Tick specimens were then stored in 70% ethanol at 4°C until molecular analysis.

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Fig 1. Geographic origin of *I. holocyclus*, *A. triguttatum*, and *Haemaphysalis* ticks used in this study.

Centre, map of Australia; Left, inset of south-west Western Australia; Right, inset of Australian east coast.

doi:10.1371/journal.pone.0145449.g001

DNA extraction

Total genomic DNA was extracted from individual ticks using the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Germany) following the manufacturer's recommendations (QIAGEN Supplementary Protocol: Purification of total DNA from insects). Before DNA extraction, the external surface of ticks was decontaminated in 10% hypochlorite solution, washed in sterile and DNA-free PBS, and 70% ethanol, and air-dried. Ticks were then frozen in liquid nitrogen for 1 minute, and homogenised by shaking with a 5 mm steel bead at 40 Hz for 1 minute. Extraction reagent blanks (EXB) (n = 20) were performed in parallel with all DNA extractions in order to establish background bacterial populations. All DNA extractions were performed in a physical isolation hood to minimise contamination by researchers and the environment, and sterile and DNA-free equipment was used for all procedures.

Bacterial 16S rRNA gene profiling

The V1-2 hyper-variable region (250–320 bp) of bacterial 16S rRNA genes in tick DNA samples were PCR amplified using the primers 27F-Y and 338R as previously described [7]. These PCR assays for *I*. *holocyclus* DNA samples also included 10 µM of a "*Ca*. Midichloria mitochondrii"-specific blocking primer [7], in order to inhibit the amplification of 16S sequences from this highly abundant endosymbiotic bacterium. No-template (NT) and EXB controls were included in all PCR runs.

Amplicon library preparation was performed according to recommended protocols (Illumina Demonstrated Protocol: 16S Metagenomic Sequencing Library Preparation) with exceptions. Individual uniquely indexed libraries were normalised to equimolar concentrations with AxyPrep Mag PCR Normaliser beads (Axygen, USA) following the manufacturer's recommendations, before pooling in equimolar amounts. Up to 96 uniquely indexed libraries were pooled per sequencing run, which were performed on an Illumina MiSeq using 500-cycle V2 chemistry (250 bp paired-end reads) following the manufacturer's recommendations. No-template and EXB controls were also sequenced to establish background bacterial populations. All pre-PCR and post-PCR procedures were performed in physically separated laboratories to minimise amplicon contamination.

Next Generation Sequencing Analysis

Sequences were first subjected to quality control procedures as previously described [7], with exceptions. Paired-end reads were merged using USEARCH v8.0.1623 [18] with a minimum overlap length of 50 bp and no gaps allowed in the merged alignments. Primer sequences and distal bases were trimmed from the ends of reads in Geneious v8.1.6 (Biomatters, New Zealand) [19] and reads shorter than the minimum previously reported length of the bacterial 16S V1-2 amplicon (< 250 bp) were removed. Singleton sequences (per sample) and sequences with a > 1% error rate were removed from the dataset using USEARCH v8.0.1623 [18]. Operational taxonomic units (OTUs) were created by clustering sequences at 97% similarity with the UPARSE algorithm [20], and taxonomy was assigned to OTUs in QIIME [21] by aligning to the GreenGenes 16S database

(August 2013 release) [22] using the UCLUST algorithm [18] with default parameters. OTUs taxonomically assigned to the family or genus-level were used for further analysis. OTUs that were present in EXB and NT controls were removed from all samples in order to eliminate potentially contaminating and background bacteria.

Following OTU analysis to assign genus level taxonomy to 16S sequences, BLAST was used to resolve the species identity of families and genera that have medical or veterinary significance, or contain members that are known, or proposed, arthropod endosymbionts or pathogens. Species-level taxonomy was only inferred when the query matched 16S sequences from only one species with a ≥ 99% pairwise identity over ≥ 99% the length of the query sequence. Bacterial genera that were deemed not of medical or veterinary significance, known or proposed arthropod endosymbionts, or otherwise previously associated with ticks, and that were detected in less than the mean prevalence of all taxa, are herein not mentioned.

Anaplasmataceae, *Borrelia*, and *Rickettsia*-specific PCR and Sanger sequencing

In order to gain more informative phylogenetic data and to verify NGS results, species-specific PCRs were used to further confirm (or refute) the occurrence of: *Borrelia* spp., Anaplasmataceae species (except *Wolbachia* spp.), and spotted fever and typhus group *Rickettsia* species in ticks. The *Borrelia*-specific assay targeted a 441 bp region of the chromosomal flagellin gene (*fla*B) and consisted of two nested PCRs, the primary reaction with primers *fla*B-280F and *fla*B-RL, and the nested reaction with primers *fla*B-LL and *fla*B-737R [23, 24], and verified previously in our laboratory to reliably amplify *B*. *burgdorferi* sensu lato, and relapsing fever group *Borrelia* spp. from tick specimens. The presence of Anaplasmataceae species in ticks was confirmed using a nested PCR assay targeting a 1.3 kb region of the 16S rRNA gene of Anaplasmataceae species (except *Wolbachia* spp.). The primary PCR contained the primers EC9 and EC12A [25, 26] and the nested reaction contained primers A17a and IS58-1345R [27]. The presence of spotted fever and typhus group *Rickettsia* species was confirmed with a qPCR assay using the primers CS-F and CS-R, and hydrolysis probe CS-P, as previously described [28].

Borrelia and Anaplasmataceae-specific primary PCRs contained 2 µl of tick DNA and the nested reaction used 1 µl of the primary PCR product as a template. PCRs contained PCR buffer, 2.5 mM $MgCl_2$, 1 mM dNTPs, 0.01 mg BSA (Fisher Biotech, Australia), 1.25 U Perfect *Taq* Polymerase (5 Prime, Germany), and 400 nM of each primer, in a total volume of 25 µl. All PCRs included NT controls and positive controls (*B*. *afzelii* or "*Ca*. N. mikurensis" from *I*. *ricinus* ticks, and *R*. *australis* from culture). All positive PCR products were electrophoresed in 2% agarose gels stained with GelRed (Biotium, USA), visualised under UV light, purified with the QIAquick gel extraction kit (QIAGEN, Germany), and sequenced with both forward and reverse PCR primers on an ABI 3730 96 Capillary Sequences using Big dye

v3.1 terminators (Life Technologies, USA).

Anaplasmataceae 16S phylogenetic analysis

Phylogenetic analysis was conducted on 1,265bp 16S sequences obtained from the Anaplasmataceae-specific nested PCR on *I*. *holocyclus* and *A*. *triguttatum* samples, and additional Anaplasmataceae 16S sequences retrieved from GenBank. Sequences were aligned with MAFFT [29] and the gapped alignment was refined with MUSCLE [30]. The most suitable nucleotide substitution model was assessed in MEGA6 [31] and selected based on the Bayesian Information Criterion. Bayesian phylogenetic analysis was performed with the MrBayes software [32] using the HKY85 substitution model and a discrete Gamma distribution with 5 categories, a total chain length of 1,100,000, burn-in length of 100,000, and subsampling every 200 iterations.

Results

Bacterial 16S rRNA gene community profiling

The tick species collected from people while attached and feeding included *I*. *holocyclus* (*n* = 279), *A*. *triguttatum* (*n* = 167), *Haemaphysalis bancrofti* (*n* = 7), and *H*. *longicornis* (*n* = 7) (Table 1). *Ixodes holocyclus* ticks were received from almost the entirety of its enzootic range along the east coast of Australia from Gladstone, Queensland (QLD) to Mallacoota, Victoria (Fig 1). *Amblyomma triguttatum* ticks were primarily collected from southwest Western Australia (WA), including many semi-rural and rural areas surrounding Perth, as far north as Kalbarri, WA, and southeast at Hopetoun, WA (Fig 1). *Amblyomma triguttatum* ticks were also received from Rockhampton and Charleville, QLD (Fig 1). *Haemaphysalis longicornis* were collected from only a single location; Urunga, NSW, and *H*. *bancrofti* was collected from four locations, Gladstone, QLD, Currumbin, QLD, Mollymook, NSW, and Tamban, NSW (Fig 1).

- TIFF original image (481KB)**Table 1. Summary of sample size, NGS coverage, and taxonomic diversity of tick species and life stages.**

doi:10.1371/journal.pone.0145449.t001

After NGS quality control procedures, 30,450,159 16S sequences from 460 tick samples and 25 NT and EXB control samples were used for analysis (Table 1). A total of 41 bacterial genera that were found in NT and EXB controls were removed from the dataset as background bacteria. All of the background taxa were either ubiquitous environmental or human-associated commensal bacterial genera that to the best of our knowledge have never been associated with tick-borne human or veterinary disease.

The most prevalent organisms identified in *I*. *holocyclus*, *A*. *triguttatum*, *H*.

bancrofti, and *H*. *longicornis* ticks were environmental or commensal bacteria that included 34 genera within Actinomycetales, Bacteriodetes, Firmicutes, Rhizobiales, Burkholderiales, and Gammaproteobacteria. The genera *Propionibacterium*, *Staphylococcus*, and *Streptococcus*, which live as commensals on mammalian skin were identified in all tick species (Fig 2). Other environmental genera identified, such as *Bacillus*, *Agrobacterium*, *Corynebacterium*, *Delftia*, *Flavobacterium*, *Methylobacterium*, *Mycobacterium*, *Pseudomonas*, *Ralstonia*, and *Stenotrophomones* are considered as either ubiquitous in the environment, or associated with soil and moist leaf litter environments in which ticks spend a large proportion of their life cycle (Fig 2). No *Borrelia* sp. sequences were identified in any of the 460 ticks.

- TIFF original image (676KB)**Fig 2. Cladogram and heat map showing the prevalence of bacterial genera in tick species and life stages.**

I.h, A.t, H.l, and H.b indicate *I*. *holocyclus*, *A*. *triguttatum*, *H*. *longicornis*, and *H*. *bancrofti* tick species, respectively. Female, Male and Nymph life stages are indicated by F, M, and N, respectively. The level of shading corresponds to the prevalence of the genera in the tick species and life stage. Blank shading indicates that bacterial genera were not detected in that tick species life stage.

doi:10.1371/journal.pone.0145449.g002

Bacterial endosymbionts in human-biting ticks.

Proposed bacterial endosymbionts were highly prevalent in all ticks studied, with each tick species having one or two predominant endosymbiont species and one to three less prevalent endosymbiotic associations. As anticipated from a previous study [7], the *Ixodes* tick endosymbiont "*Ca*. Midichloria mitochondrii" (16,519 unique sequences) was found in all *I*. *holocyclus* ticks, however, as expected (due to the use of a blocking primer during PCR) [7], 16S sequences from this abundant bacterium only comprised 4–17% of sequences per sample. In addition, *Wolbachia*, *Francisella*, and *Rickettsiella* spp. were also identified in 1.4%, 5.4%, and 11.1% of *I*. *holocyclus* ticks, respectively (Fig 2). Bacteria of the genus *Rickettsia* (7,069 unique sequences) were also identified in 27.5% of females, 44.9% of males, and 27% of nymph *I*. *holocyclus* ticks, with a total prevalence

30.5% in this tick species. Unfortunately *Rickettsia* 16S reads were unable to be given species designation due to high sequence homology (> 99%) between many *Rickettsia* species at the 16S locus analysed.

All *A*. *triguttatum*, *H*. *bancrofti*, and *H*. *longicornis* ticks studied were dual-infected with *Francisella* (12,990 unique sequences) and *Rickettsia* spp. (7,069 unique sequences) (Fig 2). *Francisella* and *Rickettsia* spp. sequences from these ticks were highly abundant in the NGS results, comprised between 12%-98% and 2%-88% of sequences per sample, respectively. *Francisella* sequences from all ticks were more than 98% similar to known endosymbiotic *Francisella* spp. from *A*. *maculatum* (GenBank: AY375407) and *Dermacentor* spp. (GenBank: AY375403, AY375401, JX101605) ticks from the northern hemisphere, and less than 94% similar to the infectious human pathogen *Francisella tularensis* (GenBank: NR074666), which has never been reported in Australia. In addition to endosymbiotic *Francisella* and *Rickettsia* spp., all *H*. *bancrofti* ticks also harboured a *Coxiella* sp., presumed to be an endosymbiont, as did 5% of *A*. *triguttatum* females. *Coxiella* sp. sequences were highly abundant in *H*. *bancrofti* ticks, comprising 23%-92% of sequences per sample. These *Coxiella* sp. sequences were more than 99% similar to *Coxiella* sp. endosymbionts reported previously from *H*. *lagrangei* and *H*. *longicornis* from Thailand and Korea (GenBank: KC170756, AY342036), respectively, but less than 94% similar to the infectious pathogen *C*. *burnetii* (GenBank: HG825990).

Novel Anaplasmataceae species identified in human-biting ticks.

The genus "*Ca*. Neoehrlichia" (11,493 unique sequences) was identified in all *I*. *holocyclus* life stages studied, with a prevalence of 76.6%, 63.3%, and 50.8% in females, males, and nymphs, respectively, and a total prevalence of 88.9%. "*Candidatus* Neoehrlichia" sequences formed two distinct clusters, herein putatively named species A and B, which were 6–7% dissimilar from each other (S1 Table). The closest known relative to putative "*Ca*. Neoehrlichia" species A and B was "*Ca*. N. mikurensis" (94.6–94.9% similarity) (GenBank: AB196304) from Japan. Putative species A and B sequence were also highly similar to "*Ca*. N. lotoris" (95.9–96.3% similarity) (GenBank: EF633744), although the sequence query coverage was only 90.8%. Putative "*Ca*. Neoehrlichia" species A was most common, being found in 68.8% of "*Ca*. Neoehrlichia"-positive *I*. *holocyclus* ticks, compared to species B (31.2%). All sequences from both "*Ca*. Neoehrlichia" putative species A and B were more than 99% similar to "*Ca*. Neoehrlichia" spp. 16S sequences recently obtained by NGS from *I*. *holocyclus* ticks from NSW, Australia [7], with species A and B most similar to "*Ca*. Neoehrlichia" sp. isolates PI808 (GenBank: KT203915), and PI800 (GenBank: KT203914), respectively. Among all of the "*Ca*. Neoehrlichia"-positive *I*. *holocyclus* ticks, there were no cases of co-infection with both putative species A and B.

Interestingly, "*Ca*. Neoehrlichia" sequences were not detected in any *A*. *triguttatum* or *Haemaphysalis* ticks; however, two other Anaplasmataceae species were identified in *A*. *triguttatum* ticks and a single *I*. *holocyclus* female. Novel *Anaplasma* sp. sequences (284 unique sequences) were identified in three *A*. *triguttatum* ticks

(1.8%), including one female (2.5%), and two nymphs (2%). These *Anaplasma* sp. sequences were most similar (98%) to an uncultured *Anaplasma* sp. (GenBank: JN862824) from southeast China, and the closest recognised species (97%) was *A*. *bovis* (GenBank: KJ659040). All three *A*. *triguttatum* ticks infected with this novel *Anaplasma* sp. originated from Yanchep National Park, Western Australia. Novel *Ehrlichia* sp. sequences (206 unique sequences) were also identified in two (1.2%) *A*. *triguttatum* ticks including one nymph, one female, and one *I*. *holocyclus* female (0.6%). These novel *Ehrlichia* sp. sequences were most similar (97%) to *E*. *ruminantium* (GenBank: DQ482921, CR925677), and another unresolved *Ehrlichia* sp. from *H*. *longicornis* ticks from Japan (GenBank: AY309970, HQ697588). The two *A*. *triguttatum* ticks infected with this novel *Ehrlichia* sp. both originated from Bullsbrook, Western Australia and the *I*. *holocyclus* tick originated from Pimpama, Queensland.

Anaplasmataceae, *Borrelia*, and *Rickettsia*-specific PCR

All 460 *I*. *holocyclus*, *A*. *triguttatum*, and *Haemaphysalis* ticks were negative for *Borrelia* spp. by nested PCR, confirming the 16S community profiling results. The spotted fever and typhus group-specific qPCR did not amplify any *Rickettsia* from *I*. *holocyclus* ticks. However, all *Rickettsia*-positive *A*. *triguttatum* and *Haemaphysalis* ticks (by NGS) were amplified with this qPCR assay, indicating the *Rickettsia* spp. in these ticks are within, or closely related to spotted fever and typhus group *Rickettsia* species. The Anaplasmataceae-specific PCR assay returned 37 positive *I*. *holocyclus* ticks (12.9%), including 19 females (11.4%), eight males (16.3%), and 10 nymphs (15.9%), and five positive *A*. *triguttatum* ticks (3%), including two females (5%), and three nymphs (2.9%). No *Haemaphysalis* ticks were positive for Anaplasmataceae species.

Anaplasmataceae Phylogenetic Analysis

Bayesian phylogenetic reconstruction of 1,265 bp 16S Anaplasmataceae sequences revealed that 36 (12.9% of all *I*. *holocyclus*) of the 37 positive *I*. *holocyclus* samples grouped with high confidence within the genus “*Ca*. Neoehrlichia”. Furthermore, the 16S sequences from these ticks clustered into two distinct groups, one containing identical sequences from 25 *I*. *holocyclus* ticks (9%) comprising putative “*Ca*. Neoehrlichia” species A (GenBank: KT803957), and the other containing identical sequences from 11 *I*. *holocyclus* ticks (4%), comprising putative “*Ca*. Neoehrlichia” species B (GenBank: KT803958) (Fig 3). Sequence from putative “*Ca*. Neoehrlichia” species A and B shared 96.2% similarity (S2 Table). The two known members of the genus, “*Ca*. N. lotoris” (GenBank: EF633744) and “*Ca*. N. mikurensis” (GenBank: AB074460, AB084582), were 98.1–98.6% similar at the 16S loci, however, putative “*Ca*. Neoehrlichia” species A and B were only 95.7–96.2%, and 97.3–98.4% similar to these species, respectively (S2 Table).

- TIFF original image (403KB)**Fig 3. Bayesian phylogenetic analysis of 1,265 bp novel Anaplasmataceae 16S rRNA sequences from *I. holocyclus* and *A. triguttatum*.**

Bayesian posterior probabilities are displayed at each node. Bold type indicates sequences from this study. Rounded parentheses indicate GenBank accession numbers, and square parentheses indicate the number of ticks from which identical sequences were obtained.

doi:10.1371/journal.pone.0145449.g003

The level of divergence at the 16S loci both between putative "*Ca*. Neoehrlichia" species A and B, and between these and known "*Ca*. *Neoehrlichia*" spp., confirms the clustering pattern observed in the NGS data, and described previously [7]. All *I. holocyclus* ticks positive here for novel "*Ca*. Neoehrlichia" spp. were also positive for "*Ca*. Neoehrlichia" spp. by NGS, although the prevalence of "*Ca*. Neoehrlichia" spp. was significantly lower as determined by nested PCR (12.9%) than by NGS (88.9%).

Three identical novel *Anaplasma* sp. 16S sequences (GenBank: KT803956) from *A. triguttatum* ticks (1.8%), including one female and two nymphs, clustered with high confidence, but were distinct (98.7% similarity) from *A. bovis* (GenBank: AB211163) (Fig 3, S2 Table). In addition, a further three identical novel *Ehrlichia* sp. sequences (GenBank: KT803959) from two *A. triguttatum* ticks (1.2%), including one female (2.5%) and one nymph (0.97%), and one *I. holocyclus* female

(0.6%) clustered with high confidence, but was distinct (98.3% similarity) from *E*. *ruminantium* (GenBank: X62432) (Fig 3, S2 Table). The level of divergence between these novel *Anaplasma* sp. and *Ehrlichia* sp. 16S sequences, and their closest relatives, is within the range of divergence among all *Anaplasma* species (94.7–99.4%) and *Ehrlichia* species (97.3–98.9%) (S2 Table). All ticks positive by nested PCR for novel *Anaplasma* sp. and *Ehrlichia* sp. were also positive for these taxa through NGS.

Discussion

This study follows a preliminary investigation of the bacterial microbiome associated with *I*. *holocyclus* in a localised region of NSW, with the aim of investigating a collection of human-biting ticks over a greater geographical range, including areas of Sydney, NSW, where numerous patients have been diagnosed with a Lyme disease-like illness. In Australia, approximately eight species of hard ticks, and one species of soft tick (*Ornithodorus capensis*) are known to bite humans [8, 17, 33]. Consistent with previously published and anecdotal reports, the Australian paralysis tick (*I*. *holocyclus*) and the ornate kangaroo tick (*A*. *triguttatum*) were most frequently associated with attachment and engorgement on the skin of people in this study [8]. The introduced 'bush' tick, *H*. *longicornis*, normally a parasite of cattle, and the native wallaby tick (*H*. *bancrofti*) are also well known to bite people in Australia [8]. Curiously, we did not receive any specimens of the brown dog tick (*R*. *sanguineus*), the common marsupial tick (*I*. *tasmani*) or the southern paralysis tick (*I*. *cornuatus*) for analysis in this study, all of which have previously been associated with human tick bites in Australia.

Although the external cuticle of all ticks was decontaminated with ethanol and 10% hypochlorite solution prior to molecular analyses, a range of common environmental and commensal bacteria were still prevalent among all ticks surveyed. This is most likely due to remnant bacterial DNA that survived the decontamination process, perhaps in bacterial plaques that may have accumulated in less accessible places such as between leg joints or underneath the tick's palps. In future studies careful dissection of the tick's main internal tissues (midgut, salivary gland, and gonads) may prove useful in distinguishing the microbiome of the internal tissues from environmental bacteria on the tick's external surfaces. Because all ticks surveyed were collected while actively feeding on humans it must be acknowledged that some bacteria in tick samples, such as *Staphylococcus* spp. and *Propionibacterium* spp., may have been from the blood and skin of the human hosts. However, most bacteria identified in the present study have been associated previously with ticks as members of genera that contain either known tick-borne pathogens, or arthropod endosymbionts.

Consistent with previous analysis [7], endosymbiotic "*Ca*. M. mitochondrii", *Wolbachia*, *Francisella*, and *Rickettsia* spp. were identified in *I*. *holocyclus* ticks. All *A*. *triguttatum*, *H*. *bancrofti*, and *H*. *longicornis* ticks studied were dual-infected with endosymbiotic *Francisella* and *Rickettsia* spp., which comprised a large proportion of NGS sequencing output for these samples. Although *Francisella* endosymbionts

have been described from northern hemisphere *Amblyomma* and *Dermacentor* ticks [34–36], and previously in *I*. *holocyclus* [7], this is the first description of *Francisella* spp. in a native Australian *Amblyomma* or *Haemaphysalis* tick. Species-specific blocking primers have been shown to be effective at inhibiting specific endosymbiont 16S sequences in *I*. *holocyclus* and *I*. *ricinus* [7], allowing the detection of less abundant bacterial taxa. It is probable that the use of *Francisella* and *Rickettsia*-specific blocking primers during 16S bacterial profiling of *A*. *triguttatum* and *Haemaphysalis* spp. ticks may similarly reveal more information about the less abundant bacterial taxa associated with these ticks.

The very high prevalence of *Rickettsia* spp. in *A*. *triguttatum* and *Haemaphysalis* ticks in this study suggest these *Rickettsia* spp. are likely endosymbiotic, and either advantageous or benign to the fitness of these tick species. The fact that these species were amplified with a qPCR assay designed to amplify only spotted fever and typhus group *Rickettsia* species and not the ancestral *R*. *bellii* species group [28], suggests these likely bacteria are more closely related to the spotted fever and typhus group than the *R*. *bellii* species group [37]. However, the spotted fever and typhus group qPCR did not amplify *Rickettsia* spp. found in *I*. *holocyclus* ticks, suggesting that these species are more closely related to the ancestral *R*. *bellii* group, which are typically endosymbionts of arthropods [37]. Further studies should include species-specific PCR and Sanger sequencing of a more informative marker gene to resolve the phylogenetic identity of *Rickettsia* spp. endosymbionts in Australian ticks, and to determine the prevalence of pathogenic *Rickettsia* spp. in Australia.

The absence of *Borrelia* sp. in the ticks studied here is somewhat unexpected considering the recent description of a single relapsing fever *Borrelia* sp. isolate found in a recent survey of *I*. *holocyclus* ticks using the same NGS method as in the present study. In that case the *Borrelia*-infected *I*. *holocyclus* tick was removed from an echidna, which is not a typical host for *I*. *holocyclus*. Surveying the microbial communities of ticks that share a close association with echidnas, such as *Bothriocroton concolor* and *B*. *hydrosauri*, may reveal more Australian *Borrelia* sp. isolates.

Based on the phylogenetic inference of 1,265 bp 16S sequences, the novel "*Ca*. Neoehrlichia", *Ehrlichia*, and *Anaplasma* detected in the present study appear to be putative species, as the levels of divergence between their sequences and those of their closest relatives, is within the range of accepted species separation at the 16S rRNA gene loci [38–41]. However, formal descriptions of them as new species will require analysis at multiple loci such as the citrate synthase gene (gltA), RNA polymerase sub-unit β (*rpoB*) and heat shock operon (groESL), or whole genomes [27, 42–46].

The overall prevalence of novel "*Ca*. Neoehrlichia" species A and B across all *I*. *holocyclus* life stages was 88.9% by NGS but only 12.9% by nested PCR. There are several reasons that may explain this discrepancy; firstly the nested PCR amplified a large fragment (the primary amplicon was approximately 1.4 kb and the secondary amplicon was approximately 1.3 kb), which is known to reduce the

efficiency of PCR [47]. For NGS, the amplicon size was much smaller (250–320 bp) and would therefore be expected to amplify with much greater efficiency [47, 48]. Secondly, NGS allows the detection of low abundant sequences, and mixed sequences that would not be detected with Sanger sequencing [48]. Further studies should include use a "*Ca*. Neoehrlichia"-specific droplet digital PCR quantitation assay targeting small amplicon sizes, as this will allow for more accurate quantitation [49, 50] and determination of the true prevalence of novel "*Ca*. Neoehrlichia" species in *I*. *holocyclus*.

All recognised members of the genera *Anaplasma*, *Ehrlichia*, and *"Ca*. Neoehrlichia" are obligate intracellular tick-borne mammalian pathogens that typically infect haematopoietic (mammalian) or endothelial (mammalian and tick) cells [25, 51–53]. There has been no confirmed transovarial transmission of *Anaplasma*, *Ehrlichia*, or "*Ca*. Neoehrlichia" species in vector-ticks or mammals, and therefore their persistence is attributed predominantly to infected mammalian reservoir populations [51–53]. Throughout Europe, Asia, and North America several Anaplasmataceae species are pathogens of veterinary significance (such as *E*. *canis* and *E*. *ruminantium*) and important emerging human pathogens, such as *E*. *chaffeensis*, *E*. *ewingii*, *A*. *phagocytophilum*, and "*Ca*. N. mikurensis" "*Ca*. Neoehrlichia" is a recently described genus that currently comprises two species, "*Ca*. N. lotoris", and "*Ca*. N. mikurensis" [27, 45]. Of these "*Ca*. N. mikurensis" is now recognised an emerging tick-borne zoonosis vectored by several tick species (*I*. *ricinus*, *I*. *ovatus*, and *I*. *persulcatus*), and is one of the most prevalent tick-borne infections in wildlife and ticks throughout Europe and Asia [27, 35, 54–68]. Clinical reports of human infections are steadily increasing, due in part to increased awareness and testing [53]. Infection with "*Ca*. N. mikurensis" (neoehrlichiosis) is typically severe, with a wide variety of non-specific symptoms reported [69–75]. In Europe, neoehrlichiosis usually manifests in immunocompromised patients, however in China, there are increasing reports of this infection in immunocompetent people, and asymptomatic infections in humans have also been reported [76, 77]. In contrast, "*Ca*. N. lotoris" is a tick-borne pathogen of racoons (*Procyon lotor*), and to date there are no reports of human infection [45, 78]. In the northern hemisphere treatment of patients suffering neoehrlichiosis with doxycycline (1 x 200 mg/day) for 3–6 weeks has been shown to be effective [71, 75, 79, 80], and may have implication if human or animals infections are found to occur in Australia.

The identification of four novel putative tick-borne Anaplasmataceae species in Australian human-biting ticks is of potential public health significance, especially the high prevalence of novel "*Ca*. Neoehrlichia" spp. in *I*. *holocyclus* ticks. Based on their phylogenetic position, as inferred here, and the disease-causing status of their close relatives, all four species are candidate human and animal pathogens, and almost certainly infective (symptomatic or asymptomatic) to Australian wildlife species. Determining whether these *Ehrlichia*, *Anaplasma* and "*Ca*. Neoehrlichia" species may cause disease in Australian humans, like their close relatives do overseas is of public health importance. Future studies should include the

development of specific digital and qPCR assays to more accurately determine the prevalence and pathogen load in ticks, wildlife, and humans. In addition, the isolation and culture of these organisms, in pure culture or infected mammalian and tick cell culture, will significantly aid in understanding the biology and potential pathogenicity of these novel Anaplasmataceae, and the development of specific diagnostic serological test and therapeutic practices.

Supporting Information

S1_Table.pdf

S1 Table. Distance matrix of the pairwise precent similarity of the 10 most

V1-2 sequences from "*Ca.* Neoehrlichia" putative species A (A1-10) and B (B

indicates > 99% similarity between sequences in putative "*Ca.* Neoehrlichia" spp.

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	B1	B2	B3	B4	B5	B6	B7
A1																	
A2	99.3																
A3	99.3	99.3															
A4	99.3	99.3	99.3														
A5	99.3	99.3	99.3	99.3													
A6	99.3	99.3	99.3	99.3	99.3												
A7	99.3	99.3	99.3	99.3	99.3	99.3											
A8	99.3	99.3	99.3	99.3	99.3	99.3	99.3										
A9	99.3	99.3	99.3	99.3	99.3	99.3	99.3	99.3									
A10	99.3	99.3	99.3	99.3	99.3	99.3	99.3	99.3	99.3								
B1	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9							
B2	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.7						
B3	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3					
B4	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3	99.7				
B5	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3	99.3	99.3			
B6	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3	99.3	99.3	99.7		
B7	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3	99.3	99.3	99.3	99.3	
B8	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3	99.3	99.3	99.3	99.3	99
B9	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3	99.3	99.3	99.3	99.3	99
B10	92.9	92.9	92.9	92.9	93.6	92.9	92.9	92.9	92.9	92.9	99.3	99.3	99.3	99.3	99.3	99.3	99

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0145449
.style0 {fill: #566471;} .style1 {fill: #A2CD3C;} .style2 {fill: #C54C59;} .style3 {fill: #5BC4BD;} fig**share 1 / 2**
Shading indicated > 99% similarity between sequences in putative "*Ca*.

Neoehrlichia" spp. A and B.

S1 Table. Distance matrix of the pairwise percent similarity of the 10 most prevalent 16S V1-2 sequences from "*Ca*. Neoehrlichia" putative species A (A1-10) and B (B1-10).

Shading indicated > 99% similarity between sequences in putative "*Ca*. Neoehrlichia" spp. A and B.

doi:10.1371/journal.pone.0145449.s001

(PDF)

S2 Table. Pairwise percentage distance matrix of 1,265 bp Anaplasmataceae 16S sequences from this study and retrieved from GenBank used for Bayesian phylogenetic reconstruction.

doi:10.1371/journal.pone.0145449.s002

(PDF)

Acknowledgments

The authors wish to acknowledge the assistance of Dr. John Stenos and the Australian Rickettsial Reference Laboratory for supplying *R*. *Australia* DNA, Kevin Stratford and the Pawsey Supercomputing Centre for technical assistance during data analysis, Frances Brigg and the Western Australia State Agriculture Biotechnology Centre for Sanger sequencing, and Sam Abraham for assistance with the methods.

Author Contributions

Conceived and designed the experiments: AWG SD UR PI. Performed the experiments: AWG SD. Analyzed the data: AWG. Contributed reagents/materials/analysis tools: AWG SD AR UR CLO AP PI. Wrote the paper: AWG SD AR UR CLO AP PI.

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RC RUSSELL1, SL DOGGETT1, R. MUNRO2, J. ELLIS3, D. AVERY1, C. HUNT4 AND D. DICKESON5
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Evidence for Ixodes holocyclus (Acarina: Ixodidae) as a vector for human lyme Borreliosis infection in Australia.

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Abstract

Ixodes holocyclus (Acarina: Ixodidae) and Ixodes cornuatus (Acarina: Ixodidae) are two tick species found in the more densely populated areas of Australia and are known to be the cause of the neurotoxic disease tick paralysis in humans and mammals. Borreliosis otherwise known as Lyme disease is an emerging infectious disease in humans in Australia. Borrelia burgdorferi sensu stricto (Spirochaetales: Spirochaetaceae) and sensu lato are closely related spirochetal species that are the causative agents of Lyme disease in humans. Clinical transmission of this tick-borne disease can be identified in several but not all cases by a characteristic rash known as erythema migrans. However, there has been no study of the tick vectors of this infection in Australia. We used morphological and molecular techniques to identify unequivocally the ticks on the patients of this study to be I. holocyclus and then show the presence of B. burgdorferi sensu stricto infection in erythema migrans biopsies. I. holocyclus has not previously been associated with erythema migrans or Lyme disease. Two patients presented to the lead author's medical practice with erythema migrans in mid and late 2012. The morphology and cytochrome oxidase 1 and ITS2 genes of the two ticks were studied. The skin at the attachment site was sampled by central biopsy for both real time and endpoint Borrelia polymerase chain reaction (PCR) analysis and subsequent sequencing. Morphologically, the two ticks were either I. holocyclus or I. cornuatus. Molecular studies and nucleotide sequencing revealed that both ticks were I. holocyclus. Real time and endpoint PCR on the central tissue biopsy samples returned positive results for B. burgdorferi DNA. Our results are evidence for transmission of B. burgdorferi sensu stricto species to humans by the tick I. holocyclus in Australia. I. holocyclus is commonly associated with human tick bites on virtually the entire eastern coastline of Australia.

KEYWORDS:

Australia; Borrelia burgdorferi; Ixodes holocyclus; Lyme disease; erythema migrans

PMID: 25434042 [PubMed - in process] **Free full text**

Int J Gen Med. 2015; 8: 15–26.
Published online 2014 Dec 23. doi: 10.2147/IJGM.S75825

PMCID: PMC4278782

Clinical determinants of Lyme borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort

Peter J Mayne
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Go to:

Abstract

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Background

Borrelia burgdorferi is the causative agent of Lyme borreliosis. This spirochete, along with *Babesia*, *Bartonella*, *Anaplasma*, *Ehrlichia*, and the *Rickettsia* spp. are recognized tick-borne pathogens. In this study, the clinical manifestation of these zoonoses in Australia is described.

Methods

The clinical presentation of 500 patients over the course of 5 years was examined. Evidence of multisystem disease and cranial nerve neuropathy was sought. Supportive laboratory evidence of infection was examined.

Results

Patients from every state of Australia presented with a wide range of symptoms of disease covering multiple systems and a large range of time intervals from onset. Among these patients, 296 (59%) were considered to have a clinical diagnosis of Lyme borreliosis and 273 (54% of the 500) tested positive for the disease, the latter not being a subset of the former. In total, 450 (90%) had either clinical evidence for or laboratory proof of borrelial infection, and the great majority of cases featured neurological symptoms involving the cranial nerves, thus mimicking features of the disease found in Europe and Asia, as distinct from North America (where extracutaneous disease is principally an oligoarticular arthritis). Only 83 patients (17%; number [n]=492) reported never leaving Australia. Of the 500 patients, 317 (63%) had clinical or laboratory-supported evidence of coinfection with *Babesia* or *Bartonella* spp. Infection with *A. phagocytophilum* was detected in three individuals, and *Ehrlichia*

chaffeensis was detected in one individual who had never traveled outside Australia. In the cohort, 30 (11%; n=279) had positive rickettsial serology.

******Conclusion**

The study suggests that there is a considerable presence of borreliosis in Australia, and a highly significant burden of coinfections accompanying borreliosis transmission. The concept sometimes advanced of a “Lyme-like illness” on the continent needs to be re-examined as the clinical interplay between all these infections. Evidence is presented for the first report of endemic anaplasmosis and ehrlichiosis on the continent.

Keywords: *Borrelia*, Lyme disease, *Babesia*, *Bartonella*, Australia, humans

Go to:

Introduction

The existence of human tick-borne disease (TBD) has been previously described in Australia by the author to comprise multiple zoonoses induced by the pathogens *Borrelia*, *Babesia*, *Bartonella*, *Anaplasma*, and *Ehrlichia* spp.[1] Other arbobacteria infections sometimes found worldwide are *Coxiella burnetii*, *Francisella tularensis*, *Yersinia pestis*, and the *Rickettsia* sp., with the latter comprising significant infection on this continent and having three unique subspecies.[1] Most grow intracellularly, but *Borrelia* sp. is also extracellular and *Y. pestis* is mainly extracellular.[2] In this case series of 500 consecutive patients presenting between October 2010 and February 2014, either referred or self-initiated regarding the possibility of “Lyme disease”, an analysis was done to define the clinical characteristics at the time of presentation of these zoonoses compared to those seen in North America, Europe, and Asia, as well as to quantify incidence figures. Patients attended from every state in Australia. In Australia, borrelial infection typically manifests with a neurological presentation, which follows the course seen in Europe and Asia, as discussed earlier.[1] Australian endemic human babesiosis with *B. microti* has been reported clinically, serologically, and at the molecular level.[1,3,4] There has been a recent nomenclature shift, with this piroplasm now classified as a *Theileria* sp. In this manuscript, the former nomenclature will be used. Babesiosis with *B. duncani* has been previously reported as an Australian endemic infection by this author.[1] Herein, the first Australian human endemic Anaplasmataceae infections with *A.*

phagocytophilum and *E. chaffeensis* are reported: three cases for the former and one for the latter pathogen. This is intended to show a complex interplay of symptoms and signs between these TBD infections, clinically necessitating a thorough assessment to be able to treat the diseases appropriately. Diagnosing borreliosis has previously been discussed, including the fact that North America singularly has a large proportion of mono or pauci arthritis characterized by extensive swelling, which can be migratory.1 This arthritic presentation is not seen in Australian borreliosis in this series. Also, it is important to reiterate that the involvement of a large number of cranial nerves will point the differential diagnoses at only borreliosis, bartonellosis, sarcoid, and Guillaine Barre disease.1 Amyloid should now be added to this list as a further causative factor, but there also appears to be a strong association with neuroborreliosis and sarcoidosis.5–13 The patient's clinical history and examination techniques used in diagnosing TBD at a clinical level are discussed and correlated to the level of laboratory-supported diagnosis that can be expected. Particular emphasis is given to diagnosing neuroborreliosis from a clinical perspective. Some early demographic and epidemiological data for this continent are presented.

Go to:

Materials and methods

Demographic and epidemiologic assessment

The data from 500 patient records were analyzed using 55 parameters that were subdivided into demographic, epidemiological, and clinical findings (see Tables 1 and
and2).
2). A patient questionnaire was obtained prior to consultation to assess the likelihood of multisystemic disease and multiple cranial nerve abnormality. This was a truncated version of a symptom list from the Burrascano Lyme disease guidelines.14 Inquiry was made into tick attachment and any associated reddening or swelling of the skin. A travel history outside Australia was sought from the perspective of ever going abroad or being born overseas.
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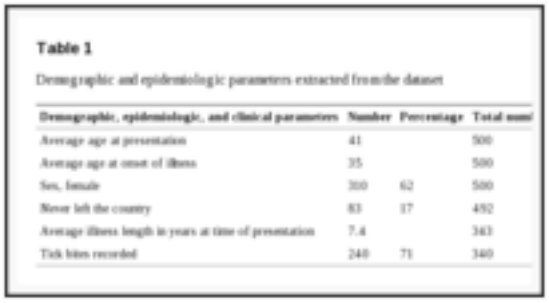

Table 1

Demographic and epidemiologic parameters extracted from the dataset

Demographic, epidemiologic, and clinical parameters	Number	Percentage	Total num
Average age at presentation	41		500
Average age at onset of illness	35		500
Sex, female	310	62	500
Never left the country	83	17	492
Average illness length in years at time of presentation	7.4		343
Tick bites recorded	240	71	340

Table 1

caption a4

Demographic and epidemiologic parameters extracted from the dataset

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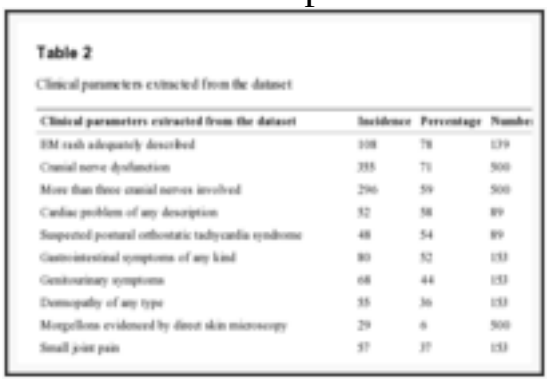

Table 2

Clinical parameters extracted from the dataset

Clinical parameters extracted from the dataset	Incidence	Percentage	Number
EM rash adequately described	108	78	139
Cranial nerve dysfunction	355	71	500
More than three cranial nerves involved	296	59	500
Cardiac problem of any description	52	58	89
Suspected postural orthostatic tachycardia syndrome	48	54	89
Gastrointestinal symptoms of any kind	80	52	153
Genitourinary symptoms	68	44	153
Dermopathy of any type	55	36	153
Morgellons evidenced by direct skin microscopy	29	6	500
Small joint pain	57	37	153

Table 2

caption a4

Clinical parameters extracted from the dataset

Clinical assessment

Symptoms and signs of cranial nerve deficit were sought using a cranial nerve assessment tool (Table 3). This tool was refined over time. Signs were sought by physical examination. The peripheral nervous system was briefly reviewed, but it was generally not helpful in pinpointing TBD, except that a pathological upwards plantar response (Babinski sign) and hypothenar wasting were not uncommon, which are possible indicators of babesiosis.15 Particular note was made of large joint pain (hip, knee, shoulder, elbow, wrist, and ankle) with or without swelling compared to small joint pain in the hands, feet, spine, and jaw. The costochondral joints were checked for tenderness. Three psychological parameters in particular were sought: panic attacks lasting more than 30 minutes is associated with borreliosis; in addition, sudden bursts of anger and

worsening obsessive compulsive behavior are both associated with bartonellosis. Certain investigation parameters were analyzed in three groups with testing performed at Australian Biologics Sydney (Table 4), IGeneX at Palo Alto, CA, USA (Table 5), and ancillary tests conducted at laboratories across Australia (Table 6; this table also shows single-photon emission computed tomography [SPECT] scans).
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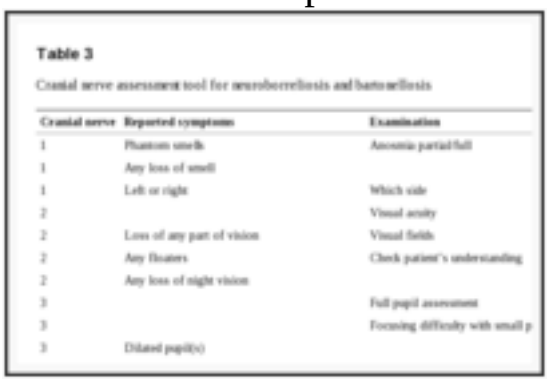

Table 3

Cranial nerve assessment tool for neuroborreliosis and bartonellosis

Cranial nerve	Reported symptoms	Examination
1	Phantom smells	Anosmia partial/full
1	Any loss of smell	
1	Left or right	Which side
2		Visual acuity
2	Loss of any part of vision	Visual fields
2	Any floaters	Check patient's understanding
2	Any loss of night vision	
3		Full pupil assessment
3		Focusing difficulty with small p
3	Dilated pupil(s)	

Cranial nerve assessment tool for neuroborreliosis and bartonellosistable ft1table-wrap mode=article t1

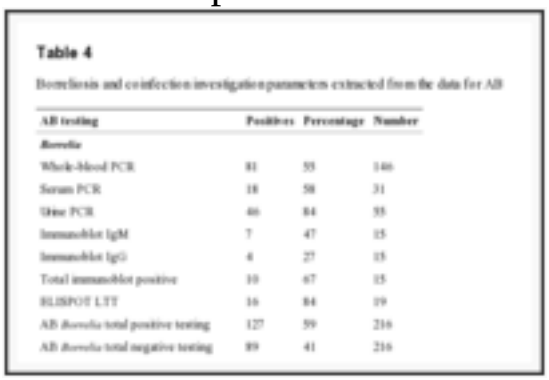

Table 4

Borreliosis and coinfection investigation parameters extracted from the data for AB

AB testing	Positives	Percentage	Number
Borrelia			
Whole-blood PCR	81	55	146
Serum PCR	18	58	31
Urine PCR	46	84	55
Immunoblot IgM	7	47	15
Immunoblot IgG	4	27	15
Total immunoblot positive	10	67	15
ELISPOT LTT	16	84	19
AB *Borrelia* total positive testing	127	59	216
AB *Borrelia* total negative testing	89	41	216

Borreliosis and coinfection investigation parameters extracted from the data for ABtable ft1table-wrap mode=article t1

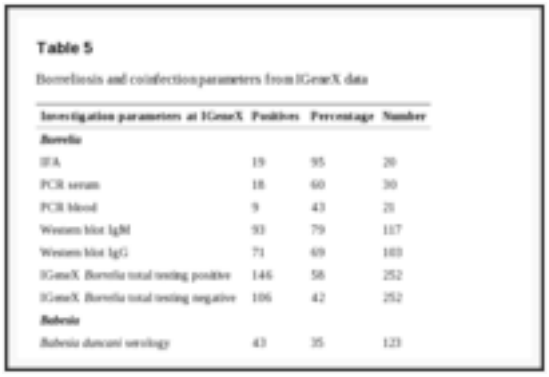

Table 5

Borreliosis and coinfection parameters from IGeneX data

Investigation parameters at IGeneX	Positives	Percentage	Number
Borrelia			
IFA	19	95	20
PCR serum	18	60	30
PCR blood	9	43	21
Western blot IgM	93	79	117
Western blot IgG	71	69	103
IGeneX *Borrelia* total testing positive	146	58	252
IGeneX *Borrelia* total testing negative	106	42	252
Babesia			
Babesia duncani serology	43	35	123

Borreliosis and coinfection parameters from IGeneX datatable ft1table-wrap mode=article t1

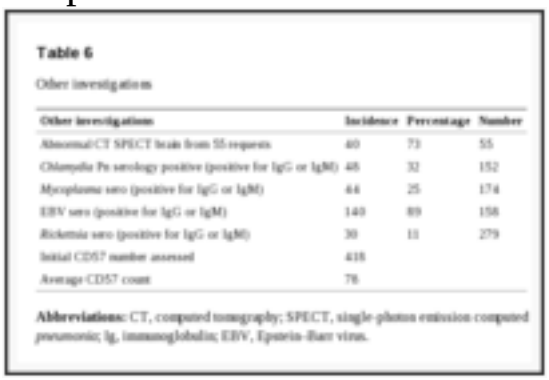

Table 6

Other investigations

Other investigations	Incidence	Percentage	Number
Abnormal CT SPECT brain from 55 requests	40	73	55
Chlamydia Pn serology positive (positive for IgG or IgM)	48	32	152
Mycoplasma sero (positive for IgG or IgM)	44	25	174
EBV sero (positive for IgG or IgM)	140	89	158
Rickettsia sero (positive for IgG or IgM)	30	11	279
Initial CD57 number assessed	418		
Average CD57 count	78		

Abbreviations: CT, computed tomography; SPECT, single-photon emission computed *pneumoniae*; Ig, immunoglobulin; EBV, Epstein-Barr virus.

			Table capti

Other investigations

Borrelia

The patients' symptoms, signs, and examination were checked for neurological problems (as indicated in Table 3), as well as for multisystemic problems over the timeline of the disease (as previously discussed) to form a clinical opinion of the likelihood of the disease based on the involvement of more than three cranial nerves and at least one other system.1 The history questionnaire was used to assess patients' symptoms in the first instance in all cases, and some points would require clarification during the consultation, which involved the use of the cranial nerve tool and multisystem review. If the review showed three or fewer cranial nerves involved, then it was determined that borreliosis was a possibility; however, laboratory testing needed to be relied upon for a diagnosis. For more than three cranial nerves involved, a clinical

diagnosis of borreliosis or bartonellosis was made. At this juncture, it should also be stated that reliance was placed on a negative computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain to exclude overt intracranial disease, and this was ordered if not done previously.

Babesia and Bartonella

Attention was also focused on blocks of symptoms in the patient questionnaire that typically occurred with these TBD coinfections (Table 7). The significant presence of more than just a few of these factors in recognized TBD illness is a harbinger for the presence of coinfection based on clinical grounds. Symptoms/signs that may indicate *Babesia* are tinnitus, chills, flushing, dysphagia, severe neurological disease, thirst, night sweats, chest wall or sternal pain, polydipsia, fatigue, severe nausea, malaise, fever, fine hemangiomas, hypothenar atrophy, papular or vesicular rash, petechiae, anemia, abnormal platelet counts, and the development of these symptoms during borreliosis therapy.15,16 Two European reviews are also helpful in understanding this disease from a clinical perspective.17,18 Symptoms/signs that may indicate *Bartonella* are brain fog, ice pick headaches, photophobia, tachycardia, bowel issues, tender subdermal masses (particularly of the neck, forearm, and ileotibial band), increased obsessive compulsive behavior, anxiety, endocarditis, retinitis, peripheral neuropathy, rapid relapse off antibiotics, immediate systemic illness following tick bite (within days), swollen joints, psychiatric issues, costal margin pain and tenderness, rapid mood shifts, and the development of these symptoms during *Babesia* therapy.15,16
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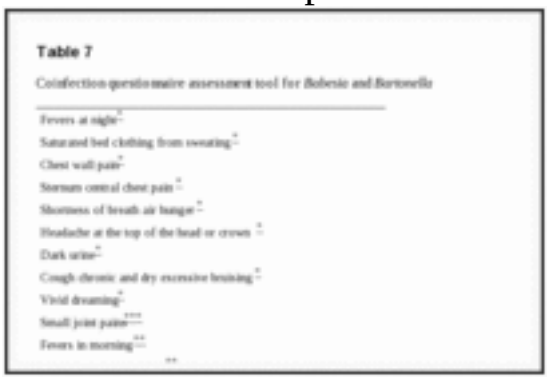

Table 7

Coinfection questionnaire assessment tool for *Babesia* and *Bartonella*

Fevers at night*
Saturated bed clothing from sweating*
Chest wall pain*
Sternum central chest pain*
Shortness of breath–air hunger*
Headache at the top of the head or crown*
Dark urine*
Cough–chronic and dry–excessive bruising*
Vivid dreaming*
Small joint pains***
Fevers in morning**

Coinfection questionnaire assessment tool for *Babesia* and *Bartonella*

Anaplasma/Ehrlichia

A. phagocytophilum and *E. chaffeensis* are the etiologic causes of anaplasmosis and ehrlichiosis, and they were formerly known as human granulocytic ehrlichiosis and human monocytic ehrlichiosis, respectively, indicating the white cell line implicated in each infection.1 The particular symptoms that may guide the diagnostician with these infections are, myalgia, tendon pain, extreme soft tissue tenderness, right upper quadrant pain, slumped posture, hepatomegaly, diaphoresis, muscle spasms, elevated liver enzymes (intrahepatic pattern) and, most importantly, a depressed white cell count.15,16 Acute infection can be lethal, and treatment must start on clinical suspicion with doxycycline before laboratory confirmation. However, when these TBDs are associated with borreliosis, there appears a much slower chronic and lingering infection with reports of extreme fatigue and feeling severely unwell. With not much in the way of distinctive symptoms, a high degree of suspicion is warranted. Testing is done by serology and polymerase chain reaction (PCR).

Other infections

Mycoplasma pneumoniae and *Mycoplasma fermentans* will lead to fatigue, fever, insomnia, headache, bowel issues, psychiatric disturbances, and arthritis (both large and small joints) with joint swelling.16 *M. fermentans* can show a negative response to prior antibiotic use with tetracyclines and macrolides. *Chlamydophila pneumoniae*, formerly designated as *Chlamydia pneumoniae* (CPN), is a known cause of respiratory illness, but it may also cause widespread arthritic pains in patients with TBD, as can *C. trachomatis*.19 Of particular note in the current study shows 68% monoarthropathy and 32% polyarthropathy. *M. pneumoniae* and *Chlamydia* serum PCR investigations returned positive findings in spite of negative serology for those infections in the patient series. *Rickettsia* infections sometimes appeared, and they routinely had neither been tested nor treated. Anecdotally, Epstein–Barr virus infection is said to be problematic in the presence of borreliosis and can flare up

during treatment. Brucellosis, human herpesvirus 6, human immunodeficiency virus (HIV), leptospirosis, parvovirus, Q fever, and syphilis must be considered when determining the status of the patient, particularly in the context of immune suppression, as will be discussed.

Imaging studies

Many patients presented with prior brain CT scans and MRIs. When absent, a CT of the brain was ordered. In some patients who appeared to have a greater degree of neurological deficit but normal prior scanning, SPECT CT of the brain was done.

Laboratory assessment

Two laboratories capable of extensive investigation of *Borrelia* and other TBD coinfections were used.

Australian biologics

Australian Biologics Laboratory Testing Services in Sydney, Australia was used initially in the series, and whole blood and serum analyses were performed for the detection of specific *Borrelia* sp. DNA using multiplex PCR for *B. burgdorferi*, *B. afzelii*, and *B. garinii*. Samples were analyzed in duplicate with positive and negative controls using primers AB–B1 (proprietary to Australian Biologics) for the *Borrelia* 16S rRNA gene target. The thermal profile for both analyses involved incubation for 2 minutes at 50°C, polymerase activation for 10 minutes at 95°C, then PCR cycling for 40 cycles of 10 seconds at 95°C, dropping to 60°C sustained for 45 seconds. The positive control used was an American Type Culture Collection (ATCC) *B. burgdorferi* genomic control. The magnitude of the PCR signal generated (ΔR) for each sample was interpreted as positive or negative, as compared to positive and negative controls. Validation of the assay was produced firstly by the use of external sequencing, and secondly through participation in quality assurance programs for the detection of *Borrelia* by PCR with Quality Control for Molecular Diagnostics (Glasgow, Scotland, UK), a joint research facility of Glasgow and Strathclyde Universities (Glasgow, Scotland, UK). Later, urine PCR analysis was added. From February 2012, some patients were also investigated by Mikrogen immunoblot for IgM and IgG, and they were also tested for borrelial T-lymphocyte transformation via the enzyme-linked immunosorbent spot test (ELISPOT). This laboratory also offered

M. pneumoniae, *M. fermentans*, and chlamydophila PCR investigations.

*****IGeneX

The other laboratory principally used for borreliosis investigation was IGeneX in Palo Alto, CA, USA. IGeneX also offered testing for *Babesia*, *Bartonella*, *A. phagocytophilum*, and *E. chaffeensis*. IGeneX is a major laboratory specializing in TBDs. It is a reference laboratory recognized by the American College of Pathologists, and it is also Clinical Laboratory Improvement Amendments (CLIA), Medicare, and Medicaid approved, thus satisfying licensing requirements for testing throughout most of the United States to perform highly complex clinical testing.20 The US Food and Drug Administration and Centers for Disease Control and Prevention (CDC) oversee the performance of the CLIA.20 IGeneX has also met licensing requirements for testing in the states that require additional licensing: California; Florida; Maryland; New York; and Pennsylvania. Statements concerning laboratory performance and validation in the area of quality assurance in borreliosis testing are available on the IGeneX Website.21 Collection of sera and timely shipment was previously discussed.1 Sera were analyzed by an immunofluorescent antibody assay (IFA), followed by IgG and IgM Western blot (WB) assays. IGeneX WB uses two different strains B-31 and B-297. OspA and OspB bands are retained in the tests.22 These bands were removed from the CDC reporting criteria because they were used in the Lyme vaccine, LYMErix™. The result is a sensitivity level >90% for the IGeneX WB test compared to 46% sensitivity for commercial tests.22 Both whole blood and sera were analyzed for the detection of specific *Borrelia* sp. DNA using multiplex PCR testing for *B. burgdorferi*-specific DNA sequences from OspA A plasmid and flagellin genes. The test is not *B. burgdorferi* specific, and it also detects *B. afzelii*, *B. andersoni*, and *B. garinii*. Simultaneously, some patients were tested for *B. microti*, *B. duncani*, *A. phagocytophilum*, *E. chaffeensis*, and *B. henselae* infection by IgG and IgM IFA serology, PCR, and fluorescent in situ hybridization.

Other laboratories

Several standard accredited laboratories across Australia were used for CD57 estimation and the several nonborreliosis serologies discussed within.

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Results

Demographic and epidemiologic factors

The average patient's age at presentation was 41 years, with a median of 42 years and a standard deviation (SD) of 18 years. Refer to Table 1 and see Figure 1 for a rather widely splayed cumulative distribution curve. There were 310 females (62%) in the study. Only 83 of 492 respondents (16.5%) reported never leaving the country. Illness length in years was an average of 7.4, with a maximum of 47 years, a minimum of 0.17 years, a median of 3.0 years, and a SD of 9.5 years from a sample size of 341. The average age of the commencement of illness was therefore 35 years, with a median of 35 years and a SD of 19 years. Thirteen patients' illness could be traced from birth or in the first year of life; six were siblings, two were from one family, and four were from another, indicating the possibility of maternal transmission. In addition, borreliosis was laboratory proven in both mothers. A recognized history of tick bites was found in 240 of 340 individuals (71%), though the time span from bite to illness could not be reliably estimated in the majority of cases.

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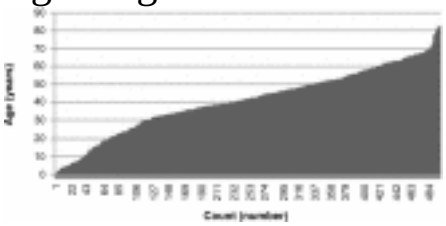

Cumulative age histogram of patients in years.**Clinical findings**

Borrelia

Certain clinical parameters are shown in Table 2. Of the 240 individuals reporting a tick bite, 108 (22% of the total cohort size) reported an associated rash large enough to have been considered as erythema migrans. Clinically, the diagnostic criterion for this rash is a minimum of 5 cm. Cranial nerve involvement was extensive and is charted as a

histogram for each individual nerve (Figure 2). Cranial nerves 5, 8, 10, and 11 were involved in at least 50% of respondents. It was previously proposed that when diagnosing neuroborreliosis clinically, only *Borrelia*, *Bartonella*, sarcoid, amyloid, and Guillain–Barre disease could affect more than two cranial nerves at a time, and that three nerves appear to be a crucial number in this series.1 Involvement of more than three cranial nerves was found in 296 patients (59%), while involvement of more than two cranial nerves was found in 310 patients (62%) (see Figures 3,
,4A,
4A, and
and4B).
4B). The incidence of clinically-supported borreliosis jumps dramatically at the involvement of four cranial nerves (refer to Table 8 and Figure 4). Cardiac history commonly revealed tachycardia or arrhythmia. All patients presented with electrocardiograms that were already performed to assess for heart block (a known complication of borreliosis), and to determine the baseline QTc for prescribing purposes.23 No incidence of third-degree heart block was found. Seriously delayed QTc was found in only a very small number of patients. There were some patients that were already diagnosed with postural orthostatic tachycardia syndrome (POTS), and adding suspected cases from the patient histories brought that number to 48. Many went on to have a positive tilt table test. POTS is a recognized complication of borreliosis.24,25 Neural involvement with lymphoplasmocellular infiltrates can be found in the autonomic ganglia.26 More centrally, cardiac rhythm has been shown to be controllable from the reticular formation in the midbrain, as well as from the posterior hypothalamus.27 There are no literature reports associating bartonellosis to POTS on a PubMed search. The author, however, suspects this association on the basis of Herxheimer-type reactions (which will be discussed) during initial *Bartonella*-targeted treatment, which is administered prior to borrelial treatment.
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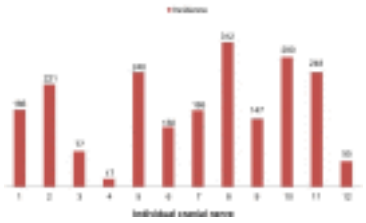
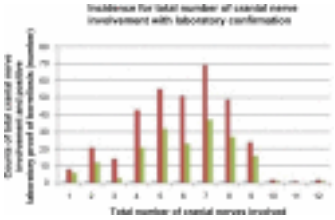
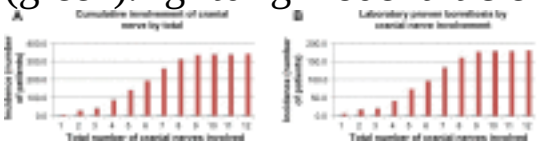


Histogram of the incident of involvement for each cranial nerve.fig ft0fig mode=article f1



Histogram showing the incidence of the total number of cranial nerves involved in the dataset (red) and respective laboratory proof of borreliosis (green).fig ft0fig mode=article f1



Cumulative involvement of the cranial nerves by total and laboratory-proven borreoliosis by cranial nerve involvement.table ft1table-wrap mode=article t1

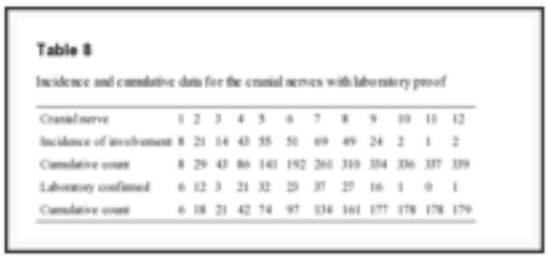


Table 8

Incidence and cumulative data for the cranial nerves with laboratory proof

Cranial nerve	1	2	3	4	5	6	7	8	9	10	11	12
Incidence of involvement	8	21	14	43	55	51	69	49	24	2	1	2
Cumulative count	8	29	43	86	141	192	261	310	334	336	337	339
Laboratory confirmed	6	12	3	21	32	23	37	27	16	1	0	1
Cumulative count	6	18	21	42	74	97	134	161	177	178	178	179

Incidence and cumulative data for the cranial nerves with laboratory proofIn addition to POTS and heart block, borreliosis can cause carditis. A PubMed search for borreliosis + carditis conducted on September 6, 2014 yielded 326 articles. Severe cardiac arrhythmia and arrest has been reported.28–33 Bartonellosis may cause carditis and also cardiac valvular disease. A PubMed search on the same date for cardiac + bartonellosis + human returned 123 articles.

In the study gastrointestinal symptoms were recorded for nausea, belching, flatus, diarrhea, constipation, and abdominal pain. *Borrelia* may cause bladder dysfunction. A key urinary symptom includes returning immediately after urinating but finding nothing to pass. Skin problems were reported in 11% of the cohort; they were usually widespread and often presented with itching. In 6% of patients, Morgellons could be demonstrated by skin microscopy. Morgellons has recently been closely associated with borrelial infection.34–37 There was no previous report of the incidence of Morgellons in a large borreliosis population cohort. Moreover, 11% of patients presented with small joint pain. Most often, one had to see which treatment worked best to abate this symptom to be sure of its diagnostic etiology. The two patients with large joint pains had returned from living in the US where they had contracted and undergone treatment for their infection. Using cranial nerve criteria of the involvement of more than three cranial nerves, there was a clinical diagnosis of borreliosis in 296 individuals and laboratory proof in 158 of those cases.

Babesiosis

Examining the possible constellation of symptoms and signs with respect to babesiosis there were 268 cases clinically suspected and 46 laboratory proven. The babesiosis figures can be further subdivided into infection by *B. microti* (number [n]=13) and *B. duncani* (n=33), respectively, from 123 patients; four individuals had both simultaneously. These coinfections are more difficult to prove.

Bartonella

For bartonellosis, when examining the possible constellation of specific symptoms and signs, it was found that there were 240 clinically-suspected cases and 23 laboratory-proven cases. With *Bartonella*, we suspect that there is more than the genus *B. henselae* involved, which is the only one tested in this series. In Australia, *B. koehlerae* has been reported to cause human disease.38 *B. bacilliformis*, *B. quintana*, and *B. vinsonii* subspecies *berkhoffii* are known to cause human disease as well.39–42 Similarly, it is suspected that in Australia, there are other *Babesia* sp. causing human infection, and *B. divergens* is known to cause human disease.40 Combined symptomatology of the two coinfections, as well as borreliosis alone, was found in 307 (61%) individuals. Finally, by aligning the data for clinical and laboratory factors for all three infections, only 50 (10%) individuals in the entire cohort had no diagnosis. This assumes that *Babesia* and *Bartonella*, where they had occurred, were transmitted with borreliosis as a TBD. For *Bartonella*, this is not necessarily true, and among the case load there were two incidences of pure *Bartonella* infection, requiring only *Bartonella*-targeted treatment to achieve full recovery.

Other infections

Clinical factors for other infections were not reliably recorded in the individual files.

Laboratory findings

The specific methods of laboratory testing for borreliosis, bartonellosis, babesiosis, *Anaplasma*, and *E. chaffeensis* have been previously discussed.1 Results showing the incidence of the respective findings are set out in Table 4 for Australian Biologics; of note, 127 of 216 individuals

were borreliosis positive (59%). In Table 5, which presents data for IGeneX, it was found that 146 of 252 individuals were borreliosis positive (58%). Also, IGeneX testing showed that 66 of 123 individuals were positive for *Babesia* (54%), 29 of 122 were positive for *Bartonella* (24%), eight of 26 were positive for anaplasmosis (31%), and five of 26 were positive for *E. chaffeensis* infection (19%). A review of the sequencing information of the data from Australian Biologics showed that the following species were found: *B. burgdorferi* CA382; N40; ZS7; *B. garinii*; *B. afzelii*; and *B. valaisiana*. The initial CD57 count was assessed in 418 individuals; the average was 78. Three were as low as zero. Normal is considered higher than 110.

Imaging

Invariably, CT and MRI were normal, except that occasionally an MRI brain scan would report flecks or spots of white in white matter, which were dismissed as unimportant, incidental, or of no clinical significance. SPECT CT of the brain is a valuable method for documenting the degree of neuroborreliosis.43 Fifty-five patients underwent the procedure. Some sites in Australia reliably reported the defects of this disease as neuroborreliosis. Other sites reported defects, but they dismissed these as nonspecific, in spite of the diagnosis on the request form. A review of the patient data indicated that 40 scans (73%) showed regional defects in perfusion, as reported by radiologists, with some reports confirming neuroborreliosis.

Anaplasma Ehrlichia infection case reports

Many patients were tested by serology and PCR for *A. phagocytophilum* and *E. chaffeensis* in Australia with completely negative results. Recently, the author requested this testing from IGeneX, and positive results were obtained again. Eight patients had *Anaplasma* infection and five had *E. chaffeensis* infection. Importantly though, four very sick individuals were found: one with *E. chaffeensis* and three with *Anaplasma*; these patients had never left Australia. This becomes the first case report of endemic human infection in Australia with these two bacteria. Two had low white cell counts of 3.4 and 2.9 by 10⁹/L, respectively. All four were markedly intolerant of doxycycline and had to start 50 mg doses, which slowly increased incrementally. All had laboratory-proven coexistent borreliosis, indirectly providing evidence for tick transmission.

Rickettsial infections

Of the 500 patients in this study, 30 of 279 (11%) individuals had positive rickettsial serology (either IgM or IgG), demonstrating past or present infection. Allowing for some degree of cross-reactivity, this is still an appreciable number.

Other parameters

Other factors known to suppress the immune system in borrelial infection are the administration of cortisone and chemotherapy.44,45 Many coexisting infections are found in borreliosis, the incidence of which is reported in Table 6. *C. pneumoniae* testing by serology was positive in 48 out of 152 individuals and by PCR in nine out of 50 individuals on serologically-negative patients. Serology detected that 44 of 174 individuals were positive for *M. pneumoniae*, while PCR detected that 30 out of 65 were positive among serologically-negative patients. *M. fermentans* by PCR detected 18 positives out of the 69 tested. Other infections that were screened for included Q fever, brucellosis, leptospirosis, and HIV; viral encephalopathies may need to be considered.46

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Discussion

Distinguishing between borreliosis and bartonellosis in a clinical setting is extremely difficult, and reliance on specific *Bartonella* symptoms is important. It appears that both diseases often coexist and are tick-borne in Australia. A comparison can be made with a recent publication by Berghoff 47 on the differential diagnoses of these infections. There is a wide age distribution and, importantly, an extensive delay in the recognition and diagnosis of neuroborreliosis. This could be improved with detection of cranial nerve symptoms and deficits. The cumulative histogram in Figure 4 shows, firstly, an exponential increase in the clinical diagnosis of neuroborreliosis with increasing cranial nerve involvement and, secondly, the number of positive laboratory findings that rise correspondingly with the involvement of four cranial nerves. The histograms show a sigma curve, which is virtually identical for both a clinical diagnosis versus a laboratory-supported diagnosis. When examining the figures for a clinical diagnosis, the X-axis simply represents an ordinal increase in count and the gradient (tan θ) is simply

the difference between each Y-axis count: 14 for 3; 43 for 4; 55 for 5; 51 for 6; and 69 for 7, before falling again as the sigma curve flattens. Linear regression analysis of the data yielded a coefficient of 0.999485. It is proposed that the involvement of three cranial nerves is thus a critical number, which could pivot the diagnosis of clinical borreliosis or bartonellosis in either direction; however, the involvement of four or more cranial nerves correlates well with a clinical diagnosis of either borreliosis or bartonellosis.

The ELISA/IFA/WB controversy has been discussed before.[1] The current CDC position statement on Lyme disease case surveillance can be found on the CDC Website.[48] It has been acknowledged by the CDC that there is a large burden of disease that can only be diagnosed clinically. Specifically, the CDC states the following “For purposes of surveillance”:[48]

Nervous system. Any of the following, alone or in combination: lymphocytic meningitis; cranial neuritis, particularly facial palsy (may be bilateral); radiculoneuropathy; or, rarely, encephalomyelitis.

In this report, there is very close concordance between the sensitivities of IGeneX and Australian Biologics running at 58% and 59% of the total tested, respectively. In Europe borreliosis is associated sometimes with acrodermatitis chronica atrophicans which is caused by *B. afzellii*. In this series, only two patients had acrodermatitis chronica atrophicans and one had never traveled outside Australia. Diagnosing the correct infections to prioritize appropriate treatment is essential. It is important to clear coinfections before treating infections caused by *Borrelia*.[15,16] Treating *Borrelia* infections first can, in fact, make the patient deteriorate further.[15,16] This is supported by observation of the emergence of the specific coinfection symptoms of bartonellosis or babesiosis during borreliosis treatment. Not only must the two coinfections be cleared, but any of the other current infections should also be treated, and sometimes this can be viral. A quick check on the CD4 count will tell if the patient is fighting a viral load. Almost surprisingly, the incidence of HIV positivity in the cohort was zero where tested. As can be seen by the data presented, pure borrelial infection is not common in Australia, and consideration must be given to a host of infections when assessing TBD. The data were

reliable for this finding from patient 300 onwards. In this subset, 17 (8.5%) patients had pure borreliosis confirmed by testing, and no clinical suspicion or laboratory proof of coinfection was observed. Fifty patients (10%) could not be diagnosed as having one of the three key infections in the series, either clinically or by investigation. It is highly concerning that 11% of patients had positive rickettsial serology with a protracted disease complex and had never been tested for this infection.

White matter flecks seen on MRI in proven neuroborreliosis can be a result of that infection.49–52 Further, Kanekar and Devgun53 state that in neuroborreliosis,

The most common abnormality seen on MR is multiple bilateral periventricular and/or subcortical foci of T2 prolongation. These findings usually mimic MS (multiple sclerosis); however, multiple enhancing cranial nerves (third, fifth, and seventh cranial nerve), nerve root, or meningeal enhancement may favor Lyme over MS.43,53–56

The incidence of SPECT abnormality in neuroborreliosis was 75% in one study.43 The current study's data of 40 out of 55 scans (73%) appear to conform to those findings if the reported areas of reduced perfusion are considered as an abnormal scan. CD57 is a measure of immune suppression in the presence of borreliosis;57 normal is considered a value ≥110 cells/mm3 using the Beckman Coulter antibody (catalog A74779; Beckman Coulter, Inc., Brea, CA, USA), with <60 considered very significant borreliosis. The potential diversity of tick-borne infections was recently enumerated at 237 pathogens in a recent study of *Ixodes perscultatus* in the People's Republic of China;58 this has modeling applications for Australia.

Jarisch–Herxheimer reaction

This reaction is well described in the literature, but particularly well by Horowitz.16 In summary, the breakdown products of the cellular components of *Borrelia*, *Babesia*, and *Bartonella* (in particular, the cell wall products of *Borrelia*) produce a temporary IL storm of IL-1, IL-6, and TNF-α. The effect is similar to having viral influenza, which induces the same storm, plus a patient will experience some of the symptom set associated with their own disease complex. The reaction can be very severe in some individuals and is dependent upon bacterial load versus treatment dosage. In treatment, a balance is sought between what a patient

can comfortably endure using as many remedial measures as possible to relieve distress, and sufficient therapy to be effective against the disease being targeted.

The vectors

Recently Mayne et al have unequivocally demonstrated evidence for *Ixodes holocyclus*, the paralysis tick, as a vector for human borreliosis.[59] The vector is found along the entire eastern seaboard of the continent and across into central Victoria. Other vectors need to be sought in this region and across Australia.

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Conclusion

Neuroborreliosis can affect large numbers of cranial nerves. Herewith, data were presented demonstrating that a thorough search for symptoms and signs of cranial nerve abnormality can clinically pinpoint neuroborreliosis and/or bartonellosis when more than three nerves are involved. The total results highlight an underestimated set of TBD in Australia that requires further research and evaluation. Borreliosis is protean in its manifestation and difficult to treat if protracted. Those in the medical profession in Australia need to be alert to the possibility of detecting this disease and its associated coinfections early to initiate effective short-lasting treatment, thus preventing a cascade into severe chronic borreliosis, which can be devastating in all aspects of life.

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Acknowledgments

The author thanks the Southern Analytics Team: Kosta Patsan; Alex Patsan; Daphne Patsantzopoulos; Richard Jennings; Irene Pappas; Costa Pappas; Emily Miklovic; and Anna Farmaki for their arduous work in preparation of the data; as well as Marianne Middelveen for manuscript review.

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Footnotes

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Disclosure

From 2014 the author has served as a medical adviser at Australian Biologics as a free service. The author reports no other conflicts of interest in this work.

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Ticks of Australia. The species that infest domestic animals and humans.

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Abstract

The book Australian Ticks by F.H.S. Roberts (1970) is a land-mark in Australian tick biology. But it is time for a new and improved book on the ticks of Australia. The present book has identification guides and accounts of the biology and diseases associated with the 16 species of ticks that may feed on domestic animals and humans in Australia. These comprise five argasid (soft) ticks: Argas persicus (poultry tick), Argas robertsi (Robert's bird tick), Ornithodoros capensis (seabird soft tick), O. gurneyi (kangaroo soft tick), Otobius megnini (spinose ear tick); and 11 ixodid (hard) ticks, Amblyomma triguttatum (ornate kangaroo tick), Bothriocroton auruginans (wombat tick), B. hydrosauri (southern reptile tick), Haemaphysalis bancrofti (wallaby tick), H. longicornis (bush tick), Ixodes cornuatus (southern paralysis tick), I. hirsti (Hirst's marsupial tick), I. holocyclus (paralysis tick), I. tasmani (common marsupial tick), Rhipicephalus (Boophilus) australis

(Australian cattle tick) and R. sanguineus (brown dog tick). We use an image-matching system to identify ticks, much like the image-matching systems used in field-guides for birds and flowers. Ticks may be identified by drawings that emphasise unique matrices of uniformly defined morphological characters that, together, allow these 16 ticks to be identified by morphology unequivocally. The species accounts have seven sections: (i) General; (ii) Differential diagnosis; (iii) Hosts; (iv) Life-cycle and seasonality; (v) Disease; (vi) Habitat and geographic distribution; (vii) Genes and genomes; and (viii) Other information. There are 71 figures and tables, including a glossary character matrices, drawings of life-cycles, drawings of genera, species, and colour photographs of tick biology.
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*****Queensland Institute of Medical Research, Brisbane, Australia

Abstract The vectors of scrub typhus and Queensland tick typhus have not previously been
positively identified in Australia. Small mammals were trapped at Mossman (north Queensland) and 4 strains of Rickettsia tsutsugamushi were isolated in mice inoculated **...**

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Range expansion of the tick Amblyomma triguttatum triguttatum, an australian vector of Q fever.

McDiarmid L[1], Petney T, Dixon B, Andrews R.

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Abstract

The tick Amblyomma triguttatum triguttatum has previously been reported from Western Australia, Queensland and New South Wales. A viable population of this species, including all developmental stages, has now been discovered on the southern end of Yorke Peninsula, South Australia. Species determination was carried out morphologically and using 18S and 16S rRNA. The data for 16S rRNA are the first published for this species. Amblyomma t. triguttatum is significant through its involvement in the natural, Australian cycle of Coxiella burnetti, the pathogen causing Q fever. The environment of Yorke Peninsula contains all of the components required for a natural Q fever cycle and three cases of this disease have been reported from this area since 1995. These findings reinforce the need to put in place effective mechanisms to monitor parasite distributions at a time of

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Borrelia burgdorferi sensu stricto and Borrelia afzelii: Population structure and differential pathogenicity.

Jungnick S[1], Margos G[2], Rieger M[1], Dzaferovic E[1], Bent SJ[3], Overzier E[4], Silaghi C[4], Walder G[5], Wex F[6], Koloczek J[7], Sing A[7], Fingerle V[1].

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Abstract

MultiLocus sequence typing (MLST) is considered a powerful method to unveil relationships within bacterial populations and it constitutes an economical and fast alternative to whole genome sequencing. We used this method to understand whether there are differences in human pathogenicity within and between different Borrelia burgdorferi sensu lato species. Therefore, 136 strains from human patients or ticks from Europe were included in MLST analyses. The scheme employed used eight chromosomally located housekeeping genes (i.e. clpA, clpX, nifS, pepX, pyrG, recG, rplB and uvrA). We investigated Borrelia afzelii, one of the predominant species in Europe, and B. burgdorferi sensu stricto (s.s.), because it allowed comparative analysis to strains from the USA. We typed 113 patient isolates as well as 23 tick isolates. For further comparative purposes an additional 746 strains from Europe and the USA were included from the MLST website http://borrelia.mlst.net. We observed an overlap of the B. burgdorferi s.s. populations from Europe and the USA isolated from human patients while there was no overlap of the populations found in tick vectors. Further results indicate that B. afzelii was significantly less associated with disseminated infection than B. burgdorferi s.s. and that B. burgdorferi s.s. from Europe caused neuroborreliosis to a significantly greater extent than B. afzelii or B. burgdorferi s.s. in the USA. Our data suggest that there may be an evolutionary basis of differential interspecies pathogenicity in Borrelia. This was not evident within Borrelia species: we found the same sequence types in patients with disseminated or localized symptoms when the number of strains was sufficiently high. We hypothesize that the finding that B. burgdorferi s.s. in Europe is much more associated with neuroborreliosis than in the USA maybe linked to factor(s) related to the human host, the tick vector or the bacterium itself (e.g. plasmid content and structure).

KEYWORDS:
Borrelia afzelii; Borrelia burgdorferi sensu stricto; Human pathogenesis; Lyme borreliosis; Multilocus sequence analysis; Population structure
PMID: 26341331 [PubMed - in process]

Abstract:
Ticks Tick Borne Dis. 2015 Dec 18. pii: S1877-959X(15)30053-4. doi: 10.1016/j.ttbdis.2015.12.012. [Epub ahead of print]

A review of reverse vaccinology approaches for the development of vaccines against ticks and tick borne diseases.

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Abstract

The field of reverse vaccinology developed as an outcome of the genome sequence revolution. Following the introduction of live vaccinations in the western world by Edward Jenner in 1798 and the coining of the phrase 'vaccine', in 1881 Pasteur developed a rational design for vaccines. Pasteur proposed that in order to make a vaccine that one should 'isolate, inactivate and inject the microorganism' and these basic rules of vaccinology were largely followed for the next 100 years leading to the elimination of several highly infectious diseases. However, new technologies were needed to conquer many pathogens which could not be eliminated using these traditional technologies. Thus increasingly, computers were used to mine genome sequences to rationally design recombinant vaccines. Several vaccines for bacterial and viral diseases (i.e. meningococcus and HIV) have been developed, however the on-going challenge for parasite vaccines has been due to their comparatively larger genomes. Understanding the immune response is important in reverse vaccinology studies as this knowledge will influence how the genome mining is to be conducted. Vaccine candidates for anaplasmosis, cowdriosis, theileriosis, leishmaniasis, malaria, schistosomiasis, and the cattle tick have been identified using reverse vaccinology approaches. Some challenges for parasite vaccine development include the ability to address antigenic variability as well the understanding of the complex interplay between antibody, mucosal and/or T cell immune responses. To understand the complex parasite interactions with the livestock host, there is the limitation where algorithms for epitope mining using the human genome cannot directly be adapted for bovine, for example the prediction of peptide binding to major histocompatibility complex motifs. As the number of genomes for both hosts and parasites increase, the development of new algorithms for pan-genomic mining will continue to impact the

future of parasite and ricketsial (and other tick borne pathogens) disease vaccine development.

KEYWORDS:
Reverse vaccinology; Tick borne diseases; Ticks; Vaccines; Veterinary
PMID: 26723274 [PubMed - as supplied by publisher]

Research into ticks and tick-borne disease

Department of Medical Entomology: Ticks

Department of Medical Entomology
Level 3, ICPMR,
Westmead Hospital AUSTRALIA

**** http://medent.usyd.edu.au/fact/ticks.htm

The female Paralysis tick, *Ixodes holocyclus*

Introduction

Ticks are bloodsucking, external parasites that are often encountered by people during activities in the Australian bush.

There are many species known to attack humans and so samples should be referred to our expert laboratory for proper identification. Over the last twelve years, the Department of Medical Entomology, ICPMR has been at the forefront of research into ticks and tick-borne disease, and has been the leading health authority for the provision of information on the ecology and control of this important public health pest.

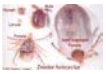
Natural History

The distribution of our most medically important tick, the Paralysis tick, *Ixodes holocyclus*, is roughly confined to a 20-kilometre band that follows the eastern coastline of Australia. As this is where much of the human population resides in NSW, encounters with these parasites are relatively common. Although most cases of tick bite are uneventful, some can result in life threatening illnesses including paralysis, tick typhus and severe allergic reactions.

Ticks belong to the order Acarina, which also contains mites. The Australian tick fauna consists of approximately 75 species that can be divided into two families. The soft ticks (Family Argasidae) are represented by only a few species in Australia and are often associated with nests or resting places of animals. These ticks have a wrinkled appearance, which is akin to soft leather. The hard ticks, (Family Ixodidae), comprise the majority of our ticks and are distinguished by a hard dorsal plate in the shape of a fingernail and elongated mouthparts that have rows of backward pointing teeth. Some species of tick use these teeth in conjunction with a cement to remain attached to the host while blood feeding.

The Paralysis tick can be found in a variety of habitats but are especially common in wet sclerophyll forests and temperate rainforests. They have very few predators, and are more likely to succumb to desiccation from high temperatures and low humidity. From the enormous numbers of eggs (2,500-3,000) deposited in the moist leaf litter by the female before she dies, only a fraction of the eggs will survive and eventually grow to become adults. The

six-legged larvae hatch after the eggs have incubated for 40-60 days. To moult to the next stage, the larval tick must obtain a blood meal. In searching for a host, they display a behaviour referred to as 'questing'; whereby the tick climbs to the top of nearest vegetation and waves its forelegs to and fro slowly, hopefully contacting a prospective passing host. This is usually a native animal such as a bandicoot, which is the main host, but also possums, kangaroos, and humans. This questing behaviour is undertaken each time a host is required for blood. Ticks usually do not climb more than around 50cm in the vegetation and there is no evidence to suggest that they fall out of trees.



Once a suitable host is found, the larvae will blood feed for 4-6 days, drop from the host and moult to the eight-legged nymphal stage. Nymphs require a further blood meal for 4-8 days before moulting to the adult stage. Both female and male ticks quest for a host, but for different reasons; the female for a bloodmeal, the males to search the host for female ticks in order to mate and sometimes feed from them. Males may actually parasitise the female ticks by piercing their cuticle with their mouthparts to feed on her haemolymph (the tick's blood) and up to 3-4 males have been found feeding on one female tick. Male ticks rarely bloodfeed on a host. The adult female Paralysis tick will feed for up to around 10 days, drop off the host and lay eggs over several weeks. The entire life cycle of the Paralysis tick, involving 4 stages and 3 hosts, will take around a year to complete. Each life stage can be present throughout the year, although for the Paralysis tick, adults are more abundant in the spring and the early summer months, larvae in mid to late-summer, and nymphs during winter.

Clinical Presentation

Tick paralysis is most likely to be seen in children. The initial symptoms of tick paralysis may include unsteady gait, increased weakness of the limbs, multiple rashes, headache, fever, flu like symptoms, tenderness of lymph nodes, and partial facial paralysis.

Tick paralysis develops slowly as the tick engorges, which will take several days. Despite the removal of the tick, the patient's condition typically will continue to deteriorate for a time and recovery is often slow. Undetected ticks are another possible reason for any prologed debilitation and should always remain a concern. Improvements in modern medicine and the development of a tick antitoxin have prevented further deaths from tick paralysis in the last 70 years. The antitoxin is available from the Commonwealth Serum Laboratories. Despite these developments, a few cases of tick paralysis in children are seen at major hospitals each year. Additionally, ticks take a high toll on pets every summer.

Tick typhus is an infection with a rickettsia (bacteria-like organism) transmitted from native animals by ixodid ticks and is confined to the eastern coast line of Australia and Bass Strait Islands. Clinical symptoms include headaches, multiple rashes, swollen glands, fever and flu like symptoms. The disease is rarely fatal and is commonly treated with antibiotics.

Lyme disease is a tick-borne infection common in the northern hemisphere and is caused by spirochaete bacteria. Symptoms are varied and may include rashes, fever, muscle and joint pain, and arthritis. The disease is not fatal and treatable with antibiotics. Despite clinical cases being reported from the early 1980's, there has been no confirmation that the disease occurs in Australia. (for more information go to the Fact Sheet on "Lyme Disease").

Allergic reactions are the most serious medical condition associated with ticks. These reactions can vary from a mild itching with localised swelling to widespread swelling with pain (**Error! Hyperlink reference not valid.**) to a severe and life threatening anaphylatic condition. Unlike with most other medical conditions associated with ticks, severe allergic reactions may occur with any tick stage. For people who develop severe allergic reactions, it is imperative that they must always avoid contact with ticks and avoid potential tick infested areas.

Laboratory Diagnosis

All stages of ticks are identified with the aid of a stereomicroscope and taxonomic keys, but many species are difficult to identify accurately. The Department of Medical Entomology provides a specialist identification service.

Treatment and Control

The best method of avoiding ticks is to stay away from known tick infested areas. If visiting such an area, light coloured clothing should be worn, as ticks will be much easier to detect. Trousers should be tucked into socks and shirts into pants. An insect repellent containing DEET or Picaridin should be applied, with a cream repellent applied to the skin and a spray repellent to footwear and clothing (note that DEET can damage some synthetic clothing). The repellent should be reapplied every few hours. All clothing should be removed on returning home and placed into a hot dryer for 20 minutes, which will kill any ticks that may still be on the clothing. Note that ticks can wander on the body for some two hours before attaching. This is how they become attached to the head (contrary to popular belief, they do not fall out of trees). The body should thereafter be searched well for ticks, especially behind the ears and on the back of the head. Children and pets should be examined for ticks after visiting bushland areas.

In locations where people live where they contact ticks in their backyard, then strategies can be undertaken to reduce the tick population and thereby minimise exposure. The Paralysis tick is very susceptible to dry conditions and so decreasing soil moisture can lessen their impact. This can be achieve through the reduction of foliage cover, which increases sunlight penetration to the ground, reducing the shrub layer, reducing mulching and watering, and ensuring that the lawn is kept mown low. Bandicoots, the main host of the Paralysis tick, can be kept out of the backyard through the use of animal exclusion fencing. This needs to go below the ground surface by 0.5m so that the animals cannot dig underneath.

If ticks continue to be a problem, then insecticide control is an option. Currently the only registered insecticide for the control of the Paralysis tick in NSW is Brigade. Only a licensed pest controller

can apply this chemical.

Tick Removal

If a tick is detected that is attached, never attempt to place any chemical such as methylated spirits onto the tick, nor should it be touched or disturbed, as the tick will inject saliva into the skin, which could make the situation worse. Rather the tick **should** be sprayed with an **aerosol** insect repellent preferably containing **pyrethrin** or a **pyrethroid** (if a repellent cannot be found which contains a pyrethroid, then **Lyclear**, a scabies cream containing **permethrin** will work fine). The combination of hydrocarbons and the pyrethrin acts as a narcotic and a toxicant, and prevents the tick from injecting its saliva. The tick should be sprayed again one minute later (or dabbed with the Lyclear) and left. After 24 hours it should drop off naturally or be gently removed with fine-tipped forceps. It is normal for a tick bite to remain slightly itchy for several weeks, however if other symptoms develop, then a doctor should be consulted immediately.

Confirmation and Enquiries

Identification of ticks, and other medically important insects, is performed through the Medical Entomology Department at ICPMR, Westmead Hospital. The Medical Entomology Department is the only NATA accredited laboratory in Australia for the identification of arthropods of medical importance.

See 'Contacts' for further information.

Revised & updated 7/Nov/2003

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Ixodes holocyclus

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Ixodes holocyclus

Ixodes holocyclus before and after feeding
Scientific classification
Kingdom: Animalia
Phylum: Arthropoda
Class: Arachnida
Order: Acari
Family: Ixodidae
Genus: *Ixodes*
Species: ***I. holocyclus***
Binomial name
Ixodes holocyclus Neumann, 1899


Ixodes holocyclus, commonly known as the **Australian paralysis tick**, is one of about 75 species of Australian tick fauna and is considered the most medically important. It can cause paralysis by injecting neurotoxins into its host. It is usually found in a 20-kilometre wide band following the eastern

coastline of Australia. Within this range *Ixodes holocyclus* is the tick most frequently encountered by humans and their pets. As this area also contains the majority of Australia's most densely populated regions, incidents of bites on people, pets and livestock are relatively common.
Paralysis ticks are found in many types of habitat particularly areas of high rainfall such as wet sclerophyll forest and temperate rainforest.[1] The natural hosts for the paralysis tick include koalas,[2] bandicoots, possums and kangaroos.[1]

Common names[edit]

The use of common names has led to many colloquial expressions for *Ixodes holocyclus*. The most generally accepted name used within Australia is *Australian paralysis tick* or simply *paralysis tick*.[3] The following table gives some of the other names used to describe various stages of *Ixodes holocyclus*. Many of these common names, such as dog tick or bush tick, are best not used for *Ixodes holocyclus* because they are also used for some of the other ticks found in Australia.[3]

Used (and misused) common names for *Ixodes holocyclus*	**Life stage/gender referred to**	**Comments**
Paralysis tick of Australia	All stages	The preferred common name for *Ixodes holocyclus*
Scrub tick	Adult female, Adult male	In Queensland, *scrub tick* is *also* used for *Haemaph*
Bush tick	Adult female, Adult male	Throughout Australia, *bush tick* is *also* used for *Hae*
Dog tick	Adult female, Adult male	In NSW, *dog tick* is more correctly used for *Rhipice*
Wattle tick	Adult female, Adult male	*Wattle tick* was used by pioneers in the Illawarra re sheep.
Common hardback tick	Adult female, Adult male	*Common hardback tick* was used in *The Northern H* emphasises that *Ixodes holocyclus* is indeed a 'har humans and animals in the Sydney region.
Bottle tick or *blue bottle tick*	Adult female	*Bottle tick* describes that the engorging tick become probably refers to a bluish hue associated with the

		animal, the marine stinger of the same name, the 'b
Shell back tick	Adult male	*Shell back tick* describes the tortoise-shell appearar the adult male.
Grass tick	Nymph and Larva	The term *grass tick* is usually used to refer to the sr information because any tick can be found in the gr
Seed tick	Larva	The term *seed tick* usually is used to refer to the sm
Shower tick	Larva	The term *shower tick* presumably refers to how hun time – this is because they have hatched from a sin by the first of three hosts.
Scrub itch tick	Larva	The term *scrub itch tick* is used in Queensland to d and animals in huge numbers, causing a rash. With missed until they engorge to an appreciable size.

Early scientific history[edit]

One of the earliest Australian references to ticks as a problem in human disease is found in the journal kept by Capt William Hilton Howell for his 1824-1825 journey from Lake George to Port Phillip. In this he remarked on "the small insect called the tick, which buries itself in the flesh, and would in the end destroy either man or beast if not removed in time" [4][5]

James Backhouse, a well-travelled Quaker of the early colonial period, gives the following account:[6] "At Colongatta, in Shoal Haven...district, which, like that of Illawarra, is much more favourable for the grazing of horned cattle than for sheep. Among the enemies of the latter in these rich, coast lands, is the Wattle Tick, a hard flat insect of a dark colour, about the tenth of an inch in diameter, and nearly circular, in the body; it insinuates itself beneath the skin, and destroys, not only sheep, but sometimes foals and calves. Paralysis of the hind quarters often precedes death in these cases. Sometimes it occasions painful swellings, when forcibly removed from the human body, after having fixed its anchorlike head and appendages in the skin. To prevent this inconvenience, we several times, made them let go their hold, by smearing them over with oil, or with wet tobacco ashes."

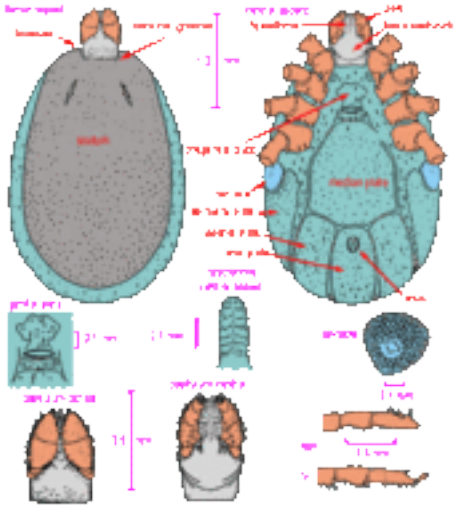
Whilst pioneering settlers knew that ticks posed a threat to their dogs and perhaps to themselves, the paralysis tick was not scientifically identified until 1899 (by Neumann[7]). It was further studied by Nuttal and Warburton (1911).[8]

By 1921 Dodd had established a definitive link between *Ixodes holocyclus* and clinical disease in three dogs. His findings were that it took 5 to 6 days from time of attachment for clinical signs to develop, with motor paralysis being the major neurological deficit.

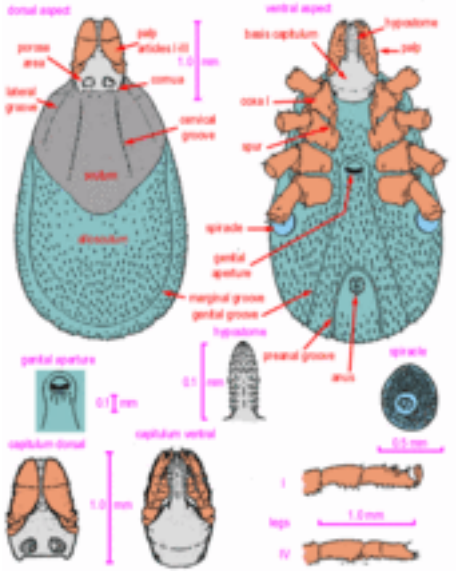
The life cycle was studied chiefly by Clunies-Ross (1924).[9] Ian Clunies Ross also demonstrated that a toxin produced by the tick was responsible for the paralysis and not some infective agent carried by the ticks.[10][11] The lifecycle was further studied by Oxer and Ricardo (1942)[12] and later summarised by Seddon (1968).[13]
In 1970 Roberts' work *Australian Ticks* [14] gave the first comprehensive description of Australian ticks, including *Ixodes holocyclus*.
The first confirmed human death due to tick venoming in Australia was reported by Cleland in 1912 [15] when a large engorged tick caused flaccid paralysis in a child, progressing to asphyxiation. Headstones at the Cooktown cemetery apparently reveal how some human deaths were attributed to ticks.[16]
In the first half of the 20th century at least 20 human deaths had been attributed to the paralysis tick. Eighty percent of the victims reported in NSW between 1904 and 1945 were children aged under four years. Many cases of 'infantile paralysis' (later known as poliomyelitis) may well have been misdiagnosed and actually been cases of tick paralysis.[17]

Anatomy, life cycle and behaviour[edit]

Life cycle of *Ixodes holocyclus*



General anatomy of an Ixodid male tick - to help with identification landmarks



General anatomy of an Ixodid female tick - to help with identification landmarks

Overview[edit]

The life cycle of *Ixodes holocyclus* consists of four (4) stages- egg, larva, nymph, adult. Ticks hatch as six-legged larvae after an incubation period of 40 to 60 days. Larvae search for a blood meal from a host, feed for four to six days, then drop from the host and moult to become an eight-legged nymph. Nymphs require a second blood meal before they can moult again to become an adult. Female adults then require a further blood meal of up to ten days before dropping off to lay up to 3000 eggs in leaf litter. Male adults will search for females on the host for mating, and to parasitise the females for blood meals. This life cycle takes around a year to complete[1] (average 365 days, minimum 135 days, maximum 437 days).

Larvae have 3 pairs of legs and the nymphs and adults 4 pairs. *Ixodes holocyclus* requires three hosts to complete its life cycle, thus it is a 'three-host tick'. To moult to the next stage a blood meal must be obtained by the larva or nymph. Moulting is known as *ecdysis*.
To find a host, ticks use a behaviour known as 'questing' - climbing onto vegetation and waving forelegs slowly until a host comes within reach. When on the host, they may not attach immediately, but wander for up to two hours until attaching on the back of the host's head or behind an ear.[1] Certain chemicals such as carbon dioxide (hence the use of 'dry ice' baits) as well as heat and movement serve as stimuli for questing behaviour.

Male adult *Ixodes holocyclus*

Female adult *Ixodes holocyclus* with eggs

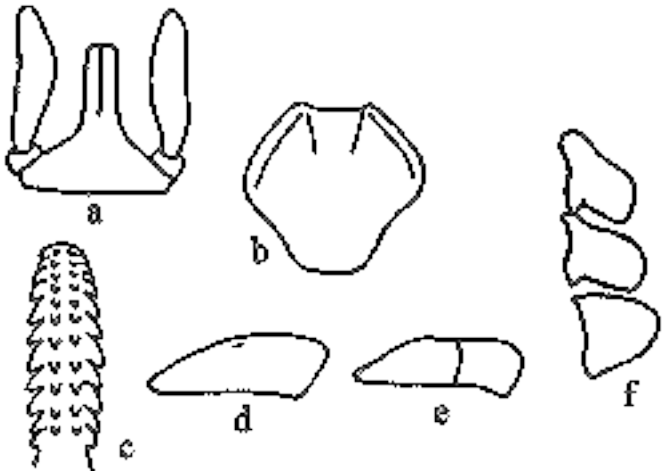
Both female and male ticks quest for a host, but for different reasons. The female quests for a blood meal, the male to search the host for a female tick in order to mate and feed from her. Males may parasitise the female ticks by piercing their cuticle with their mouth parts to feed on the haemolymph (up to 3-4 males have been found feeding on one female tick). Adult male ticks rarely blood-feed on a host. The outside surface, or cuticle, of hard ticks actually grows to accommodate the large volume of blood ingested, which, in adult ticks, may be anywhere from 200 to 600 times their unfed body weight.[18] When a tick is fully engorged it is said to be *replete*.

Egg[edit]

Adult females lay large numbers of eggs (between 2000 and 6000) in leaf and branch litter, under the scaly or fibrous bark of certain trees and shrubs, or in dense fine foliage near the tips of branches. They utilise a wax-like substance to make a cohesive mass of eggs and attach these at the selected site. A small fraction of the eggs survive and hatch to larvae after 40–110 days incubation. Development occurs with suitable warmth and high humidity (e.g. moist leaf litter).

Larva[edit]

Larvae, also known as 'seed ticks' and sometimes 'grass ticks', emerge from the eggs and move towards lateral branches, and across grassy areas during humid weather in order to find and attach to their host. Larvae undergo 7–44 days of hardening and then climb vegetation (e.g. the tips of leaves), from where they attach to a passing host. Larvae feed for 4–6 days then drop to the ground. Over a further 19-41 day period larvae then moult to become nymphs. The overall period in the larval stage (hatch to moult) is temperature dependent. It may, for example, take 20 days at 27.5 °C and 40 days at 21 °C, but may extend to 36 weeks. Larvae are just visible to the naked eye. Under laboratory conditions unfed larvae may survive for 162 days.

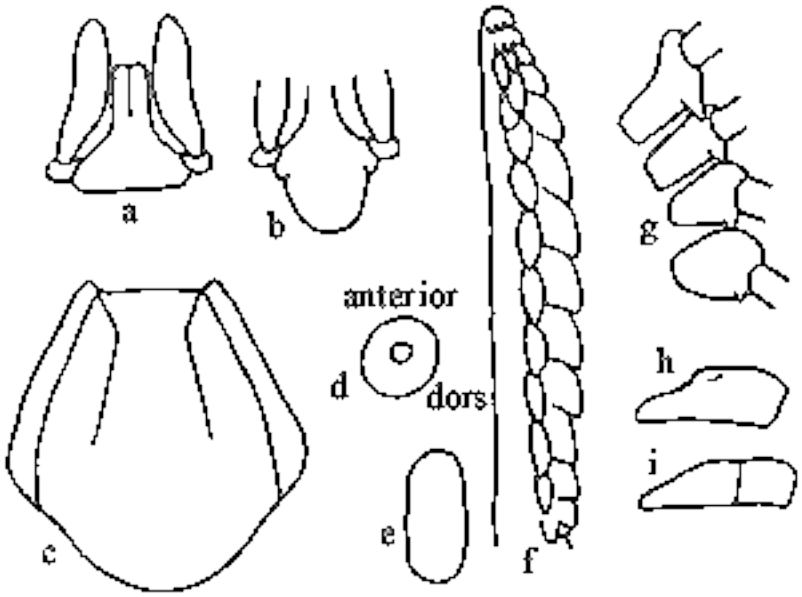


Ixodes holocyclus larva: a, capitulum (dorsal view); b, scutum; c, hypostome; d, tarsus I; e, tarsus IV; f, coxae

Diagnosis: Capitulum with slender palpi, hypostome rounded apically, dentition 2/2; scutum about as long as wide, with faint lateral carinae; all coxae with small, external spurs. **Body**: Broadly oval, 0.5 x 0.4 mm (unfed) to 1.15 x 1.0 mm (engorged) **Capitulum**: About 0.2 mm in length, basis triangular, about 0.16 mm wide, palpi elongate and slender. Hypostome apically rounded, 0.14 mm in length, dentition 2/2 of 10-12 teeth, the teeth of the inner file blunt and small, some minute denticles apically. **Scutum**: About as long as broad, 0.31 by 0.32 mm and widest a little anterior to mid-length, lateral carinae present but faint; anterolateral margins usually convex and posterolateral margins concave; cervical grooves short but well defined. **Anal grooves**: Ill defined anteriorly and do not converge behind **Legs**: Coxae with small external spurs; tarsus I 0.14 mm in length, tarsus IV 0.14 mm in length [14]

Nymph[edit]

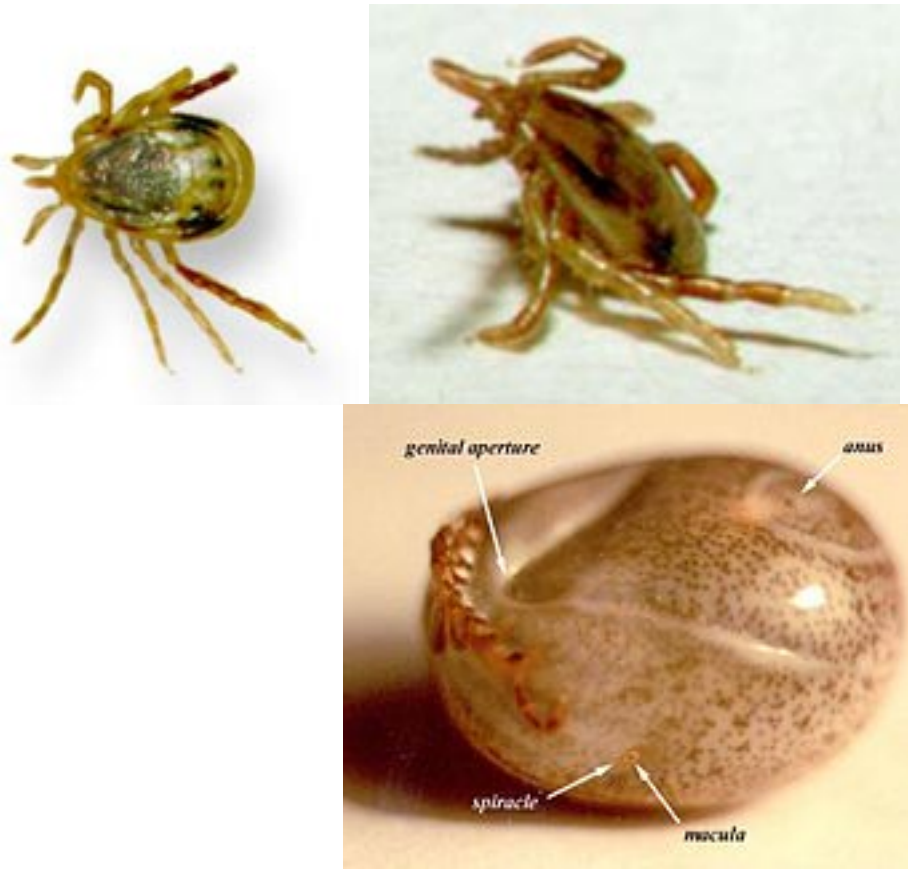
Nymphs are very active and on average 5–6 days (but possibly up to 31 days) after moulting attach to another host. Nymphs feed for 4–7 days, then drop to the ground. After a further 3–11 weeks the nymphs moult to become adult males or females. Again the period is temperature dependent: it may, for example, take 20 days at 24-27 °C but 53–65 days at 10-21 °C. Dry conditions also prolong this period and can actually kill nymphs. Under laboratory conditions unfed nymphs can survive for 275 days.



Ixodes holocyclus nymph: a, capitulum (dorsal view); b, capitulum (ventral view); c, scutum; d, spiracular plate; e, sternal plate; f, hypostome; g, coxae; h, tarsus I; i, tarsus IV

Diagnosis: Capitulum as in female, hypostome dentition mainly 2/2, 3/3 distally; scutum about as long as wide with lateral carinae; sternal plate present, oval; anal grooves converging posteriorly but remaining narrowly open; legs as in female. **Body**: Oval with fine parallel striae and some scattered pale hairs; 1.2 x 0.85 mm (unfed) to 3.5 x 2.5 mm (engorged); marginal grooves well developed and complete in unfed specimens. **Capitulum**: Length 0.40- 0.43 mm. Basis dorsally 0.23- 0.25 mm in width; posterior margin straight; posterolateral angles not salient; auriculae well defined. Palps as in female; articles 2 and 3 0.30- 0.32 mm in length. Hypostome lanceolate and bluntly pointed; dentition 2/2, 3/3 distally. **Scutum**: About as wide as long, 0.61 x 0.63 mm - 0.71 x 0.70 mm. Lateral carinae well developed. Punctations few, shallow, scattered. Cervical grooves apparent, continuing to mid-scutal region as superficial depressions. **Sternal plate**: Oval, 0.27- 0.30 mm in length and a little more than twice as long as wide. **Spiracular plate**: Subcircular, greatest diameter about 0.14 mm. **Legs**: Coxae armed as in female. Tarsus I tapering gradually, other tarsi more abruptly; length of tarsi I and IV about 0.28 mm.[14][19]

Adult female[edit]

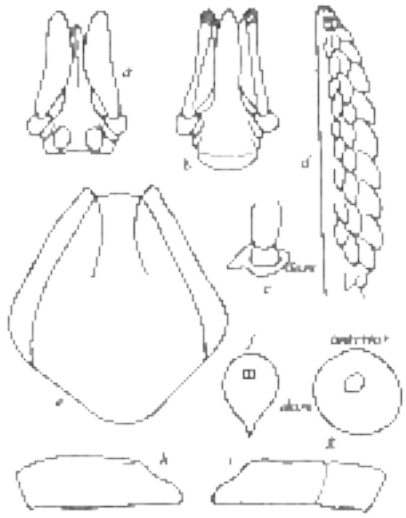


Engorged adult female of *Ixodes holocyclus*. This lateral view shows the breathing hole (*spiracle*) as well as the genital and anal grooves which are useful with species identification (see below)

Adult female of *Ixodes holocyclus*, ventral view showing the genital aperture (upper opening), anus (lower opening) and breathing spiracles (on the sides). One can also distinguish a faint but fully encircling (*holocyclus*) groove around the anus

The newly moulted adult female becomes increasingly active for the first 6–7 days and seeks a host. It attaches to the final host after 7–9 days (but possibly up to 77 days). After insemination by a male tick, or sometimes before, a female feeds on blood in order to gain the nutrients for the egg mass to mature. Adult females engorge for a period of 6–30 days, the time again being temperature dependent. (The 30 day engorgement time is derived from laboratory culture colonies.) Under natural conditions, the time taken for an adult female to engorge while on the host varies from 6 to 21

days, the period being longest in cool weather. When fully engorged (replete), the adult female drops off the host to the ground. After 11–20 days the gravid female starts to lay a batch of 2000 to 6000 eggs (20-200 eggs per day over 16–34 days) into moist vegetation such as leaf and branch litter, under the bark of trees and shrubs, or in foliage near the tips of branches. The eggs are attached as a mass utilising a wax-like substance. The female tick dies 1–2 days after egg laying is complete. Under laboratory conditions, female ticks have been kept alive for more than two years.
Due to the variation in time taken for the female to engorge, a host may carry a tick for up to three weeks without the tick being significantly engorged or causing paralysis. However, in warm weather the female engorges rapidly, and at the same time, injects her toxin into the host, thus causing paralysis if the host is susceptible. The adult female does not usually inject detectable amounts of toxin until the 3rd or 4th day of attachment to the host, with peak amounts being injected on days 5 and 6.



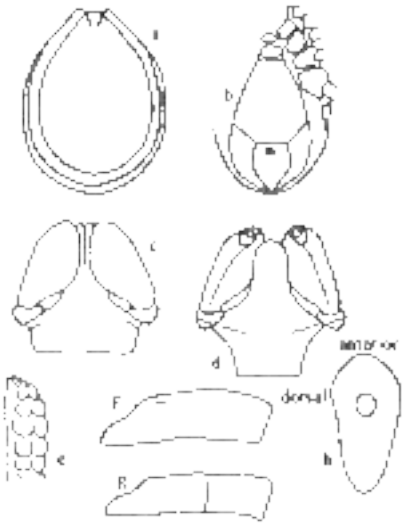
Ixodes holocyclus adult female: a, capitulum (dorsal view); b, capitulum (ventral view); c, palpal article I (lateral view); d, hypostome; e, scutum; f, anal grooves; g, spiracular plate; h, tarsus I; i, tarsus IV.

Diagnosis: A very large tick when fully engorged; scutum about as long as broad and broadest a little posterior to mid length, with strong lateral carinae; capitulum relatively long porose areas deep, cornua usually absent, but when present at most only mild and rounded; auriculae present; hypostome lanceolate, dentition mainly 3/3; no sternal plate; anal grooves meeting at a point behind; all coxae with an external spur decreasing in size posteriorly; trochanters III and IV usually with small, pointed ventral spurs. **Body**: Unfed specimens, oval, flat, yellowish, 2.6 x 1.1 mm - 3.8 x 2.6 mm; marginal groove well developed and continuous; hairs small, scattered, most numerous in region of marginal fold. Semi-engorged specimens frequently

with body widest behind coxa IV and with a waist at level of spiracles. Fully engorged specimens broadly oval, attaining 13.2 x 10.2 mm, living ticks with blue-grey alloscutum, the dorsum light in colour, a dark band in region of marginal groove. **Capitulum**: Length 1.00- 1.035 mm. Basis dorsally 0.60- 0.68 mm in width, the lateral submarginal fields swollen and frequently delimited from the depressed, median field by ill-defined carinae; posterior margin sinuous, posterolateral angles swollen, sometimes mildly salient; porose areas large, deep subcircular or oval, the longer axis directed anteriorly, interval frequently depressed, at most about the width of one; basis ventrally with posterior margin rounded and with well-defined, blunt, retrograde auriculae. Palps long and slender, some long hairs ventrally; article I rounded and somewhat salient laterally, inner 'ring' with dorsal tongue-like prolongation and ventrally semicircular and plate-like, the posterior margin of the plate extending beyond the palp; articles 2 and 3 with no apparent suture, 0.75- 0.85 mm in length and about four times as long as wide, narrowly rounded distally. Hypostome lanceolate and bluntly pointed; dentition mainly 3/3, the innermost file of small, spaced teeth, basally 2/2. **Scutum**: As wide as or a little wider than long, widest a little posterior to mid length, 1.6 x 1.7 mm- 2.4 x 2.4 mm, flat medianly, convex external to the long, strong lateral carinae; anterolateral margins practically straight, posterolateral margins mildly concave; posterior anle broadly rounded. Punctations numerous, fine, sometimes a little coarser medianly and laterally, shallow rugae frequently present posteriorly. Cervical grooves well defined but short. Emargination moderate. Scapulae blunt. **Genital aperture**: On a level with coxa IV, but in engorged specimens sometimes just posterior to this level. **Anal grooves**: Rounded anteriorly, curving behind anus to meet in a somewhat elongate point. **Spiracular plate**: Subcircular, greatest dimension 0.40- 0.45 mm. **Legs**: Coxae smooth, I and II sometimes with mild rounded ridges externally, each with a row of long hairs posteriorly and an external spur, longer and more pointed than in male, and decreasing in size posteriorly. Trochanter IV (and sometimes III) frequently with a small, ventral spur. Tarsi tapering a little abruptly; length of tarsus I 0.70- 0.80 mm, and of tarsus IV 0.60- 0.78 mm.[14]

Adult male[edit]

The newly moulted male seeks a host. Male ticks do not engorge from the host, they wander over the host searching for unfertilised females with which to mate. The male dies after fertilising the female although some males can endure for longer if they parasitise the engorging female.



Ixodes holocyclus adult male: a, body (dorsal view); b, body (ventral view); c, capitulum (dorsal view); d, capitulum (ventral view); e, hypostome; f, tarsus I; g, tarsus IV; h, spiracular plate

Diagnosis: Body measurements less than 3.0 x 2.5 mm; lateral grooves completely encircling scutum, no lateral carinae; punctuations fine; basis capituli punctate dorsally, palpi short and very broad; hypostome dentition 2/2, with rounded teeth; anal plate bluntly pointed behind; adanal plate curving inwardly to a point; coxae with well-defined spurs decreasing in size posteriorly; trochanters III and IV frequently with small, ventral spurs. **Body**: Oval, sometimes broadly so, 1.9 x 1.6 mm- 3.2 x 2.3 mm; marginal body fold narrow but prominent; hairs dorsally sparse medianly, more numerous on marginal body fold. **Capitulum**: Length 0.51- 0.65 mm in width, surface punctate; posterior margin straight; no cornua; posterolateal margins slightly divergent anteriorly; basis ventrally narrowing to the straight posterior margin, surface with a short anterolateral ridge. Palps short and broad; article 1 rounded and a little salient laterally, ventrally with a transverse rounded flange continuous with ridge on basis; articles 2 and 3 with no apparent suture, 0.33- 0.40 mm in length, almost twice as long as broad, rounded distally, hairs moderate in number, some long hairs ventrally. Hypostome short and broad, 0.25- 0.28 mm in length, narrowing and shallowly rounded distally; dentition 2/2 of large rounded teeth, some small teeth distally and crenulations basally. **Scutum**: Oval, convex, only a little smaller than body. Lateral grooves deep and completely encircling the scutum, anteriorly somewhat linear and may simulate mild lateral carinae. Punctations fine, usually most numerous submarginally and anteromedianly; pseudoscutum sometimes faintly apparent. Cervical grooves, short, shallow. Emargination moderate. Scapulae blunt. **Genital aperture**: On a level with anterior margin of coxa III, sometimes at level of 2nd intercoxal space.

Ventral plates: Pregenital plate wider than long; median plate 1.5 x 1.2 mm, the width posteriorly about 3/4 of the length; anal plate 0.75 x 0.50 mm, anterior margin straight or mildly curved, pointed posteriorly; adanal plates curving to points near the point of the anal plate; plates with scattered punctuations and hairs. **Spiracular plate**: Elongate, oval, narrow posteriorly, the longer axis directed anteriorly, about 0.50- 0.53 mm in length. **Legs**: Length moderate. Coxae practically contiguous, with a row of long hairs near posterior margin; posterointernal angles of coxae I and II may be somewhat sharp but not salient; all coxae with an external spur, strongest and bluntly pointed on coxa I, smallest on coxa IV. Trochanters III and IV with a small, dark ventral spur, only a tuberosity on II. Tarsi ending somewhat abruptly; length of tarsus I 0.65- 0.71 mm, and of tarsus IV 0.62- 0.70 mm.[14]

Distinguishing *Ixodes holocyclus* from other Australian ticks[edit]

Engorged adult female *Ixodes holocyclus* showing the darker brown 1st and 4th pairs of legs

Engorged adult female *Ixodes holocyclus* showing the complete pear-shaped

encirclement of the anus by the anal groove. This feature gives the tick its species name *holocyclus*, meaning 'complete circle'.

This is NOT *Ixodes holocyclus* - it is *Haemaphysalis longicornis* (the 'Bush tick') and shown here for comparison only. Unlike *Ixodes holocyclus* all pairs legs of *Haemaphysalis longicornis* are of the same shade, the legs are finer, the mouthparts are much shorter, and the shield is rounded

The two features which are most easily recognised and which are characteristic of *Ixodes holocyclus*:

- The first and last pairs of legs are distinctly darker than the 2 middle pairs of legs
- The anal groove forms a complete, though somewhat pear-shaped oval, around the anus (this feature gives the tick its species name *holocyclus*, meaning 'complete circle')

Other ticks which commonly need to be differentiated from *Ixodes holocyclus* include *Rhipicephalus sanguineus* (Brown dog tick), *Haemaphysalis longicornis* (Bush tick) and *Rhipicephalus microplus* (Cattle tick). The Medical Entomology Department of Westmead Hospital, Sydney provides professional identification of ticks and other medically important insects.

Common hosts[edit]

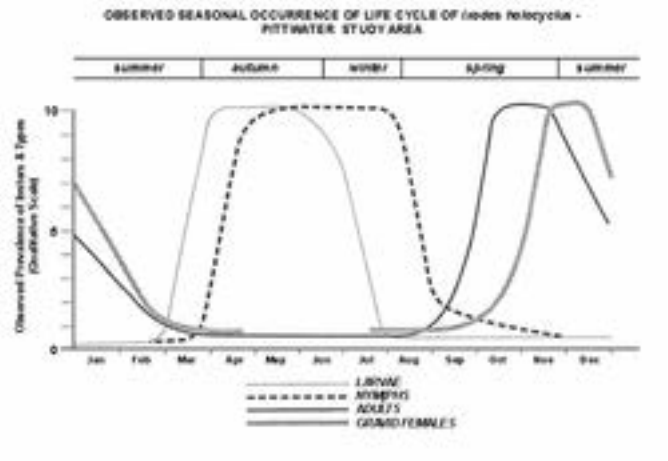
Common hosts include long nosed bandicoot (*Parameles nasuta*), giant brindle bandicoot (*Isoodon torosus*), echidnas and possums. Potential hosts include many species of mammals, birds and occasionally reptiles. Because of continuous infestation the native animals are usually immune to tick toxins. Most mammals such as calves, sheep, goats, foals, pigs, cats, cavies, rats, mice and humans can be infested by the Australian Paralysis Tick. Fatalities resulting from a single engorged adult female tick are mostly reported in the young animals of the larger species and all ages and sizes of the pet species (dogs and cats). Larvae and nymphs can also produce toxic reactions in the host. Fifty larvae or five nymphs can kill a 40 g rat, larger numbers of either can induce paralysis in dogs and cats. They can be quite easy to find on

short-haired animals, but very difficult to find on long-haired animals like Persian cats. If you live in a tick-prone area, and your pets are allowed outdoors, it is suggested that you physically check your pets daily for ticks. Unfortunately they are often not discovered until the tick is large enough to be felt. By this time the tick has subjected the animal to a large amount of toxins. One adult female can kill four susceptible rats during engorgement. Although it is not typical, an engorging adult female apparently can re-attach several times to different dogs.[20]

Natural predators[edit]

Natural predators of *Ixodes holocyclus* include insectivorous birds and wasps.[21]

Seasonality[edit]



Seasonality of *Ixodes holocyclus* in the Pittwater area north of Sydney, 1995.

Humid conditions are essential for survival of the paralysis tick. Dry conditions, relatively high temperatures (32 °C) and low temperatures (7 °C) are lethal for all stages after a few days. An ambient temperature of 27 °C and high relative humidity is thought to be optimal for rapid development.[22] Very dry or very wet conditions are not ideal.

The tick population in a given year is probably governed by the rainfall in the previous year if the temperature variations have only been moderate. North-easterly weather, with moisture laden sea breezes on the east coast of the continent provide ideal conditions. This pattern usually sets in during spring and early summer. Tick venoming in animals (especially pets) peaks in spring to mid-summer.

Typically, larvae appear late February to April/May, followed by nymphs from March to September/October and then gradually the adult population from August to February, peaking around December. Under favourable conditions a second cycle may result in a second peak in May. However, infestation by adults can occur at any time of the year when conditions are suitable, even in

mid-winter. Only in the very hot summer months are they difficult to find.
The graphs show seasonal occurrence for one species only i.e. *Ixodes holocyclus*. The extent to which other species found in the area parallel *Ixodes holocyclus* in this and other aspects of their biology is not known.
The graphs show the average seasonal prevalence of instars and types, observed over an eight-year period, which includes six years of detailed field observation, and collection, supported by information and specimens from many people.
Ixodes holocyclus emerged from this survey as the dominant acarine ectoparasite of mammals and avians in the study area, its population dwarfing those of other tick species, and various species of mites.
As distinct from instars (the life cycle stages separated by metamorphosis), only one particular type within an instar has been graphed at this stage - gravid females. It is probable that further separation of types will become possible with additional survey and observation, for example it appears that at certain times in spring and summer adult males greatly outnumber females, in proportions as high as five to one. These and other indications need further checking to ensure that they are not merely coincidences.
As the graphs show, small numbers of each instar are present throughout the year, with the numbers in each life cycle segment rising to plateau levels at particular times and then falling away again. These pictured results are from observations covering a period when climate in the whole South West Pacific Area was subject to a major weather anomaly. Continued observations may well result in a change in the shape of the curves. One graph line, that of the gravid female type within the adult group, has been shown as incomplete. Positive recordings of winter occurrences of gravids have varied from nil to very small numbers in two of the eight years.[23]

Relative sizes[edit]

Comparison of Non-engorged ticks

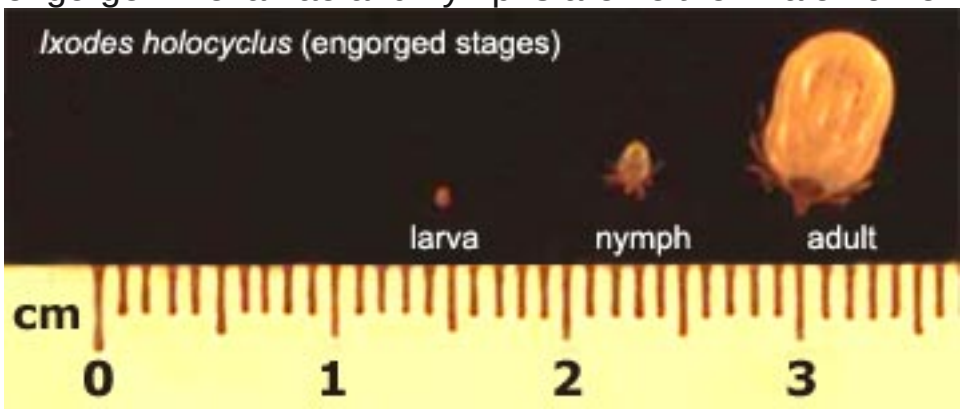
Unengorged Larvae (6 legs)	Unengorged Nymph (8 legs)	Unengorged Adult (8 legs)	
			
0.5 mm long, 0.4 mm wide	1.2 mm long, 0.85 mm wide	3.8 mm long, 2.6 mm	1

		wide	w

Measurements refer to the body section only (i.e. legs are not included)

Comparison of Engorged ticks

Larval, nymph and adult (female) stages. The adult male tick does not engorge. The larvae and nymphs are neither male nor female.



Sexual dimorphism[edit]

Only the final adult stage of *Ixodes holocyclus* shows obvious sex variation. Larvae are neither male nor female. Nymphs are sexually immature (have no genital aperture).

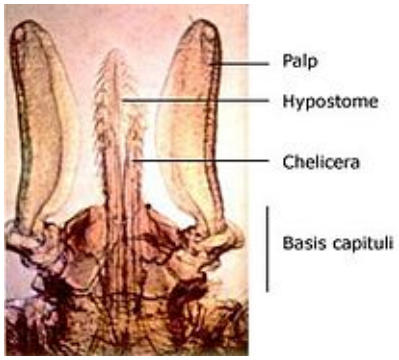
The male does not engorge. The shield (*scutum*) covers the entire dorsal body. It has a tortoiseshell pattern. The mouthparts section (the *capitulum*) is very short because it is not required for feeding from the host (though it may be used to feed from the female tick). The male tick does not pose a medical risk to humans or animals - the significance of finding a male is that it is looking for a female - so humans and animals should be checked fully for the possible presence of an adult female tick somewhere.

Adult male

The female does engorge. The shield (*scutum*) covers only the front part of the dorsal body. The mouthparts section (the *capitulum*) is very long because it is required for feeding.

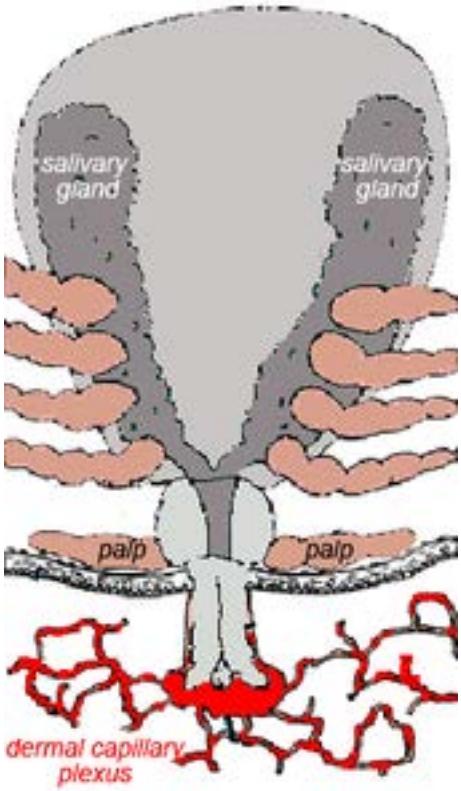
Adult Female (with early engorgement)

Feeding process[edit]



The capitulum of *Ixodes holocyclus*

The capitulum of *Ixodes holocyclus* (scanning electron microscopy)



The feeding process of *Ixodes holocyclus*

Ticks generally are obligate blood feeders. All active stages (larvae, nymphs and adults) require blood as a source of nutrition (except for a few Argasid genera in which the adult mouthparts are non-functional, i.e. Antricola, Otobius and Nothoaspis). Adults also require the blood for sperm or egg production. The feeding process of Ixodid ticks has first a slow phase for several days followed by a fast phase in the last 12–24 hours before detachment. There may be a tenfold increase in fed: unfed weights by the end of the slow phase, but there is an additional tenfold increase by the end of the final fast phase. Leaving the full engorgement as late as possible reduces the chances of detection and removal by the host . The process of feeding is called engorging. The hypostome has a groove along its dorsal surface forming a food canal (also known as the preoral canal) through which blood is drawn from the host and passed on to the mouth and pharynx. During blood feeding by Ixodid ticks, the liquid portion of the meal is first

concentrated by removal of water and excess ions, which move across the gut epithelium and enter the ticks body cavity (hemocoele). These components are then taken up by the salivary glands which produce a watery saliva that is injected back into the host [24]

Blood meal digestion in ticks is similar in all species. The digestive system in both Ixodid and argasid ticks is histologically divided into foregut, midgut and hindgut. The foregut comprises the sucking pharynx and the oesophagus. The midgut contains a ventriculus with a valve, a variable number of blind diverticula (caeca), and a rectal tube. The hindgut is divided into a rectal bulb and the rectum itself.

The **mouthparts** section of the tick is known as the *capitulum* (Latin: 'little head'). The capitulum is not really a true 'head' in the sense that the structures one normally associates with the head (primitive brain, salivary glands) are not contained within it - these structures are located in the 'body' of the tick. Incidentally, *Ixodes holocyclus* has no eyes.

The *palps* are the paired tactile and positioning limbs which are not inserted into the skin but splayed out on the surface. The *chelicerae* are the paired cutting jaws which cut a channel into skin for the hypostome. The *hypostome* is the single feeding tube which also anchors the tick by its reverse barbs. The *basis capituli* forms the basal ring of cuticle to which the palps, chelicerae and hypostome are attached. The basis capituli moves in the dorsoventral plane, and articulates with the body proper.

Once it has chosen a feeding site a tick positions itself with its legs so as to elevate its body at a sharp angle.

Guided by the palps, the *chelicerae* cut into the skin with their horizontal cutting action. These rip and tear at the epidermal layers and a small pool of blood is formed. The *hypostome* is inserted and this provides the initial attachment strength. In the case of *Ixodes holocyclus* the *hypostome* is inserted very deep into the dermis. The palps remain spread apart on the surface. The process by which Ixodid and Argasid ticks feed is termed **telmophagy** (= pool feeding). (This contrasts with the process of solenophagy, used by mosquitos, in which feeding is direct from a small venule.) The resultant pool expands as a result of the anticoagulants released from the salivary glands. In some Ixodid ticks a cementum is secreted into the wound within 5–30 minutes of cutting into the skin. This material hardens quickly into a latex-like covering around the mouthparts but excluding the palps that remain flattened out on the skin surface.[25] *Ixodes holocyclus*, however, is one of the Ixodid ticks which does not produce cementum.[26]

The host reacts against the tick lesion by haemostasis, inflammation and cell mediated immunity (CMI). An array of pharmacologically active substances is

injected with the saliva of the tick, including anticoagulants, PGE2, prostacyclin, apyrase and in certain tick species antihistamines. PGE2 and prostacyclin inhibit platelet aggregation and dilate blood vessels. Feeding is almost continuous with pulses of salivation alternating with periods of feeding to ensure continued suppression of host defences.
There is a concentration of saliva and presumably toxin in the granulomatous reaction around the tick mouth parts. It is thought by some experimenters that the residual toxin located in this granuloma is at least partially responsible for the increasing paralysis which occurs after the tick is removed. By comparison, the North American paralysis tick *Dermacentor andersoni* (found in the Rocky Mtns) does not produce a granuloma at the site of attachment, and in this case the paralysis rapidly regresses after the tick is removed.[20] Unlike *Dermacentor andersoni*, *Ixodes holocyclus* is a deep feeder with a long hypostome (which may penetrate as deep as 1689 um).

Engorgement[edit]

The following images all depict the **adult female** of *Ixodes holocyclus*. Colour and markings change markedly as engorgement progresses. It is the third tick, the *moderately engorged* adult female (width, at level of the spiracles, more than 4 mm) which is most commonly removed from dogs with tick venomning. If the fully engorged tick were found on a dog it suggests that the dog has a degree of immunity.


Adult female - No engorgement


Adult female - Early engorgement


Adult female - Moderate engorgement

Adult female - Full engorgement

These pictures are not to scale with each other. Because the size of the 'shield' (*scutum*) does not change as the female tick engorges, you can use it to compare the relative sizes.

Distribution and habitat[edit]

Ixodes holocyclus is found mainly along coastal eastern Australia - from near Cooktown in Far North Queensland to Lakes Entrance in Victoria. In places, it is found more than 100 km inland, particularly in areas of moist escarpments and ranges such as the *Bunya Mountains* (QLD) and the *Lower Blue Mountains* (NSW).[27]

Approximate distribution of *Ixodes holocyclus* - note that a similar tick in Tasmania is also shown as causing tick paralysis.

The distribution map gives only an *approximation* of the Paralysis Tick's distribution as the situation is not static. There are reports of paralysis ticks from inland Victoria, including north-eastern suburbs of Melbourne. This may reflect general movement of insects and ticks from equatorial to polar latitudes which in turn may be related to global warming.

The need for humid conditions largely determines the botanical niche of Australian paralysis ticks. Low, leafy vegetation provides higher humidity levels by reducing the desiccating effects of wind and direct sunlight. This

environment also suits the tick's major host, the bandicoot, as it seeks out grubs and worms in the leaf litter. Certain vegetation may be conducive to paralysis ticks.
Paralysis ticks can and do climb trees. In fact nymphs and adults climb many meters to the very tops, but descend in windy or dry weather. Larvae appear to remain closer to ground. They reportedly will fall near and onto people sitting underneath.

Tick bites[edit]

Overview[edit]

The kinds of effects caused by bites of *Ixodes holocyclus* vary in their frequency according to the type of host and whether the tick is at the stage of larva, nymph or adult.

- Humans are most notably affected by local irritation, allergic reactions and tick-transmitted infectious diseases. Tick paralysis is possible but now uncommon.
- Domestic animals (pets and livestock) are most notably affected by tick paralysis. Allergic reactions and tick-transmitted infectious diseases are possible but rarely diagnosed.
- Native animals are most notably affected by anaemia caused by carrying heavy burdens of ticks drawing large quantities of blood. Tick paralysis is possible but mainly recognised in captive animals where there has been a discontinuity in exposure and so a drop in immunity.

Although there is the possibility of innate differences between species, the variations in effects can largely be explained by how often and how heavily hosts are exposed.

Humans[edit]

Ixodes holocyclus Adult female tick - early attachment on human skin. The palps are clearly seen to be splayed out on the surface of the skin.

Ixodes holocyclus Adult female tick - early attachment on human skin behind ear at hair line. Note swollen lymph node on neck below the tick

At the site of a bite by an adult female *Ixodes holocyclus* one can expect there to be an itchy lump which lasts for several weeks.

Although most cases of tick bite are uneventful in humans, some can produce life-threatening effects including severe allergic reactions,[28] tick-transmitted infectious diseases such as Rickettsial Spotted Fever (also known as Queensland tick typhus), and tick paralysis.

Larvae and nymphs, as well as adults are capable of causing very dramatic allergic reactions. Dramatic local redness (*erythema*) and fluid swelling (*oedema*) may develop within 2–3 hours of attachment of even one larva. Apparently many people have experienced spectacular allergic reactions when they have come into contact with both live and dead tick products. Having a tick simply walk over a person's hand produces in some people an intense discomfort and itching – what particular components of the tick body cause these reactions is unknown, but it could be a water-soluble component that is excreted through the cuticular canals.[29]

In southeast Queensland a 'maddening rash' (known locally as 'scrub itch') is caused by infestation by many tick larvae. This especially affects people clearing leafy bushland such as lantana scrub. Not infrequently a single tick embedded over an eyelid will result in gross facial and neck swelling within a few hours. The person can go on to develop very severe signs of throat (tracheopharyngeal) compression within 5–6 hours after the first onset of symptoms.

Whilst systemic paralysis is possible in humans, it is now a relatively rare occurrence. This is because an engorging adult female tick needs to remain attached for several days. This was more likely to occur in the past because there was less medical and public awareness of the problem and perhaps because it was more likely to be misdiagnosed as 'infantile paralysis' (poliomyelitis or 'polio'). Paralysis is more likely to occur in children and in situations where ticks are attached in places they are not easily detected (e.g. under bandages). Up to 1989, 20 human fatalities had been reported in Australia.
If an unusual black scab (*eschar*) develops at the site of a tick bite, or if there are any other signs of illness occurring within a few weeks of a tick bite (especially 'flu-like' symptoms, fever, generalised skin rash, muscular or joint pain), it is recommended that a doctor be consulted and informed about the tick bite – a Rickettsial infection (*Rickettsia australis*, or *Rickettsia honei*) may be the cause. Whilst many such Rickettsial infections are self-limiting, early treatment with antibiotics can prevent longer-term problems in some individuals. Doctors in Australia may also wish to investigate the possibility of a Lyme-like disease, particularly if there is also a 'bullseye' or 'target' shaped skin rash (known as erythema migrans).
Further information for Australian doctors is available at Tick-transmitted Diseases (Australia).

Domestic animals[edit]

Moderately engorged *Ixodes holocyclus* attached to a dog (severely paralysed). Whilst reactive swelling around the tick can make it appear as though it is penetrating deeper, only the mouthparts penetrate the epidermis of the skin. Even when the tick has fallen off or removed there is usually a readily palpable skin lump remaining for days to weeks.

The tick's paralysing toxin has been estimated to affect as many as 100,000 domestic animals annually, with up to 10,000 companion animals being referred to veterinary surgeons for treatment.

Allergy[edit]

All stages of *Ixodes holocyclus* are commonly responsible for allergic reactions in humans.

Allergic reactions to larvae[edit]

Attachment of a few larvae to a non-sensitised host provokes little or no response, even though the contact is prolonged over several days. However, towards the end of feeding some pruritus, erythema and localised oedema may develop at the site of attachment.

Repeated infestation with the larva, as occurs in rural and wooded suburban areas where bandicoots are common, rapidly leads to the development of hypersensitivity. Dramatic local erythema and oedema and pruritus may develop within 2–3 hours of attachment of even one larva if a person has been sensitised by a previous bite. In this case attachment of the larvae leads to formation of intra-epidermal blebs and eventually vesicles. These eventually rupture and the larvae detach and are lost. Frequently, a tick embedded over an eyelid will result in gross facial and neck swelling within a few hours. The person can go on to develop very severe signs of tracheopharyngeal compression within 5–6 hours after the first onset of symptoms.[20]

During damp summers any disturbance of taller plants - e.g. clearing lantana, can produce a shower of tick larvae. When large numbers of larvae attach to a sensitized person, severe allergic dermatitis may result. The maddening rash that results is commonly referred to as 'scrub-itch'. Such outbreaks are seasonal in southeast Queensland and occur most commonly during January, February and March when larval populations are at their peak. Dermatitis is most commonly encountered in rural workers.[30]

Allergic reactions to nymphs and adults[edit]

Attachment of the nymphal and adult stages produce variable responses, many of which are clearly allergic in nature. Often, attachment may provoke little or no response and the patient may be quite unaware of the presence of a tick for some days until eventually minor itchiness leads to its discovery. Conversely, there is sometimes a locally heightened sensitivity or hyperaesthesia.[31] Such attachment sites are surrounded by erythema.[30]

After the tick is removed pruritus may recur at the site of attachment at intervals over some weeks, and a small firm lump usually forms within a day

or so of the tick's removal. This again may persist for many weeks. There may be some discoloration of the area of the bite. In other cases the skin reactions may be severe with marked pruritus and considerable erythema, oedema and induration.[30] People also report headaches.[31]

Food allergy[edit]

An association between tick bite reactions and red meat allergy in humans has also been reported. This represents a potentially novel cross-reaction between an arthropod and a food protein.[32] The induced allergy is unusual in that the onset of the allergic reaction, which ranges from mild gastric symptoms to life-threatening anaphylaxis (skin rashes, swollen tongue and serious drop in blood pressure), can occur up to 3 to 6 hours after eating meat (beef, lamb or pork) and often many months (e.g. 3 to 6 months) after the tick bite.

All mammals, except humans and higher apes, have a carbohydrate, commonly known as *alpha-gal* (alpha-1, 3-galactose) in their tissue fluids. When a tick feeds on the blood of a mammal (bandicoot, possum, cat, dog etc.) it takes alpha-gal into the tick's digestive system. When the same tick attaches to the next host (e.g. a human) it transfers the alpha-gal to the tissues of that next host. The immune system of some humans recognises alpha-gal as foreign and so produces antibodies against it. In this case the antibody produced is IgE, which is the type of antibody responsible for most allergic reactions. Thus the human is primed for a delayed allergic reaction to subsequent ingestion of *mammalian* meats (not chicken or fish).

Alpha-gal in a human can now be recognised through a blood test. Note that those individuals allergic to tick bites and who have an immediate reaction are reacting to *proteins* in the tick's saliva (not the *carbohydrate* alpha-gal). Such individuals, however, may have a greater predisposition to also developing the allergy to mammalian meats several months later.[33]

Tick paralysis[edit]

Main article: Tick paralysis

Toxins[edit]

It is thought the toxins of *Ixodes holocyclus* cause a failure of secretion of neurotransmitter acetylcholine at neuro-muscular junctions. In experiments where nerve-muscle preparations were incubated in solution containing toxin the paralysis effect was delayed for six to seven hours after the addition of toxin. As in live test animals, this effect was temperature dependent,

paralysis being more rapid at higher temperatures. Live mice injected with toxin did not develop signs of paralysis for some 8 to 12 hours after the injection.[34] The cause of the delayed toxicity is unknown.[35]
Several toxic fractions have been isolated from the salivary glands of *Ixodes holocyclus* but there has been variation in the reported molecular size of the principle paralysing toxin or toxins.
Early work suggested that the most neurotoxic fraction was a protein (molecular weight 40000-60000 D, stable when freeze dried, originally named *holocyclotoxin*) [36][37][38][39][40][41][42][43][44] Another toxin was found to be apparently lethal but non-paralysing.[45]
A more recent study isolated three *low* molecular weight toxins, all three of which could cause hind limb paralysis in baby mice. Only one, however, could be isolated in quantities permitting further research. The molecular weight was relatively small at 6000 D with approximately 50 amino acids (which is homologous to scorpion and spider toxins).[46][47][48]
The evolutionary purpose and benefit of the paralysing toxin is not clear. Being arachnids, ticks are related to predatory arthropods (spiders, scorpions and mites). Spiders and scorpions have retained toxins and developed specialised delivery structures (fangs and telsons) while mites and ticks have lost this feature. Of the 800 species of ticks, 40-64 species (across 10 genera) have been reported as causing a form of toxosis.[49][50][51][52] So as ticks have moved from a predatory existence to a parasitic existence, most species have lost their toxin which would have been a disadvantage for a parasitic lifestyle (parasites in general do not want to kill their host). Gene sequence of the tick toxin shows high homology to scorpion toxins.[53]
The saliva of *Ixodes holocyclus* also contains anti-coagulant.[54]

Domestic pets[edit]

Dogs and cats on the East Coast of Australia commonly succumb to the tick paralysis caused by *Ixodes holocyclus*. A similar tick species, *Ixodes cornuatus*, appears to cause paralysis in Tasmania.[55] The paralyzing toxin (or toxins) is produced in the salivary glands and injected as part of the feeding process.
The adult female tick is usually attached for a minimum of 3 days before the very earliest signs are noticed although a *very* observant person might begin to notice a slight change in behavior after 48 hrs of attachment (in a warm climate). Typically, however, a person would not notice obvious signs until at least the 4th day of attachment. In colder weather the feeding process is slowed down considerably and some animals may not show significant signs of paralysis for as long as two weeks. Furthermore, dogs rarely show

significant signs until the adult female has engorged to a width of at least 4 mm (measured at the level of the spiracles, see photo of lateral view of *Ixodes holocyclus*).

Left untreated, the outcome is often, if not usually, fatal. The toxin or toxins paralyze muscle tissue - in particular:

- **Skeletal muscles**. This results in the overt paralysis for which the tick is named. Typically the paralysis starts in the pelvic limbs and subsequently ascends to affect the pectoral limbs and then the axial muscles.
- **Respiratory muscles**. Initially this results in rapid, shallow breathing with an inability to cough. In advanced stages it is associated with a slower exaggerated breathing pattern.
- **Laryngeal muscles**. This results in an altered 'voice' and an increased risk of aspiration pneumonia (inhalation of food, saliva or vomitus into the lungs). Aspiration pneumonia in this situation results in labored breathing with a distinctively foul breath.
- **Oesophageal muscle**. This results in drooling (of saliva) and regurgitation. It increases the risks of choking and aspiration pneumonia. Megaoesophagus is commonly recognized in dogs and may be diagnosed on thoracic radio-graphs.
- **Heart muscle**. This results in congestive heart failure and pulmonary edema, seen also as labored breathing.

Spring is the peak season for tick paralysis because this is when the ticks molt and develop into the final adult stage of their life cycle (it is the adult stage that also produces the most toxins during feeding). Sporadic cases of tick paralysis, however, can occur all year, even in mid-winter.

Once attached the female draws blood and gradually swells. In typical warmer weather conditions the initial engorgement is slow but after four or more days it becomes very rapid. The rapid engorgement phase is also associated with the greatest injection of the toxic saliva.

The dog or cat may show a great variety of signs that may be very subtle in the early stages. Early signs include lethargy, loss of appetite, apparent groaning when lifted, altered voice (bark/meow), noisy panting, coughing, drooling of saliva, gagging, regurgitation (dogs) and enlarged pupils (cats). Occasionally a single limb may appear to be weak or lame. A tick attached to the side of the face may cause loss of blink reflex, corneal ulceration and an ocular discharge. A tick attached near the anus can cause anal incontinence. As toxicity progresses the combination of signs becomes more characteristic of tick envenoming. There is progressive limb weakness which is seen first in the hind legs. A dog may appear to walk as though 'drunken' (ataxic). There may be an inability to climb stairs or an inability to turn in tight circles without

stumbling. The respiration may become slower and have a grunt at the end of expiration. Some animals are easily panicked at this stage and should be handled calmly (especially cats).

Ultimately paralysis becomes so severe that an animal is unable to stand or even lift its head. The breathing becomes slow, exaggerated and gasping. A foul odor on the breath can signal aspiration pneumonia. Although pain is not regarded as being a feature of tick paralysis animals would appear to be in severe distress. Finally, the mucous membranes develop a bluish hue (*cyanosis*) and a state of coma indicates that death is imminent.

It is important to note that when the Australian paralysis tick is removed, the signs usually continue to *worsen* for up to 48 hours, though worsening is usually most pronounced in the first 12–24 hours after removal.

The primary treatment for tick paralysis is the careful administration of anti-tick serum. The effectiveness of anti-tick serum is most dependent on how early it is administered. Early treatment offers the best chance of full recovery. Unlike snake bite, tick envenoming has a relatively slow onset. Despite this slow onset the fatality rate can be very high (even higher than snake bite) if antiserum is not given sufficiently early, *before* the signs are advanced.

Ancillary treatments may include:

- supporting respiration (ensuring airway patency, ventilation, supplying enriched oxygen, treating pulmonary edema, reducing risk of further aspiration, treatment of secondary pneumonia)
- minimizing stress and reducing oxygen demand
- maintaining core body temperature
- maintaining hydration and blood pressure (not usually necessary initially)
- protecting eyes if eyelids paralyzed
- assisting urination if unable to urinate

Prevention of tick paralysis is mostly achieved by a combination of daily searching and the use of tick-repelling or tick-killing agents. These may be topical sprays, rinses and collars or systemic oral insecticides. Some owners decide to clip the fur short to help detect ticks.

Daily searching usually gives a person a few days to find an attached tick. However ticks at the early stage of attachment are small and flat and so easily missed. Whilst most ticks on dogs are found around the head, neck and shoulders, they can be anywhere on the dog's surface. They are easily missed on the face, legs and between the toes. Occasionally they are found inside the lips, ear canals, prepuce or vulva, and anus.

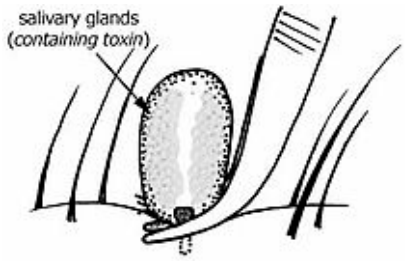
Cats mostly have ticks where they cannot reach to groom themselves - often on the back of the neck or between the shoulder blades, under the chin, on the head, or upper leg. Ticks can attach elsewhere, even the tail, hard palate

or inside the anus.[55]
Long haired cats that venture outdoors are more at risk.[55]
Matted coats and skin with lumpy lesions also make it more difficult to find ticks. Some veterinarians perform a close clip of the entire coat to help find attached ticks.
The incidence of tick toxicity on cats is unknown, and they may be less susceptible to paralysis than dogs.[55]
Development of a vaccine to protect dogs against the tick toxin has been attempted but not yet been successful.[56]

Horses[edit]

The number of paralysis ticks required to paralyse a horse is unknown but in one study [57] there were cases of large horses with only one to two ticks being paralysed and unable to stand. Horses of any age and size can be affected by tick paralysis. In the same study, 26% of the horses died and of the surviving horses, 35% developed one or more complications including pressure sores, corneal ulcers, pneumonia and sepsis. The relatively high mortality rates in horses in this study could be due to a range of factors including horses being badly affected before vets are called, difficulties associated with nursing a recumbent horse, difficulties with owners needing to deliver the bulk of nursing care and lack of information to veterinarians managing the disease in horses.

Tick removal[edit]



Tick removal. Compressing the body of the tick could cause more noxious substance (allergens, salivary toxins and infectious micro-organisms) to be injected into the host.

Debate around the best method of tick removal is based around two main concerns. The first is that the method of removal could cause further injection of noxious substances (allergens, paralysing toxins and infectious micro-organisms). The second is that the method may leave the 'head' (really just the tick's mouthparts) embedded in the skin.
The tick's salivary glands and gut contents are together the main source of

the noxious substances and these are located in the main body of the tick. It is thought that any irritation of the tick might cause it to either inject saliva or regurgitate gut contents or that any compression the body of the tick might *squeeze* saliva and gut contents into the host.
Specifically, application of methylated spirit, nail polish remover, turpentine or ti-tree oil is thought to irritate the tick and make it inject more of the noxious substances. Spreading butter or oil over the tick is no longer recommended either. Application of pyrethrin (or pyrethroid) insecticides has been suggested 'as a narcotic and a toxicant, which prevents the tick from injecting its saliva' [58] but with this method the tick may remain physically attached for 24 hrs before it drops off dead.
Leaving the mouthparts (incorrectly referred to as 'the head') embedded in the skin is usually of lesser concern. If left embedded the mouthparts cause a foreign body reaction and are usually eventually sloughed like a splinter. It has been noted that when *Ixodes holocyclus* is forcibly extracted the feeding tube (the *hypostome*) is usually damaged which suggests that part of its tip remains embedded in the skin.
In sensitive areas of the body (e.g. eyelids) touching the tick can make its presence become suddenly painful.
Precautionary principles for tick removal:[59]

- If a person has difficulty removing a tick, or has reason to be concerned about allergic reactions, it is best to seek professional medical attention. The process of removing ticks in humans has been associated with anaphylactic reactions and so it is best to have appropriate medical supplies (oxygen, adrenaline) ready.
- Instruct children to seek adult help for proper tick removal
- Wear thin disposable gloves if available
- Avoid unnecessary touching of the tick's body
- Grasp the tick's mouthparts as close to the skin as possible
- Grasp *very firmly* (because the long feeding tube of *Ixodes holocyclus* is deeply embedded and has reverse barbs)
- After removal disinfect the bite site (and the tick removal instrument)
- Larval ticks ('grass ticks' or 'seed ticks') are usually present in large numbers - it is considered safe to soak for 30 minutes in a bath to which 1 cup of bicarb soda has been added, then scrape off the dead larvae.
- Save the tick in a small airtight container with moist paper or a leaf or blade of grass. Label with the date removed and the locality where the tick was acquired. The tick can be identified later if you develop illness, especially in the following 4 weeks. (Note that an engorged female will deposit eggs within a few weeks and these will hatch into thousands of

larvae which can escape the container if not properly sealed.)
- Look for more ticks (both on humans and on pets)

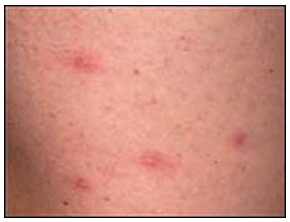
Methods of grasping the tick:
- A pair of fine curved tweezers (preferably angled). The traditional method used in humans.
- Loop of thread. This can sometimes be difficult to place without disturbing the tick.
- A specialised tick removal tool. Tools include tick hooks, tick scoops, tick tweezers, and tick loops. These are usually inexpensive and highly recommended in areas where ticks are prevalent.[60][61][62]

Vector competence[edit]

For hard (Ixodid) ticks it is virtually impossible for mechanical transmission to occur on its own (i.e. without some replication), as they *tend* to not take multiple blood meals from different hosts in the one stage. Passing an organism between tick stages (trans-stadial transmission) seemingly requires that an organism can also replicate within the tick.

Bacterial diseases[edit]

Spotted fevers[edit]



Rickettsial spotted fever caused by *Rickettsia australis* - can be mistaken for Chicken Pox.

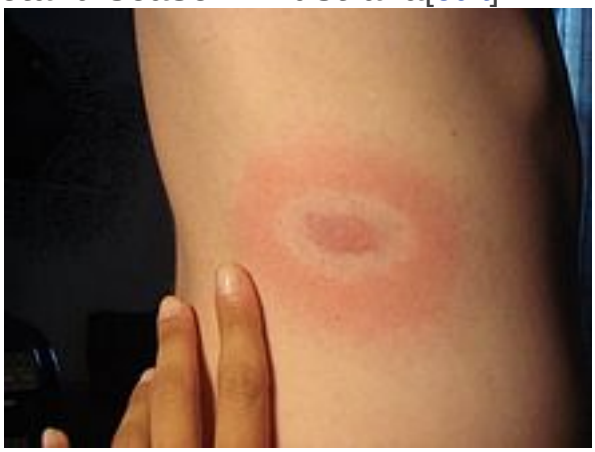
These days there is not much disputing that *Ixodes holocyclus* is the main vector for **Rickettsial Spotted Fever** (also known as **Queensland tick typhus**) (causative organism the bacteria *Rickettsia australis*) and **Flinders Island Spotted Fever** (causative organism *Rickettsia honei*). *Rickettsia australis* is an obligate, intracellular bacterial parasite that proliferates within the endothelial cells of small blood vessels, causing a vasculitis. Spotted Fever is probably more common than the Lyme-like Disease. Sometimes infection with *Rickettsia australis* occurs without a rash - there may just be a high fever. Usually (65% of cases) there is a black spot (known as an *eschar*, usually 2–5 mm in diameter) at the site of the tickbite. This looks like a scab with redness and swelling around it. Usually there is only one eschar unless there has been more than one tick bite. Often lymph glands nearby are enlarged and painful. Fever starts 1–14 days (usually 7–10 days) following

the tickbite, followed within a few days by a rash. The rash can look like chicken pox as the spots may contain some fluid. Other symptoms include headache, stiff neck, nausea, vomiting, mental confusion, aching muscles and joints. The illness may be more severe in adults and elderly persons. Spotted Fever is diagnosed by two blood tests (IgM and Weil-Felix tests) taken 10 days apart. PCR analysis of skin biopsies is a more recent testing method. The disease runs its course in two weeks or so but can be cured more quickly with antibiotics (tetracyclines). As antibiotic treatment is very effective, many persons feel so well that they do not return to have the second blood sample collected. But this second blood test is important as it is the only way of proving the diagnosis of Spotted Fever. Spotted Fever rarely, if ever, occurs more than once because the body usually becomes immune for life. In rare instances there may be an apparent repeated infection after recovery - it is not known whether this is caused by another strain. It is also presently unknown if chronic infections occur. It is rarely fatal. Australian Spotted Fever was first described in 1946 when 12 soldiers contracted the disease during training exercises in north Queensland - it was at that time known as Queensland Tick Typhus (QTT). Infections generally arise in rural areas, but 10% of reported cases appear to have been acquired in major cities. It is apparently not uncommonly seen in tick collectors in southern Queensland. It mostly occurs during the winter and spring, but can occur at any time in temperate areas.

Q-fever[edit]

Ixodes holocyclus is also commonly mentioned as a potential vector of **Q-Fever** (*Coxiella burnetii*). The ornate kangaroo tick / wallaby tick *Amblyomma triguttatum s.l.* has also been implicated in Q-Fever.[63]

Lyme-like spirochaetal disease in Australia[edit]



Erythema migrans, Bullseye or Target lesion - typical of Lyme disease but not always present

Lyme Disease was first described in Europe in 1883, the 1970s in North

America and 1982 in Australia. It is an illness caused by a spiral bacterium called a spirochaete. The most common name for this spirochaete is Borrelia burgdorferi, but many different Borrelia species cause Lyme Disease worldwide. An Australian spirochaete has not been found in humans yet, and may be quite different from *Borrelia burgdorferi*. Despite clinical case reports, it is still controversial as to whether Lyme disease can be contracted in Australia. Some doctors and health authorities believe it does, while others are adamant that it does not. Until the controversy is resolved patients with suspected Lyme-like Disease should be treated with an appropriate course of antibiotics because early treatment of Lyme Disease invariably results in a complete cure.

Some vector competence studies have been undertaken on *Ixodes holocyclus* with respect to the Lyme disease pathogen *Borrelia burgdorferi sensu stricto* (with a United States strain). These suggested that the tick can not transmit this strain of spirochaete (.[64] Despite this, there is a strong belief that some kind of Lyme-like spirochaete causes a **Lyme-like disease** in Australia and that it is carried by *Ixodes holocyclus*. At the Royal North Shore Hospital in Sydney the Tick-borne Diseases Research Unit continues its study into this issue. Cases of Lyme-like disease are being diagnosed on the basis of clinical signs (often musculoskeletal, chronic fatigue, neurological and dermatological), exclusion of other infections, serology (which is supportive but not conclusive), and response to antibiotic treatment. Initially, antibiotics may cause a worsening of symptoms (the Herxheimer reaction), as spirochaetes are destroyed, which in part supports the diagnosis. This Australian form of Lyme-like borreliosis is associated with a different clinical picture from true Lyme disease of North America.[65][66][67][68]

Skin lesions of Lyme-like disease. Early symptoms in the first four weeks after a tickbite include a rash or red patch that gradually expands over several days. It may get quite large (up to 50 mm or more in diameter). This rash is called *erythema migrans* or EM. It can be difficult to distinguish from an allergic reaction at the site of the bite. Allergic rashes usually occur within 48 hours of the bite, then fade. EM usually first appears after a delay of 48 hours following the tickbite and gets bigger over a few days or weeks. As it expands, EM rash often has a 'target' or 'bullseye' appearance, clearing in the middle with a more obvious red edge. EM can persist for months or years, coming and going in that time. Sometimes many small EM rashes occur. EM may only occur in as few as 20% of persons with Lyme disease.

Other body systems affected by Lyme-like Disease. More common than EM are symptoms due to invasion by the spirochaete of the nervous system, heart, muscles and joints. These may start weeks or months after the tickbite. Initially, they may include flu-like illness, fever, headache, sore

throat, fatigue, aching muscles and joints. More serious are meningitis, Bell's palsy (weakness of the face muscles), swelling of joints, and heart problems with palpitations and breathlessness. Lyme disease is difficult to distinguish from many other illnesses like Chronic Fatigue Syndrome (CFS) because the symptoms may be similar. If you have symptoms that could be Lyme disease, even if you do not remember a tickbite, see your doctor. Diagnosis is helped by a blood test called a Western Blot test, but your doctor will consider whether any other illness could be causing your symptoms and may do some other tests.

Treatment of Lyme-like Disease. Early treatment with antibiotics is important to prevent more serious problems. Pregnant women bitten by a tick should see their doctor. Some data suggests that Lyme Disease can affect the foetus, but two large studies in the US and Europe showed no increased risk of adverse effects on the foetus.

Viral diseases[edit]

So far, no viruses have been isolated from *Ixodes holocyclus*.[63] This does not however exclude the possibility that such diseases may be found in the future.

Protozoal diseases[edit]

So far no protozoa have been isolated from *Ixodes holocyclus*. This does not however exclude the possibility that such organisms may be found in the future.

See also[edit]

- Ticks of domestic animals

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- **Tick bite causes meat allergy**

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Some people develop an allergic reaction to meat between one and six months after being bitten by an Australian paralysis tick (Source: Ryan Wick/ Flickr, Creative Commons)

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- **Audio: Tick bite causes meat allergy (Science Online Audio)**
- **Tick allergy**, ABC Health & Wellbeing

There's no doubt that there are many health benefits to a well-planned vegetarian diet — and you live longer as well. But in most parts of Western society, vegetarians are in a minority — say between 2 and 10 per cent.

But this could change, thanks to the rapidly increasing spread of an Australian tick. For some people, one bite from this tick makes them allergic to meat for the rest of their lives.

The tick is the Australian paralysis tick. It lives along the east coast of Australia from Lakes Entrance in Victoria to up to Cooktown in Far North Queensland. The adult ticks are about four millimetres across.

They attach themselves to plants, and jump on a passing bandicoot or human. They'll crawl up inside your clothing and get some blood from you by biting your skin usually on the head and neck.

In most cases, you'll get a little local itching and swelling — now this is **not** an allergic reaction. Sometimes there will be inflammation at the site of the tick bite combined with a large swelling, and both the swelling and the inflammation will last for several days. In this case, you have had a mild allergic reaction to the tick bite. But every now and then you will have the major life-threatening allergic reaction known as anaphylaxis.

Anaphylaxis can cause a rash all over your body. Swelling of your throat and tongue which makes it difficult to breathe, vomiting and diarrhoea, and a serious fall in blood pressure. In short, you are at major risk of dying. Anaphylaxis is a medical emergency.

Back in the year 2008, Associate Professor Sheryl van Nunen, an immunologist at Sydney's Royal North Shore Hospital, wrote up her remarkable observations. Her paper discussed a small cluster of 25 patients who had been bitten by the Australian paralysis tick. Several months after the bite, suddenly out of the blue, they all had an allergic reaction to eating meat. Seventeen of the 25 patients had one or more serious symptoms of swelling of the tongue, constriction of the throat, difficulty in breathing plus an audible wheeze when they tried to breathe.

Surprisingly, none of them recorded a major allergic reaction to the original tick bite.

Finally, after a lot of work, the immunologists (and don't forget they're the really clever doctors) seem to have worked out the cause of the meat allergy.

It starts with a not-very-sweet sugar called 'galactose'. We humans can eat it just fine. When you combine galactose with a sugar called glucose you get lactose — the sugar in breast milk.

The trouble begins when you combine two galactose molecules together in a rather special way to make a bigger sugar called 'galactose-alpha-1,3-galactose', commonly called alpha-gal. All mammals carry alpha-gal, except for humans and the higher primates. In fact, it turns out we can be allergic to it.

So here's a scenario, a bandicoot or another cute animal is playing happily somewhere on the east coast of Australia. The Australian paralysis tick bites it to get a meal of blood and some of the alpha-gal from the bandicoot gets into the gut of the tick. After a while, the tick feels hungry again and bites a human and some of that alpha-gal from the bandicoot gets transferred into the human.

Now, many Australians get bitten by ticks, but very few then go on to get the allergy to meat. So something happens in this human — we don't know what — and their immune system slowly cranks into action.

After a delay — somewhere between one and six months after the initial tick bite — they have another regular meal involving meat, but on this occasion they get an allergic reaction. In some cases they can die from a full-blown anaphylactic reaction unless they can get medical treatment in time.

This allergic meat reaction will be set off by pork, beef, lamb and even whale meat, but not by fish or chicken because they are not mammals. It can also be set off by some marshmallows if they contain beef gelatin.

Today, Dr van Nunen sees about two cases each week of tick-caused meat allergy, and has over 500 patients with this condition on her books.

In more ways than one these people could literally — and I do mean literally — die for a steak.

Tags: diet-and-nutrition, allergies, invertebrates-insects-and-arachnids

Published 25 February 2014

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Tick Allergy

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Distribution map of the Australian Paralysis Tick (Ixodes holocyclus)

Map adapted from Roberts FHS (1970) Australian Ticks. Yeerongpilly, QLD, Australia by TAGS Inc., Bill Conroy & Norbert Fischer.

Introduction

Like other arachnids (such as spiders, scorpions and house dust mites), ticks have eight legs. They pass through a number of life stages from egg to larva to nymph and then finally, the adult.

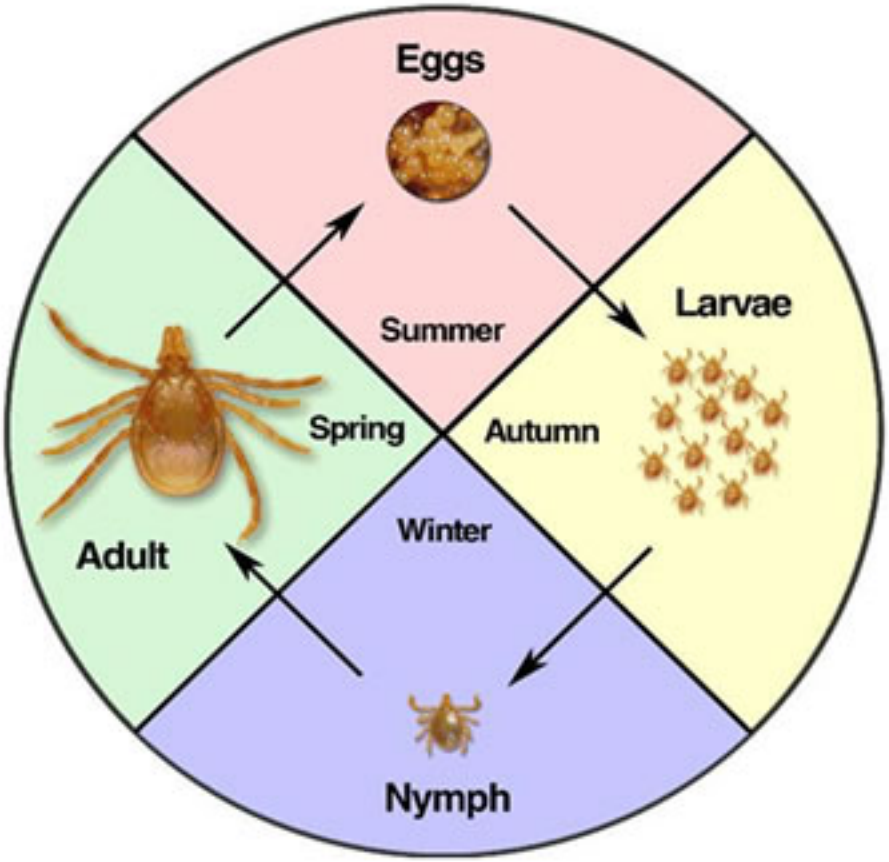

Illustration courtesy of Stephen Doggett,
Dept of Medical Entomology,
University of Sydney, Westmead

Health problems associated with tick bites include:
• Allergic reactions to tick bites;
• Allergic reactions to red meat and gelatin;
• Transmission of infections (less common than allergic reactions); and
• Tick paralysis (rare in humans, more likely to occur in children).
The focus of this article is *allergic reactions provoked by tick bites*.

Adult ticks cause the majority of health problems

Ticks are present mainly on the east coast of Australia, with known populations of ticks in several non-coastal areas (see map). Adult ticks cause the majority of the health problems in humans. All stages of ticks, however, are capable of provoking allergic reactions.
Larvae are very small, approximately 1mm in size and can be difficult to see, nymphs are slightly larger at approximately 2mm diameter with adult ticks (before a blood feed) being approximately 4mm in size.

Illustration courtesy of Stephen Doggett,
Dept of Medical Entomology,
University of Sydney, Westmead

Adult ticks attach to the tips of grass blades and vegetation, and from there transfer themselves to passing animals or humans. The tick usually crawls up inside clothing and attaches strongly to their host by biting through the skin, generally lodging in the skin of the head or neck or scalp of their host. The most common reaction is local irritation and swelling.
While the "tick season" is often considered to range from around February to August, ticks may be present at any time, therefore the risk of exposure remains throughout the entire year.

Allergic reactions to ticks

- Minor local itching and swelling is common at the site of a tick bite and is not due to allergy.
- Sometimes large local swelling and inflammation can arise at the site of a tick bite and last several days. Such reactions are usually due to mild allergy to the tick.
- Severe allergic reactions (anaphylaxis) have also been described to the Australian paralysis tick, *Ixodes holocyclus*.

Severe allergic reactions (anaphylaxis) occur when the tick is disturbed ,for example, after inadvertently disturbing the tick by scratching something which can't be seen, by deliberate attempts at tick removal or by application of irritant chemicals such as methylated spirits or kerosene to the tick.
Disturbing the tick may cause the tick to inject more allergen-containing saliva.

General strategies for managing reactions to ticks

Regardless of the type of reaction experienced after tick bites, the principles of management are:

- Try to reduce the risk of accidental tick bites (see below);
- Do not scratch anything you can't see if you live in a tick-endemic area;
- Know what to do if you find a tick lodged in your skin (and how best to remove it);
- Know how to manage *allergic reactions* (including anaphylaxis) to tick bites;
- Have your tick allergy confirmed by a doctor. This may require referral to a clinical immunology/allergy specialist, particularly if you are at risk of anaphylaxis;
- Be aware of the association between previous tick bites and the development of allergic reactions to mammalian meats and/or mammalian meat-derived gelatin.

Unfortunately, allergen immunotherapy (commonly known as desensitisation) is currently not available to "switch off" tick bite allergy.

Reducing the risk of tick bites

The following measures may reduce the risk of tick bite:

· Wear long-sleeved shirts and long trousers when walking in areas where ticks occur;

- Tuck shirt into trousers;
- Tuck trouser legs into long socks;
- Wear a wide-brimmed hat;
- Wear light-coloured clothes, which makes it easier to see ticks;
- Brush clothing before coming inside to remove ticks;
- Undress and check for ticks daily, checking carefully in the neck and scalp;
- An insect repellent may help, particularly ones containing DEET (such as RID®, Tropical RID® or Tropical Aerogard® or Bushmans®);
- Consider using permethrin-treated clothing when exposed to tick habitat (e.g. gardening in tick endemic areas); and
- In those with *recurrent dangerous allergic reactions* to tick bites, relocating to an area where ticks are not endemic is an option to consider.

What to do if you find a tick lodged in your skin and you are NOT allergic to ticks

The aim is to first kill the tick with an ether-containing spray and then remove it as soon as is practicable and in as safe a setting as is possible. Doing so may

reduce the possibility of you becoming allergic to ticks and may also reduce the risk of you contracting a tick-borne infectious disease or developing tick paralysis.

Common advice is to insert fine forceps or tweezers between the skin and the tick mouthpiece and lever the tick out. This method, however, does not prevent anaphylaxis in tick allergic individuals and therefore *ASCIA specifically advises against this method.*

What to do if you find a tick lodged in your skin and you are ALLERGIC to tick bites

If you are allergic to ticks, you should carry emergency medication (e.g. an adrenaline autoinjector such as EpiPen®) and a means of summoning medical assistance (such as a mobile telephone).

- If you know you are allergic to ticks and you are having an allergic reaction to a tick bite, follow your ASCIA Action Plan, including the use of an adrenaline autoinjector (EpiPen®) if symptoms of anaphylaxis occur.
- If you find a tick, do *NOT* forcibly remove the tick, but rather ***kill the tick first*** by using a product to rapidly freeze the tick to prevent it from injecting more allergen-containing saliva.
- In a tick allergic person, the tick should be killed and removed in a safe place (e.g. an emergency department of a hospital) until it is established that the process of killing the tick and removing it can be safely performed by the tick allergy sufferer. Once this is established, ticks may be killed and removed without necessarily attending an emergency department, depending upon the individual circumstances and after consultation with your medical specialist. Some tick allergic individuals are so highly allergic that medical support should always be sought. Your medical specialist will advise you as to which approach will be safest for you.
- If suffering your first allergic reaction to a tick, seek urgent medical attention. The tick can then be removed under medical supervision where facilities are available to treat the allergic reaction.
- Ether-containing aerosol sprays are currently recommended for killing the tick. Aerostart® and other similar products have been used extensively to kill ticks in allergic patients. It should be noted that these products are not registered for use in humans and contains benzene but there is long term experience with these products which have been shown to be very effective in treating those with serious tick allergies.
- The use of other ether-containing sprays such as Wart-Off Freeze® (and similar e.g. Elastoplast Cold Spray®) has also been effective. These products will continue to be studied and advice updated as experience increases.
- If available, liquid nitrogen applied by a doctor should also (in theory) be effective.

It is important to note that:

- This advice is based on the clinical experience of those treating patients with

tick allergy.
- Some of these products are not "registered" for use as therapeutic products for humans.
- All of these products are highly flammable, and thus should not be used near a naked flame or when smoking.
- Rapid cooling of the skin and thus skin irritation may occur.
- More information on these products may be obtained from manufacturers and distributors.
- Pending future studies of the effectiveness of various tick removal and killing methods, such advice is based on a consensus of "expert opinion" rather than derived from results of formal clinical studies.
- Freezing the tick (regardless of whether one is concerned about transmission of infection, tick paralysis or tick allergy) may also have the advantage of reducing the risk of tick sensitisation and later development of tick allergy or related allergic syndromes, as discussed below.

Confirming a diagnosis of possible tick allergy

At this time, there is no reliable skin or blood allergy test to confirm a diagnosis of tick allergy. Australian researchers have identified that the allergens causing problems are proteins in tick saliva. Diagnosis is currently largely based on the history of the reaction but some allergy test results have been associated with exposure to tick bites. Researchers have identified that the following blood allergy tests are positive in the majority of those with serious allergic reactions to tick bites, and that testing may assist in confirming the diagnosis:
- Mammalian meats Immunocap® .
- Alpha-galactose Immunocap®, a sugar molecule present in meat from mammals other than humans, great apes and Old World monkeys, as well as being found in the gut of ticks.
- Tryptase (an enzyme that is increased in those with a condition called mastocytosis, which is associated with an increased risk of allergic reactions to a number of allergic triggers including insect stings and tick bites and with more severe anaphylactic reactions to those insect stings and bites).

It is important to note that while positive red meat allergy tests are frequently seen in those with isolated tick bite allergy, routine avoidance of red meat and gelatin is *not* advised *unless* a patient has an allergic reaction to one of these foods as well. Nonetheless, patients should be aware of this possibility and informed by their doctors of the potential risk.

Tick Bites and Mammalian Meat Allergy

Australian allergic diseases physicians first described an association between tick bites and the development of mammalian meat allergy and these findings have since been confirmed by researchers in the USA and in Europe. A subgroup of

these patients will also be allergic to mammalian milks and animal-derived gelatin (present in some food products, as a binding agent in some medications as well as in intravenous blood substitutes known as gelatin colloid (e.g. Haemaccel®, Gelofusine®). The target allergen associated with these allergic reactions appears to be a sugar molecule known as alpha-galactose, present in the gut of ticks (and probably tick saliva) and all mammalian meats except for humans, great apes and Old World monkeys (e.g. beef, pork, lamb, kangaroo, venison, buffalo) and some more exotic meats eaten in some countries (e.g. guinea pig) in South America and ethnic specialty restaurants in North America and even Australia; and probably even whale meat (e.g. in Japan) as well as gelatin.

Researchers have identified that the following *blood allergy tests* are positive in the majority of those with serious allergic reactions to mammalian meat, and that testing (which can be ordered by any doctor) may assist in confirming the diagnosis:

- Beef, lamb, pork Immunocap®.
- Alpha-galactose Immunocap® a sugar molecule present in mammalian meats (but not in humans, great apes or Old World monkeys), as well as the gut of ticks.
- Elevation of tryptase (an enzyme that is increased in those with a condition called mastocytosis, which is associated with an increased risk and severity of allergic reactions to a number of allergic and non-allergic triggers including insect stings and tick bites).
- By contrast, blood allergy testing to gelatin is usually negative (even in patients who have had clear allergic reactions to gelatin orally or by injection).

In contrast, skin allergy testing to commercially available mammalian meats is much less reliable unless performed with raw, organic mammalian meats for confirmation (and very occasionally, even using raw meats, the diagnosis may not be confirmed). Gelatin skin testing results are highly variable, with often minor reactions on skin prick testing with gelatin, whilst intradermal injection skin testing is more reliable in diagnosing gelatin allergy.

Those with allergic reactions to mammalian meats are best advised to avoid all mammalian meats (beef, lamb/mutton, pork, goat, horse meat, kangaroo, venison and probably other more exotic mammals) and artificial blood (made from beef) as well as all forms of gelatin and to wear a medical bracelet warning of potential allergy to intravenous gelatin colloid (an intravenous preparation used as a blood substitute) as well.

Further advice on dietary avoidance strategies and tick-induced allergies may be found on the TiARA website.

Web links

Tick-induced Allergies Research and Awareness (TiARA): www.tiara.org.au
University of Sydney Department of Medical Entomology:

medent.usyd.edu.au/fact/ticks.htm

The Australasian Society of Clinical Immunology and Allergy (ASCIA) is the peak professional body of clinical immunology and allergy specialists in Australia and New Zealand.

Website: www.allergy.org.au

Email: projects@allergy.org.au

Postal address: PO Box 450 Balgowlah, NSW Australia 2093

Disclaimer

ASCIA Education Resources (AER) information bulletins have been peer reviewed by ASCIA members and represent the available published literature at the time of review. It is important to note that information contained in this bulletin is not intended to replace professional medical advice. Any questions regarding a medical diagnosis or treatment should be directed to a medical practitioner.

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J Accid Emerg Med. 1998 Mar;15(2):111-3.

Tick bite anaphylaxis in Australia.

Brown AF[1], Hamilton DL.

Author information

- [1]Department of Emergency Medicine, Royal Brisbane Hospital, Queensland, Australia.

Abstract

Tick bite anaphylaxis has rarely been reported. It may follow the bite of any of the different tick life cycle forms, is related to the release of salivary juices, and may range from mild itch to severe wheeze or shock. Data obtained suggest that it is more common and potentially life threatening than tick paralysis, which is more widely reported. Emergency physicians should recognise this possibility following a tick bite and be prepared to give treatment such as adrenaline rapidly. Patients

should be referred to an allergist after recovery.
PMID: 9570054 [PubMed - indexed for MEDLINE] PMCID: PMC1343038 **Free PMC Article**

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About Ticks

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- What are ticks and where do they live?

What are ticks and where do they live?

Ticks are blood-feeding parasites that are often found in tall grass where they will wait to attach to a passing host such as humans or dogs. Ticks can be found in most wooded or forested regions throughout the world and will often latch on to shoes or clothes, and then work their way up your clothing until they find a prime piece of exposed skin. When they find exposed skin they will use their cutting mandibles to cut your skin and insert their feeding tube to feed on your blood; often inserting a small amount of natural anesthetic simultaneously, which is why many people do not remember a tick bite.

Generally the ticks that cause Lyme Disease in the USA carry the *Borrelia Burgdorferi* bacteria in their gut, whilst in Europe it is often the *Borrelia Afzelii*; these bacteria cause Lyme disease. Currently research is underway in Australia to isolate exactly the type of tick and the specific bacteria that is causing Lyme disease here (there are at least 14 different types of bacteria that cause Lyme disease identified world wide).

Most of the ticks that infect people with Lyme disease are in the nymph, or immature stage of development and are about the size of a poppy seed, which means that many people do not remember a tick bite.

This is a nymph tick – it's bite is often missed, because it is so small.

This is an engorged adult tick – after it has fed.

Ticks have been found on every continent of the earth, except Antarctica. There are certain places in Australia that are known Lyme disease tick hot spots. In New South Wales (NSW), areas such as around Coffs Harbour, in the northern beaches area of Sydney (as well as the Western suburbs of Sydney), and the South Coast of NSW. The LDAA is in the process of mapping instances of Lyme disease and known locations of tick bites that led to Lyme disease & hope to be able to better identify endemic areas of infection in ticks (& humans).

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How do you remove ticks?

It is important that ticks are not covered in methylated spirits, rubbing alcohol, bi-carbonate of soda, vaseline, or burnt with a match in an attempt to remove. Such action can induce a tick to release spirochetes containing the bacteria known to cause Lyme disease into its host's blood stream. Other bacteria and parasites can also be transmitted concurrently. For more detailed information on prevention, visit our Prevention page. Briefly though:

- Use fine-point tweezers or a special tick-removing tool. Grasp the tick as close to the skin as possible. If you don't have tweezers, protect your fingers with a tissue.
- Pull the tick straight out with steady, even pressure. View a Tick's Mouth and why it is so important to pull out the tick correctly.
- Avoid squeezing the tick, breaking it, or allowing any blood to remain on your skin.
- Place the tick in a small plastic bag or vial with blades of grass, leaf, or moist (not wet) piece of tissue.
- Label the bag with your name, date, site of bite and how long tick was attached.
- Have the tick identified and tested by a lab, health department or veterinarian.
- Wash your hands, disinfect the tweezers and bite site.
- Educate yourself about tick-borne diseases and consult a doctor to see if treatment is warranted.

(Thank you to the California Lyme Disease Association for their Tick Bite removal instructions)

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Nymph Size Ticks

Nymph size ticks are tricky to remove – the above instructions will not work as they are simply too small. Pittwater City Council and the bush regenerators group (on the northern beaches of Sydney where ticks are pretty endemic) suggest, "mass infestations of small larval or nymph stage ticks are best removed by soaking for 20 minutes in a warm bath with 1 cup of bicarbonate of soda added."

NSW Government: Ticks

Human Parasites
Head Lice
Scabies
Bed bugs
Ticks

Ticks are parasites that feed on the animal and human blood. Ticks occur in humid, moist bushy areas. They are not very mobile but rely on passing animals to both feed on and transport them. Ticks are known to inject toxins that cause local irritation or mild irritation, however most tick bites cause little or no symptoms. Tick borne diseases, tick paralysis and severe allergic reactions can pose serious health threat.
Tick-borne diseases occurring in Australia are Australian Tick Typhus or 'Spotted Fever' (along the coastal strip of eastern Australia from North Queensland to Victoria) and 'Flinders Island Spotted Fever' (in Victoria, Tasmania and Flinders Island in Bass Strait).

Early symptoms of tick paralysis can include rashes, headache, fever, flu like symptoms, tenderness of lymph nodes, unsteady gait, intolerance to bright light, increased weakness of the limbs and partial facial paralysis.
As the tick engorges on more human blood the tick paralysis symptoms may intensify including after the tick has been removed. Clinical diagnosis is confirmed by specific blood tests. Tick typhus is treatable with antibiotics, although fatalities have been known to occur. In some susceptible people tick bite may cause a severe allergic reaction or anaphylactic shock, which can be life threatening. If swelling of the face and throat causes breathing difficulties, seek urgent medical attention.

- Ticks Factsheet
- Avoiding Tick and Spider Bites Factsheet

Tick
Alert Brochure : http://www.health.nsw.gov.au/environment/Publications/tick_alert_brochure.pdf

Tick bite prevention

Department of Health Australia

This page provides information about tick bites

Page last updated: 25 November 2015

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PDF printable version of *Preventing and treating tick bites* - PDF 231 KB

What are ticks?

Ticks are parasites that feed on animal and human blood. There are more than 800 species of ticks around the world, with 70 found in Australia and 16 species have been reported as feeding on humans.

There are two major groups of ticks: hard ticks and soft ticks.

Hard ticks (family: *Ixodidae*) have a hard flat body and elongated mouthparts

with rows of backward pointing teeth. This group includes the most important species that bite humans.

Soft ticks (family: *Argasidae*) have a wrinkled leathery appearance. Only a few species of this type are found in Australia and they rarely come into contact with people.

The most important tick in Australia is the Paralysis Tick, *Ixodes holocyclus*, and over 95% of tick bites in Eastern Australia are due to this species. Most tick-borne illnesses are due to this species.

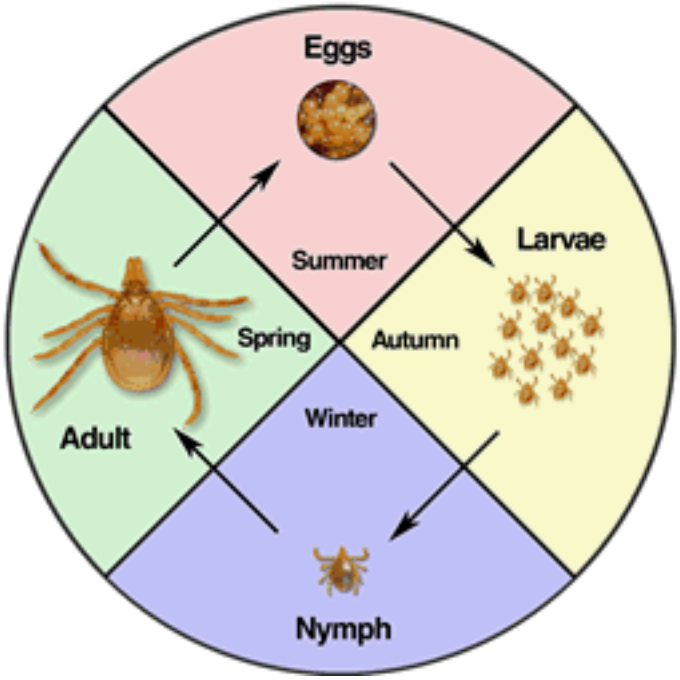
The Paralysis Tick

The Paralysis Tick, *Ixodes holocyclus,* is found along the eastern seaboard of Australia east of the Great Dividing Range, and possibly into Tasmania. It is commonly referred to as the grass tick, seed tick and bush tick depending upon its stage of development. It is not known to occur in South Australia, Western Australia or the Northern Territory.

While *I. holocyclus* is the most common, there are two other *Ixodes* species in Australia which cause paralysis: *I. hirsti*, which occurs in South Australia and also has been documented in NSW and Tasmania, and *I. cornuatus*, which occurs in Tasmania and Victoria.

There are four stages in the life cycle of a tick; the egg, larvae (around 1mm and light brown in colour when not full of blood), nymph (around 2mm and pale brown) and the adults (4–5mm in length, without blood). The Paralysis Tick needs to feed on blood to develop through its lifecycle from the larvae stage to a nymph and to an adult. The adult female takes blood to obtain protein for the laying of eggs.

When fully engorged it is grey-blue in colour up to around 1cm in length.



Tick life cycle (S.L. Doggett, Department of Medical Entomology, Westmead Hospital) as described above.

The Paralysis Tick is most common in moist, humid coastal areas with abundant native animals that serve as hosts for the tick. Long grasses and bushland provide ideal environments for ticks, and if you live close to these areas, it is not uncommon to have Paralysis Ticks in your garden. This tick has a distinct seasonality; the larval stage is most active during the autumn months, the nymph during winter and the adult during the spring. This tick is most active during periods of high humidity, especially after rain, and this is when you should take particular care to avoid tick bites.

Paralysis Ticks are not particularly mobile, and rely on passing animals for a blood meal. The Paralysis Tick will crawl up the stems of grasses or along branches and 'perch' ready to latch on to a passing animal, including humans. They rarely climb higher than 50cm in their habitat, so do not drop out of trees, despite this common belief. However, after landing on a person or animal they can walk up the body and attach to the head area.

Female *Ixodes holocyclus* (S.L. Doggett, Department of Medical Entomology, Westmead Hospital) photograph above

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How does the Paralysis Tick affect humans?

A tick attaches itself by piercing its sharp mouthparts into skin. It then injects an anticoagulant (a substance that prevents blood from forming clots) saliva which allows it to feed without the blood clotting. In the case of the Paralysis Tick, the saliva may be highly toxic to some animals and, potentially, humans. Most tick bites pose no medical problems apart from some localised swelling and redness at the bite site if the tick is removed promptly. However, in some cases people can experience more severe conditions such as tick paralysis or allergic reactions including anaphylactic shock. Early symptoms of tick paralysis may include rashes, headache, fever, influenza like symptoms, tenderness of lymph nodes, unsteady gait, intolerance to bright light, increased weakness of the limbs and partial facial paralysis. Tick paralysis, while rare, is usually seen in children rather than adults. Allergic reactions can result in swelling of the throat, and may lead to breathing difficulties or collapse. It is important to seek

medical attention quickly if such symptoms occur. If you have had similar symptoms in the past after being bitten by a tick, then it is a good idea to always be prepared.

Some serious tick-borne diseases also occur in Australia including, Queensland tick typhus and Flinders Island spotted fever. There are concerns that other serious illnesses, such as a Lyme disease-like syndrome, may be caused by exposure to Australian ticks, however there is no evidence yet this is the case (Lyme Disease).

Recently a new syndrome known as "tick-induced mammalian meat allergy" has been described, whereby people bitten by the Paralysis Tick, which is found in coastal Eastern Australia, can subsequently develop an anaphylactic reaction to consuming meats and animal by-products such as gelatine. This syndrome has also been described overseas.

How to prevent tick bites?

The best way to prevent tick bites is to avoid tick-infested areas.

If this is not possible, wear appropriate clothing such as:

- a long sleeved shirt
- long pants tucked into socks
- light coloured clothing to make it easier to see ticks on clothes before they attach to the skin

Before entering possible tick infected environments apply an insect repellent containing diethyl-meta-toluamide (DEET) or picaridin to the skin. The repellent should be applied and re-applied according to the manufacturer's instructions.

Clothing treated with permethrin is also recommended.

Permethrin wash kits for treating clothes can be obtained from outdoor recreational stores and it is important to follow the label directions. Permethrin-treated clothing is considered the most effective means of preventing tick bite in tick infested areas.

All clothing should be removed after visiting tick infested areas and placed into

a hot dryer for 20 minutes to kill any tick that could be still on the clothing. The entire body should be then checked for ticks of all sizes and stages, paying particular attention to areas behind the ears and the back of the head or neck, especially on children.

Removing ticks

If you suffer from allergic reactions to ticks, only attempt to remove a tick whilst at a medical facility such as an Emergency Department.

In non-allergic individuals or for larval or nymphal stage ticks:

- When removing a tick with fine tipped forceps (not household tweezers unless fine tipped forceps are not available), grasp the tick as close to the skin's surface as possible. Pull upwards with steady pressure and avoid jerking or twisting the tick.
- Prior to removal, the tick may be sprayed with an aerosol insect repellent containing pyrethrin or a pyrethroid chemical, although there is currently no evidence to suggest that this is of benefit. Permethrin based creams, which are available from chemists may also be used. Apply at least twice with a one minute interval between applications.
- If you have difficulty removing the tick or suffer any symptoms after removal, seek medical attention urgently.

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Tips

- Use only *fine tipped forceps* and avoid squeezing the body of the tick.
- Don't use folklore remedies such as matches or pins because they will irritate the tick and make it harder to completely remove.
- Avoid scratching and do not use irritant chemicals such as methylated spirits or kerosene.

Note

The Australasian Society of Clinical Immunology and Allergy has recently recommended killing an adult tick in place by using an ether-containing spray

to kill the tick by freezing it. These products are normally used for the treatment of warts and skin tags and are readily available from chemists. This document does not recommend this method until evidence-based research becomes available. When new evidence is published this document will be reviewed.

Further information

If you are concerned about ticks, contact:

- your Medical Practitioner
- the Poisons Information Centre: 13 11 26
- your state or territory health department
- your local council Environmental Health Officer
- Australasian Society of Clinical Immunology and Allergy (www.allergy.org.au).

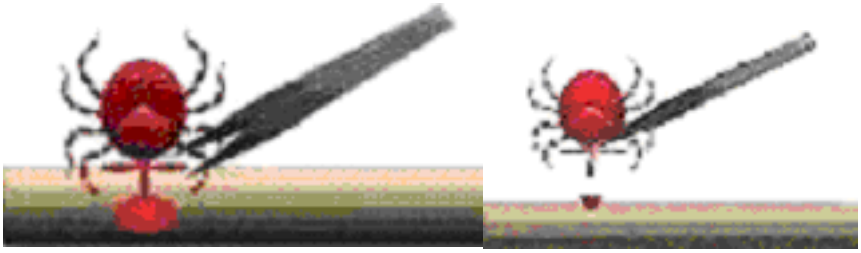
Ticks

Queensland Health:

Ticks are widespread in Australia. Tick bites generally cause minimal discomfort, but rarely humans can

experience allergic reactions, paralysis and tickborne diseases.

The key treatment of tick bite is prompt and complete removal of the tick. Use fine tipped tweezers possible. Gently pull the tick straight out with steady pressure.



Removal with Tweezers
http://www.lowchensaustralia.com/bites.htm

Alternatively a tick may be removed using the knot method. Make a loose half-hitch in a thread such as a piece of dental floss. The open knot is slipped over the tick as close as possible to the skin and then pulled taut. The embedded tick then usually somersaults out. **If you have difficulty removing all parts of the tick, seek medical attention.**

Other methods of removal, involving irritants such as kerosene or a hot match, are not recommended. Once removed, follow general first aid for bites and stings.

Upon removal of the tick, conduct a thorough search of the body for other ticks, especially body folds and creases.

Paralysis can result if the tick remains undiscovered on the body. Symptoms include general unsteadiness, tiredness, visual difficulties and weakness of the arms, legs or parts of the face. **If symptoms of tick paralysis are present, seek medical attention immediately.**

Tickborne bacterial infections are rare. Symptoms include headache, fever, joint and muscle pain and a spotted rash. **If unwell after tickbite, seek medical attention.**

Preventative measures to avoid tick bites:

- Use insect repellant containing diethyltoluamide (DEET) or picaridin before going into tick infested areas.
- Wear light coloured clothing including long pants tucked into socks, a long

sleeved shirt as well as a wide brimmed hat.

After returning from a tick area, thoroughly check the body of all members of the party (especially children) for ticks. Pay particular attention to the back of the head and neck, groin, armpits and back of the knees.

Karl Mc Manus Foundation:

http://www.karlmcmanusfoundation.org.au/ticks-in-oz

The research must identify the causative agents of Lyme Disease in Australia. Lyme Disease Association with Ticks and other vectors must be resolved.

Australian Museum: Australian Paralysis Tick: Ixodes holocyclus

http://australianmuseum.net.au/australian-paralysis-tick

Tick allergy

IMPORTANT The information provided is of a general nature and should not be used as a substitute for professional advice. If you think you may suffer from an allergic or other disease that requires attention, you should discuss it with your family doctor.

Summary Problems associated with tick bites include tick paralysis, transmission of infectious disease and occasionally, allergic reactions.

Introduction

Like other arachnids (such as spiders, scorpions and house dust mites), ticks have eight legs. They pass through a number of life stages from egg, to larva, nymph and then finally, the adult.

Health problems associated with tick bites include
•Transmission of infection
•Tick paralysis
•Allergic reactions to tick bites; and at times,
•Allergic reactions to red meat and gelatin.

The focus of this article is on allergic reactions to tick bites, and related allergic syndromes

Adult ticks cause the majority of health problems
Adult ticks (present mainly on the east coast of Australia although it is likely that other areas are also infested) cause the majority of the health problems in humans. Nonetheless, all stages of ticks are capable of provoking allergic reactions.

Larvae are very small ~ 1mm in size and can be difficult to see, nymphs slightly larger at ~ 2mm diameter with adult ticks (before a blood feed) being ~ 4mm in size, and are larger after a feed.

Adult ticks attach themselves to the tips of grass blades and vegetation, and transfer themselves to passing animals or humans. When humans are infested, the tick usually crawls up inside clothing. Adult ticks attach themselves strongly to their host by biting through the skin, and generally lodge in the skin of the head or neck or scalp of their host. The most common reaction is local irritation and swelling. While the “tick season” is often considered to range from around February to August, stages of the tick are present all year round, so there is really no one time when the risk of exposure completely disappears.

Tick paralysis
Tick saliva can cause paralysis in animals and humans (children are more susceptible), usually after the tick is embedded for 3-5 days. Symptoms are due to injected toxic proteins / neurotoxins. Symptoms include blurred vision, weakness of the limbs, and problems with coordination or speech. Treatment centres on removal of the tick. There is no vaccine to prevent reactions in humans.

Ticks as a source of infectious disease

Ticks can transfer infection from animals to human hosts, such as scrub typhus and Queensland tick typhus (spotted fever). Information on these disorders is beyond the scope of this article, but useful information can be found by contacting:

•TAGS (Tick Alert Group Support Inc., PO Box 95 Mona Vale, NSW 1660 (http://www.lowchensaustralia.com/pests/paralysis-tick/tick-alert-group-support.htm

•The Tick-Borne Diseases Research Unit, Royal North Shore Hospital, Pacific Highway, St Leonards NSW 2065.

•University of Sydney, Medical Entomology: http://medent.usyd.edu.au/fact/ticks.htm

•Victorian Health Department: http://ideas.health.vic.gov.au/bluebook/rickettsial.asp

•NSW Health Department: http://www.health.nsw.gov.au/environment/Publications/tick_alert_brochure.pdf

Allergic reactions to ticks

•Minor local itching and swelling is common at the site of a tick bite. That does not represent allergy.

•Sometimes large local swelling and inflammation can arise at the site of a bite and last several days.

•Serious allergic reactions (such as anaphylaxis) have also been described, in response to a number of species of tick species world-wide, including the so-called Australian paralysis tick, Ixodes holocyclus.

Serious allergic reactions occur when ticks are disturbed

Whereas most advice is to remove the tick as soon as possible using a fine pair of forceps, doing so in those with TICK ALLERGY is NOT advised. Almost invariably the allergic reaction occurs when the tick is disturbed, typically after attempts to remove it or, for example, after inadvertently disturbing the tick by scratching, deliberate attempts at removal or application of irritant chemicals like methylated spirits or kerosene may cause the tick to inject more allergen-containing saliva. This has implications for what should be done when a tick is

discovered. See below for specific advice in those with tick allergy.

Managing reactions to ticks

Regardless of the type of reactions experienced after tick bites, the principles of management are:

•Try to reduce the risk of accidental tick bites;
•What to do if you find a tick lodged in your skin (and how best to remove);
•How to manage allergic reactions to tick bites;
•How to confirm a diagnosis of possible tick allergy;
•The relationship between tick bites and allergic reactions to red meat and gelatin in some unlucky individuals.
•Unfortunately, there is currently no vaccine to “switch off” tick bite allergy.

Reducing the risk of tick bites

The following measures may reduce the risk of tick bite:

•Wear long-sleeved shirts and long trousers when walking in areas where ticks occur
•Tuck trouser legs into long socks
•Wear a “coolie” style hat
•Wear light clothes, which makes it easier to see ticks
•Brush clothing before coming inside to remove ticks
•Undress and check for ticks daily, checking carefully in the neck and scalp
•An insect repellent may help, particularly ones containing DEET (such as RID, Tropical RID or Tropical Aerogard or Bushmans).
•In those with recurrent dangerous allergic reactions to tick bites, relocating to an area where ticks are not endemic is an option to consider.

What to do if you find a tick lodged in your skin and you are NOT allergic

The best advice varies according to whether or not you have had serious allergic reactions to tick bites. If you have minor irritation/local swelling only, the aim is to remove the tick as soon as possible to reduce the risk of tick paralysis or tick borne infectious disease. Thus remove the tick as per common advice to use fine forceps or tweezers

between the skin and the tick mouthpiece. http://www.health.qld.gov.au/poisonsinformationcentre/bites_stings/bs_ticks.asp

What to do if you find a tick lodged in your skin and you are known to be ALLERGIC to tick bites

Eg. rash all over, anaphylaxis with difficulty breathing or a drop in blood pressure)

•If you are allergic to ticks, you should carry emergency medication (eg. an adrenaline autoinjector such as EpiPen or Anapen) and a means of summoning medical assistance (such as mobile telephone).

•If you find a tick, do not forcibly remove the tick*.

•Do not try to kill the tick using insecticide or chemicals (such as oil, turpentine, Kerosene, methylated spirits)*. Attempts to remove the tick or using these products can irritate the tick, do not result in immediate death of the tick and increase the risk that more allergen containing saliva may be injected. Indeed, most allergic reactions to ticks occur when an attempt is made to remove or poison the tick, or when it is disturbed (such as scratching).

•Instead, the authors of this educational article currently recommend that the tick is killed first using a product called “Aerostart”, and that medical attention be sought immediately after doing so. Additional information on this product is listed below.

•Seek urgent medical attention in case additional treatment is required. If you have not already removed the tick, it can then be removed under medical supervision where facilities are available to treat an allergic reaction.

•Follow you anaphylaxis action plan including use of an adrenaline autoinjector (EpiPen or Anapen) if potentially dangerous allergic symptoms occur.

Additional information on using Aerostart to kill ticks

Aerostart is a spray containing ether that is commonly used by mechanics to clear carburettors. Spraying this product onto ticks freeze-dries the tick and kills it instantly. This allows the tick to fall out without being able to inject saliva-containing allergen. Aerostart can be purchased from hardware stores and some service stations. Since Aerostart is a highly flammable product, it is wise to use it away from

naked flames and to not smoke around the area of use.

It is important to note that:
•This advice is based on clinical experience of those treating patients with tick allergy.
•This product is not “registered” for use as a therapeutic product for humans.
•This product is highly flammable, and thus should not be used near naked flame or when smoking
•Rapid cooling of the skin and thus skin irritation may occur
•This advice is based on a consensus of “expert opinion” from those treating patients with tick bite allergy rather than derived from results of formal clinical studies.
•More information on Aerostart can be obtained at: http://www.unitedpetroleum.com.au/docs/in-store-msds/aerostart--msds.pdf
•This advice will be updated if formal studies are done in this area.

Confirming a diagnosis of possible tick allergy
At this time, there is no reliable skin or blood allergy test to confirm a diagnosis of tick allergy. Australian researchers have identified that the allergens causing problems are proteins in tick saliva. Diagnosis is largely based on the history of the reaction but some allergy test results have been associated with exposure to tick bites and clinical symptoms. Researchers have identified that the following blood allergy tests (RAST, ImmunoCap) are positive in the majority of those with serious allergic reactions to tick bites, that testing may assist in confirming diagnosis and that these tests can be ordered by any doctor:
•Beef and other red meat
•Alpha-galactose, a sugar molecule present in non human red meat as well as the gut of ticks
•Tryptase (an enzyme that is increased in those with a condition called mastocytosis, which is associated with an increased risk of allergic reactions to a number of allergic triggers including insect stings and tick bites)Document2

Tick Bites and Red Meat Allergy
Australian allergic diseases physicians have described an association

between tick bites and the development of red meat allergy, of whom a subgroup will also be allergic to meat and bone-derived gelatin. These finding have been confirmed by researchers in the USA and in Europe. The target allergen associated with these allergic reactions appears to be a sugar molecule known as alpha-galactose, present in tick guts, some worms, non-human red meat (eg. beef, pork, lamb, kangaroo) as well as gelatin. The degree of the allergic immune response to alpha-galactose goes up after tick bites.

Symptoms of red meat allergy

Symptoms of tick bite allergy may range from itchy rash/hives, through to abdominal pain, difficulty breathing and collapse. Symptoms often occur at night, when larger amounts of meat are more likely to be consumed, but may not occur every time red meat is eaten. The reasons behind this remain uncertain. The onset of symptoms may also be delayed 3-8 hours after consumption. Around one third will react to gelatin, which also contains alpha-galactose. Reactions to animal milks are relatively rare but have been described.

Confirming a diagnosis of possible red meat allergy

Researchers have identified that the following blood allergy tests (RAST, ImmunoCap) are positive in the majority of those with serious allergic reactions to red meat, and that testing may assist in confirming diagnosis:

•Beef and other red meat
•Alpha-galactose, a sugar molecule present in non human red meat as well as the gut of ticks
•Tryptase (an enzyme that is increased in those with a condition called mastocytosis, which is associated with an increased risk of allergic reactions to a number of allergic triggers including insect stings and tick bites)
•By contrast, blood allergy testing to gelatin is usually negative (even in patients who have had clear allergic reactions to gelatin orally or by injection)

Skin allergy testing to red meat and gelatin is highly variable, with often minor reactions on skin prick testing using commercial extracts of red meat or gelatin, although testing with real red meat may give more

reliable results, as may other skin testing techniques.

Dietary advice in those with red meat allergy

Those with allergic reactions to red meat are best advised to avoid all red meat (beef, lamb, pork, goat, sheep meat, horse meat, kangaroo) as well as all forms of gelatin. Gelatin is present in some food products, occurs as a binding agent in some medications as well as in intravenous blood substituted known as gelatin colloid (eg. Haemaccel, Gelofusine). Those with allergy to red meat/gelatin are also advised to wear a Medicalert bracelet warning of potential allergy to gelatin colloid as well. Further advice on dietary avoidance strategies may be found on the TIARA website (http://www.tiara.org.au)

Additional Web links

Tick Alerts Support Group (TAGS, Australia) http://www.lowchensaustralia.com/pests/paralysis-tick/tick-alert-group-support.htm

CSIRO Australia Entomology www.ento.csiro.au/ and http://www.publish.csiro.au/?act=view_file&file_id=NB11025.pdf

University of Sydney Department of Medical Entomology http://medent.usyd.edu.au/fact/ticks.htm

Tick Induced Allergies Research and Awareness (TIARA) http://www.tiara.org.au

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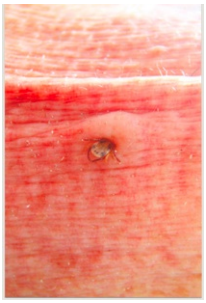
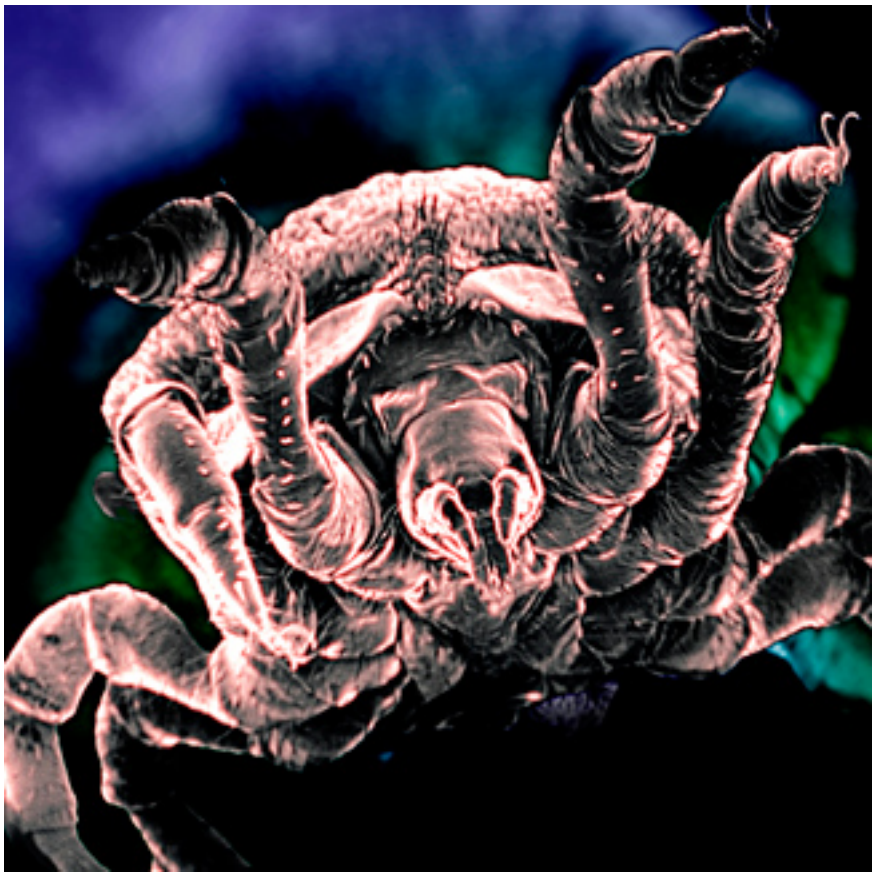
Last reviewed October 2013

The Aerostart spray, commonly used to clear carburettors but also useful for tick allergy first aid (see text below)



Tick buried into the skin of the neck



An electron microscope false-coloured image of a tick. (Credit: Getty)

Ticks poisonous hitchhikers

- BY CAROL BOOTH |
- JULY 26, 2011

Stealthy stowaways from a day in the bush, paralysis ticks are superbly adapted to feast on their hosts.

SUE BLOOMFIELD WAS ENJOYING a summer day mulching in her garden on the Atherton Tableland, in far north Queensland. She loved gardening for its intimacy with earth and the feeling of being connected to nature. And for at least one part of nature, the desire to meld was mutual. Below her busy hands, a creature was poised on the edge of a leaf, and as Sue brushed past, it latched on.

For a few hours the questing creature trundled about, until it settled on Sue's forehead. There, it administered a drop of liquid, probably analgesic, tore the flesh open with two knife-edged appendages, and wedged a barbed, syringe-like feeding tube in the opening.

From giant salivary glands (the biggest of the tick's organs, which increase in mass 25-fold during feeding), it secreted clot-inhibiting enzymes into the wound to create a feeding pool of Sue's seeping blood. She didn't feel a thing; other secretions prevented inflammation - the itching and swelling would otherwise alert her. If all went well, it could be there a week or more, growing from the size of a grain of rice to that of a pea as it ingested enough protein to trigger reproduction and the creation of up to 3000 offspring.

The blood-sucking parasite burrowing into Sue's forehead was a female *Ixodes holocyclus*, known as the Australian paralysis tick or shellback, indigenous to the forests and moist woodlands of eastern Australia.

But it's not her bloodlust that's the problem: we can certainly spare the tick the trickle she needs to reproduce.

Rather, it's what she dribbles back in exchange that can harm. Tick saliva is a biochemist's delight - vasodilators (which dilate blood vessels), anti-clotting agents and immunosuppressants keep the blood flowing and the host's immune cells at bay. Research on overseas species has found more than 300 proteins in tick saliva, but immunologist Kevin Broady, head of the department of medical and molecular biosciences at the University of Technology, Sydney (UTS), says the compounds in Australian species have been barely studied.

Tick's: saliva the real killer

At 2.30a.m. the next morning Sue was awoken by a slight burning sensation on her forehead, and discovered the tick. "When I touched it, it felt like poison shot to my feet," she says. "They started tingling. Then I started getting blotches all over my body." When her throat started swelling, she woke her husband. By the time they got to Cairns Base Hospital at 4a.m., her swollen air passages had almost closed.

What came close to killing Sue was not the tick, but anaphylactic shock brought on by her body's extreme allergic response to proteins in the tick's saliva. Medical entomologist Dr Stephen Doggett says allergic reaction is the
most widespread and severe consequence of tick bites in Australia. While the majority of reactions are merely irritating - the bites of multiple tick larvae can provoke an intense allergic dermatitis - anaphylactic shock is life threatening. Elsewhere in the world, Lyme disease has a profound impact on human health. But contrary to popular

perception, "there's not one good piece of scientific evidence for Lyme disease in Australia," Stephen says.

The most notorious impact of the paralysis tick gives it its name. "A tiny compact protein in its saliva interferes with transmissions from nerve to muscle cells," says Kevin, whose UTS team isolated the neurotoxin. Of more than 800 species of tick worldwide, only Ixodes holocyclus causes significant paralysis requiring medical or veterinary treatment. At least 20 Australians, mostly children, have died (from respiratory failure), although none since 1945.

Evolutionary mystery of why ticks so lethal

Its toxicity is an evolutionary mystery. "It's unusual for an ectoparasite [one external to the body] to kill its host," Kevin says. Ticks are arachnids, related to spiders and scorpions, for which there is an advantage in being able to kill.

Kevin thinks the toxin may be remnant from that evolutionary past. University of Queensland Professor Rick Atwell, who is investigating genetic variability in ticks, wonders whether it might have been advantageous when megafauna roamed Australia (more than 20,000 years ago), by causing localised paralysis and decreasing the parasite's chances of being dislodged, thus allowing it to feed.

For the tick's natural hosts, paralysis isn't usually a problem. Bandicoots (the most common host), koalas and other marsupials develop immunity through regular exposure. But less-preferred hosts, such as dogs and cats, often don't get the chance to acquire immunity. An estimated 10-20,000 are paralysed annually, with hundreds

dying. Other animals are also affected, including spectacled flying-foxes, a threatened species native to north Queensland, which have died in their thousands.

Despite the tick's toll, there's little success finding a vaccine. Kevin started work on it a decade ago, but the task has proved difficult. With new techniques now available, he thinks there's potential for a vaccine to protect against ticks during the attachment stage, but funding is now a problem.

Globally, only mosquitoes spread more human and animal diseases than ticks.

But the pathogens spread by Australian ticks are less dangerous than those overseas. Stephen estimates there are 100-200 cases of tick typhus - Queensland tick typhus (also known as scrub typhus) and Flinders Island spotted fever - each year caused by *Rickettsia australis* (an intracellular bacterial parasite).

While being part of a tick's lifecycle is unpleasant, sometimes dangerous, and for many animals the suffering is horrific. But try to spare a drop or two of sympathy for ticks. Their blood-sucking lifestyle isn't easy. Probably fewer than one in 1000 reach breeding age: they often succumb to desiccation. Good hosts are hard to find.

Giving ticks the flick

First and foremost, avoid tick habitat. Wear light-coloured clothing (so you can see them); tuck your shirt inside your pants, and pants inside socks; check your body regularly; and use repellents (20-30 per cent DEET or picaridin).

Different experts recommend different methods for removal of ticks, and there have been no studies to compare them. Health departments advise pulling ticks out with tweezers close to the skin. To minimise an allergic reaction, some specialists advise not to manipulate the tick, but spray it with a synthetic pyrethroid or dab it with Lyclear (a scabies treatment) and allow it to die and drop off. Those who may suffer a severe allergic reaction should only remove a tick under medical supervision.

Source: *Australian Geographic*, issue 94 (April - June, 2009)

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Lives almost lost after tick bites

Ripley Perkins became very sick from a tick.
Renee Pilcher
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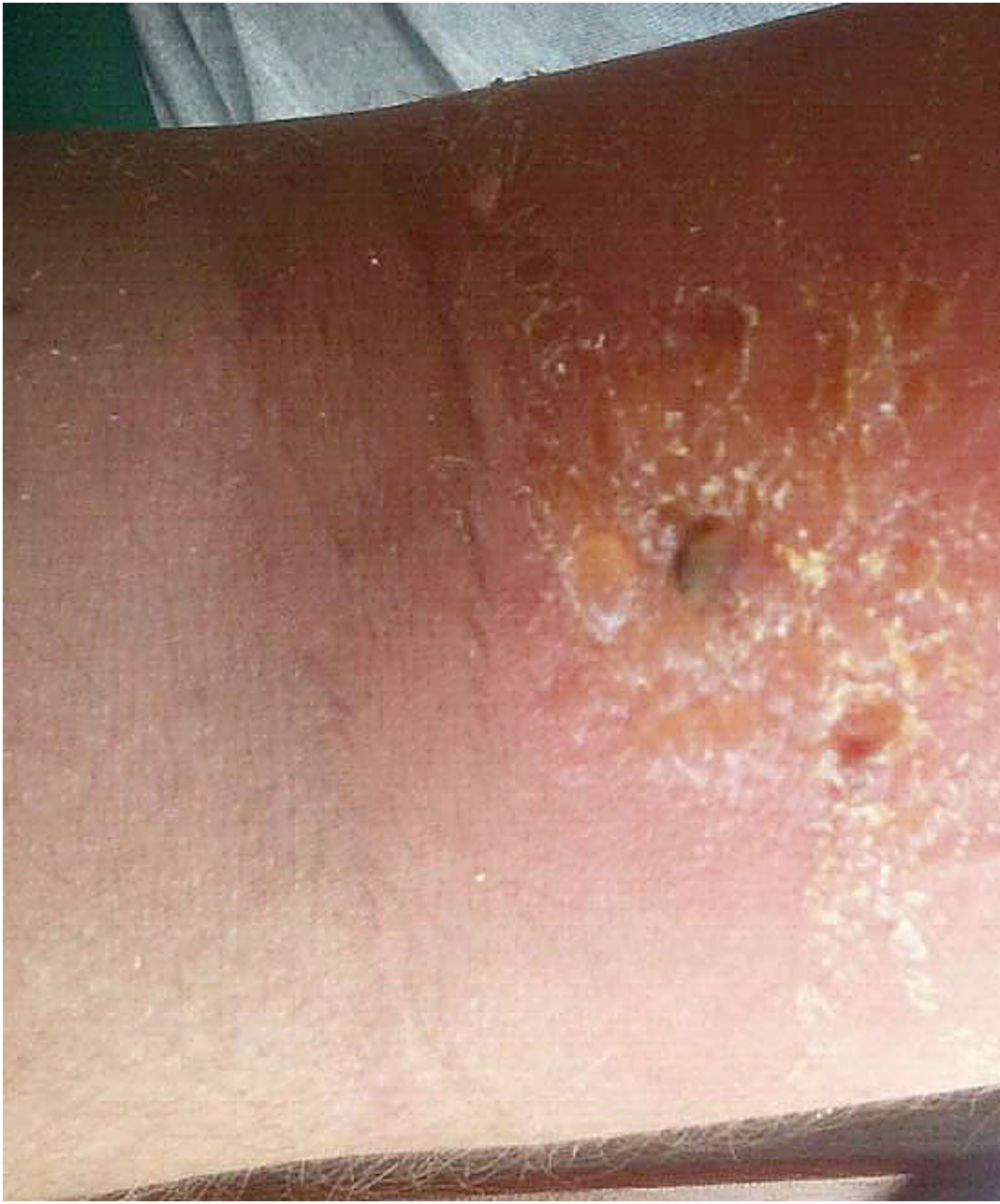
AUTHORITIES are blaming South-East Queensland's warm winter for the surge in tick bites that have recently put a Gympie father and a teenager in hospital, and almost cost a Mary St solicitor his life.

Fitness enthusiast Ripley Perkins, 41, was on the brink of organ shutdown when he was finally diagnosed with rickettsia, tick-transmitted bacteria also known as Queensland tick typhus or tick bite fever, in Nambour Hospital in June.

By the time Ripley was diagnosed, a full week after being admitted to Gympie Hospital with a mystery "virus", he had lost 14kgs, was in isolation with suspected meningococcal disease, and on continuous intravenous fluids and oxygen.

His blood pressure, heart rate and fever were all at dangerous levels, and he was in a delirium, seeing visions of dead friends.



Once the correct diagnosis was made and treatment began, it was a month before Ripley could return to work (and then only part-time), and three months before he was back training on his mountain bike.

He now knows he was bitten by a tick, most probably at his East Deep Creek home, though the tick had fallen off, leaving two bite marks and some swelling.

It was only the sharp eyes of the Nambour infectious diseases' officer that finally identified the deadly symptoms of rickettsia.

A Pomona resident who works in Gympie was bitten by a tick several months ago and sent his blood to the United States to be tested for lyme disease when Australian treatments failed to improve his ailing health. He tested positive to the condition despite it being officially non-existent in Australia.

Just over a week ago, Calico Creek landowner Ben Cameron, also in his early 40s, spent five nights on intravenous antibiotics in Gympie Private Hospital for an unspecified bacterial infection picked up from a tick.

Ben had discovered the tick just above his elbow the previous Thursday morning, and pulled it out, thinking nothing more of it.

"I've had heaps of them," he said. "But this year they do seem to be worse."

By the following night Ben's arm had puffed "to the size of my shoulder" and was "a bit sore". He went to the doctor the next day and was immediately put in hospital.

In September, Gympie State High School Year 12 student Ben Jones, 17, spent four nights in Gympie Hospital after picking up a tick while digging a veggie patch with his mum Tanya on their 10-acre Southside property.

The tick bit Ben just above the right knee. Tanya found it that night and pulled it out, but two days later the bite had turned red and swollen, and a day later it was the size of a tennis ball and quite painful.

Ben was admitted to hospital with "cellulitis", a different complication of tick bite, and watched in horror with the medical staff as the area around the bite continued to swell to the size of a football.

"I felt dizzy and it was very painful, and I started to get blurry sight," he said.

It took four nurses to hold Ben down while an intravenous line was put in his vein, "but the food in hospital was good" and the service so good that Ben and Tanya wrote a thank-you letter.

Ben said he had had "heaps" of ticks in the past, on his back and belly button, but he'd never had a reaction like this.

Sunshine Coast Local Medical Association president Dr Wayne Herdy said doctors had treated dozens of people for ticks recently and Gympie medical officer Dr Rod Day said his practice had treated at least two people a week for the last 12 months.

Ticks were "very, very prevalent", he said.

Dr Herdy said the real concern this year was that the little parasites were also much larger than normal.
"Because we've had a warmer winter than usual, we're seeing more ticks around than usual and they're large, juicy healthy ticks too," he said.
"They're not the tiny little ones we normally see at this time of the year.
"Some people are saying that the scrub ticks they're seeing are almost as big as the old cattle ticks.
"They're several millimetres across, sometimes up to half-a-centimetre or so."
Dr Day said most people were able to locate and remove any ticks on their body, but that the reaction from the toxin could persist and annoy them for up to three weeks.
Ticks live in bushland. They can attach to your skin when you're out in the bush and then feed on your blood for several days.

To deter ticks:

- Wear light-coloured clothing when out in the bush. Ticks will be much easier to see. Tuck trousers into socks and shirts into pants. Use an insect repellent containing DEET or picaridin on skin, shoes and socks.
- Children and pets should be examined for ticks after visiting bushland areas.
- When you get home, remove clothing and search carefully for ticks. Search especially behind the ears, the back of the head, neck, groin, armpits and back of the knees.
- If bitten, you may see an itchy crater-like swelling on the skin around the tick. Remove the tick with tweezers by grasping the head and rotating. Do not pull the tick by the body as this may make it release more toxin. Apply an antiseptic cream to the bite.
- Seek medical advice if you experience muscle weakness, paralysis or feel generally unwell after a tick bite.

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- **Rickettsial infections**

Victorian Government:

Rickettsial infections include scrub typhus and Queensland tick typhus (spotted fever).

Notification requirement for rickettsial infections

Notification is not required.

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Primary school and children's services centres exclusion for rickettsial infections

Exclusion is not required.

Infectious agents of rickettsial infections

Rickettsiae are obligate intracellular organisms, and there are numerous species of concern to humans. They are divided into three groups: spotted fever, typhus and scrub typhus. Rickettsiae (and their associated diseases) of particular importance in Australia are *Rickettsia australis* (Queensland tick typhus, spotted fever), *Orientia tsutsugamushi* (scrub typhus), *R. honei* (Flinders Island spotted fever) and *R. typhi* (murine typhus).

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Identification of rickettsial infections

Clinical features

There is great variation in the severity of illness produced by each organism. Infection most commonly begins with formation of a papule at the site of the bite where the infection was introduced. This usually becomes necrotic and forms a typical

black eschar (scab). Four days to 2 weeks after the bite, symptoms begin with fever and malaise, followed by adenitis in the lymph nodes draining the bite site. As the organisms spread throughout the body, fever, malaise and headache increase, and general lymphadenopathy occurs in most cases. About a week after onset, the main features are continuous fever, signs of bronchitis or pneumonia, photophobia, conjunctivitis, generalised adenopathy, delirium, deafness and a maculopapular rash, most commonly over the trunk and proximal limb parts. Splenomegaly occurs in some cases.

Fever may persist for 14 days without antibiotic treatment. The fatality rate in untreated cases is 1–40 per cent. This increases with age and depends on the infection site, the type of *Rickettsia* involved and previous exposure.

Diagnosis

In endemic areas, the clinical picture is sufficiently distinctive for a clinical diagnosis, and empirical treatment should be instigated because of the frequent delay in laboratory diagnosis. Definitive diagnosis can be made by isolation of *Rickettsia* after inoculation of the patient's blood into mice, although this requires a specialised laboratory and is not used routinely. Serological methods are the current mainstay of diagnosis; however, the results need to be interpreted with caution because of cross-

reactivity between strains. A biopsy of the eschar can be used to demonstrate *Rickettsia* by immunofluorescence or polymerase chain reaction (PCR). PCR may also be run on serum specimens.

Incubation period of rickettsial infections

The incubation period is 2–14 days. The variation in incubation period may in part be related to the inoculum size.

Public health significance and occurrence of rickettsial infections

The epidemiology varies in different parts of the world. Disease occurrence is often associated with the modification of natural habitats by humans, such as felling of a forest and its replacement by a secondary growth of scrub.

R. australis occurs along the eastern side of Australia, *R. honei* has been recognised on Flinders Island near Tasmania, and *R. typhi* occurs throughout many states of Australia. Scrub typhus occurs in Queensland, and parts of the Northern Territory and Kimberley region. The public health impact on lives or productivity lost is largely unmeasured, but is suspected to be high.

Reservoir of rickettsial infections

Humans are incidental hosts and are not useful in propagating the organism in nature. Many rats, mice and other small mammals act as reservoirs, as does transovarial transmission

within ticks and mites. An exception is louse-borne typhus (*R. prowazekii*), which does not occur in Australia. Humans are the principal reservoir for louse-borne typhus, and the human body louse (*Pediculus humanus* var. *humanus*) is the vector.

Mode of transmission of rickettsial infections

The disease is not directly transmitted from person to person. Humans are infected by the bite of an infected larval tick, flea, louse or chigger mite.

Period of communicability of rickettsial infections

People are at risk of infection for as long as they remain in infected areas. In the case of louse-borne typhus, a person is infective for lice during the febrile illness and probably 2–3 days after their temperature returns to normal.

Susceptibility and resistance to rickettsial infections

All nonimmune people are susceptible to infection, depending on environmental exposure. Long-lasting immunity probably follows infection.

Control measures for rickettsial infections

Preventive measures

There is no vaccine available. People who enter infected areas can be protected by impregnating their clothing with pesticide and renewing personal insect repellent frequently.

Chemoprophylaxis can be successfully used short term; for this, a

consultation with an infectious diseases specialist is recommended. Campers can also help to prevent tick bites by using camp beds for elevation from the floor and wearing clothing that minimises exposed skin.

Control of case

Treatment is generally oral doxycycline. Consult the current version of *Therapeutic guidelines: antibiotic*.

In severe disease, consultation with an infectious diseases specialist is recommended.

Control of contacts

Consider active case finding if other people were exposed to the same setting as the case, such as a camping holiday or military exercise.

Control of environment

Not applicable. The mites themselves act as reservoirs, so no immediate effect is achieved by rodent control.

Outbreak measures for rickettsial infections

Except in the case of an epidemic of louse-borne typhus, no outbreak measures are necessary.

International measures

In the event of an epidemic of louse-borne typhus in Australia,

the department will notify the World Health Organization and neighbouring countries of this occurrence in an area previously free from the disease.

Further information

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ALZHEIMER'S AUSTRALIA DEMENTIA RESEARCH FOUNDATION

Could a tick bite cause Alzheimer's disease?

- Jul 30, 2014
  Dr Ian McDonald Invasive Animals Cooperative Research Centre (IACRC)
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- Understand, Human Study, Lyme disease, Alzheimer's disease, Tick bite, bacteria
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Over time, there have been reports of a potential link between Lyme disease (transferred via a tick bite) and Alzheimer's

disease.

In 2011, a review by Dr MiKlossy, published in the *Journal of Neuroinflammation*, found that spirochetes (a family of bacteria, some of which cause Lyme disease) were observed in the brain, in more than 90% of Alzheimer's disease cases.

Since infection of spirochetes is treatable with antibiotic and anti-inflammatory treatments, it lead to the question – could treating spirochetes in the brain prevent or even eradicate Alzheimer's disease? In this special article, Dementia News investigates further into the link between these two diseases.

Firstly, let's take this back a step and find out more about Lyme disease

According to the Lyme Disease Association of Australia's website:

"Lyme disease is an infection caused by a bacteria (Borrelia burgdorferi) that infects humans from the bite of ticks, which are infected with the bacteria. Lyme disease is often called "The Great Imitator" because it can imitate symptoms of many other diseases such as, Parkinson's disease, Chronic Fatigue Syndrome, Juvenile Rheumatoid Arthritis, and even Alzheimer's disease."

A fact sheet produced by the NSW government states that:

"In later stages of Lyme disease the infection spreads through the bloodstream and can cause infection in the brain and membranes surrounding the brain (meningoencephalitis) and infection in or around the heart (endocarditis, myocarditis or pericarditis). The disease can also cause inflammation of joints and cause joint pain and long-term neurological involvement."

It is important to note that there is little evidence that Lyme disease actually occurs in Australia.

So, the question remains - does Lyme disease cause Alzheimer's disease?

Professor O'Day and Dr Catalano, authors of a recent article published in the *Journal of Alzheimer's disease* hypothesised that if Lyme disease does in fact cause Alzheimer's disease, then areas with a high prevalence of Lyme disease should also have a high prevalence of Alzheimer's disease.

In their paper, they compared the 13 US states with the highest prevalence of Lyme disease to the 13 US states with the highest prevalence of Alzheimer's disease. They found **no statistical correlation between deaths from Alzheimer's disease and people who had/have Lyme disease**. In fact, they found the complete opposite. Many of the states which had a high prevalence of Alzheimer's disease, had a low prevalence of Lyme disease. To put this into perspective, the top 13 states with Lyme disease were completely different from the top 13 states with Alzheimer's disease. While this is only a small sample of one country, the current available data reveals that there is no evidence to suggest that these two diseases are linked in any way.

The authors of the paper were initially intrigued to discover studies and popular professional opinion supporting a potential link between Lyme disease and Alzheimer's deaths.

"Because of the growing impact Alzheimer's disease will have world-wide, we need to understand the true factors that underlie the disease. We need to quickly rule out concerns, like Lyme disease, that unnecessarily cause widespread fear and interfere with attempts to fully understand the causes of Alzheimer's disease," said Professor O'Day.

So where did the hypothesis 'that the two diseases are linked' come from?

Multiple research papers have suggested that infection with the bacteria *Borrelia burgdorferi* (which causes Lyme disease) can also induce production of amyloid beta and Tau proteins (associated with Alzheimer's disease), however, this has only been proven in tissue cell cultures and not specifically seen in a human brain. In 2009, a study published in *Journal Advances in Psychiatric Treatment*, titled 'Rare and unusual dementias', suggested that the major reason that Lyme disease is sometimes thought to be associated with Alzheimer's disease, is that cognitive impairment, inattention and even delirium are common symptoms (if left untreated). It brings back the point that Lyme disease imitates many of the symptoms of Alzheimer's disease,

but the two diseases are very much separate.
In summary, the most important fact to remember is that Lyme disease is treatable and can be cured, while Alzheimer's disease currently has no cure and only a handful of treatments available which can alleviate some of the symptoms.

Sources:

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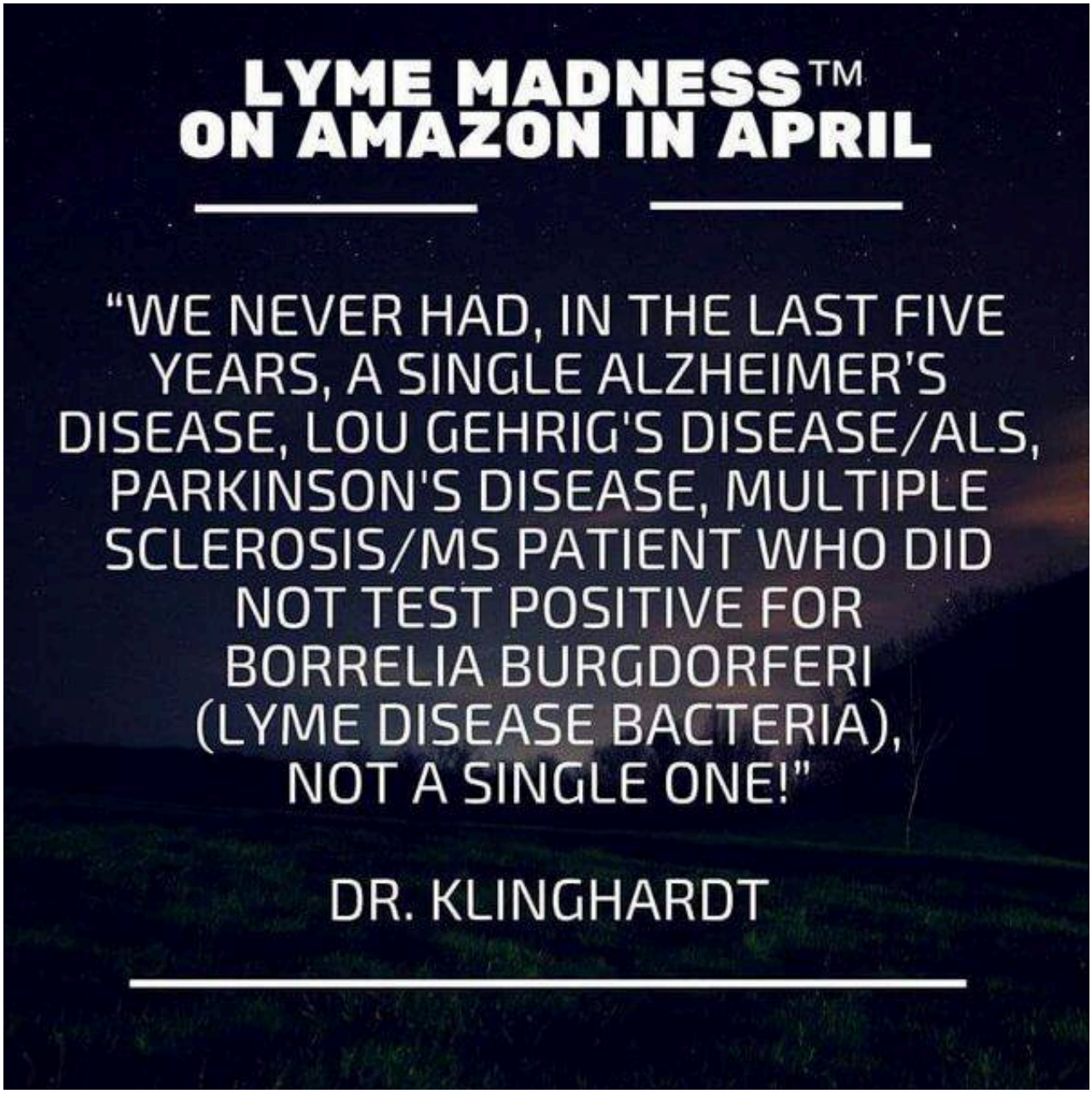

Lyme disease: A Look Beyond Antibiotics

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In the last decade the majority of outcome-oriented physicians observed a major shift: we realized that it was neither the lack of vitamins or growth

hormone that made our patients ill. We discovered that toxicity and chronic infections were most often at the core of the client's suffering. We watched the discussion, which infection may be the primary one: mycoplasma, stealth viruses, HHV-6, trichomonas, Chlamydia pneumoniae, leptospirosis, mutated strep, or what else?

The new kid on the block is Borrelia burgdorferi (Bb) and some of us have looked at it for a long time as possibly being the bug that opens the door for all the other infections to enter the system. Lyme disease has become a buzzword in the alternative medical field. Since none of the recommended treatments are specific to either one of the microbes, we can never assume that we really know what we treated once a patient has recovered.

Microbiologist Gitte Jensen, PhD had shown, that the older we get, the more foreign DNA is attached to our own DNA. Somewhere along the line pathogenic microbes invade the host's DNA and become a permanent part of it. Since we use only 2% of our DNA, it may not be a problem. In fact, it may make us who we finally become. It may also cause a number of symptoms and chronic illness. Genius Guenther Enderlein's discoveries take us off the hook: if one microbe can change into another given the right environment, why bother to find out, who we are infected with? The book "Lab 257" suggests that Bb is an escaped man-made US military bio-warfare organism (just like myoplasma incognitus and HHV 6).

Other authors suggest that different subtypes of Borrelia, which cause illnessin humans, such as B. afzelii and B.garinii have probably existed longer then B.burgdorferi and occur naturally (1, 2) and have been with us for a long time, maybe centuries or much longer then that.

Neurologist Prof. J. Faust MD, PhD of the Albert-Ludwig University in Freiburg, Germany (3) related many neurological and psychiatric illnesses to spirochete infections as early as the 1960s. He was so skilled in his clinical knowledge that he could – based on clinical neurological symptoms –accurately predict which valley in the Black Forest the infected patient was from! This clearly was a time before Bb - showing that non-syphilis spirochete infections were around earlier then the famous Bb outbreak in Connecticut in the mid seventies. It also makes a strong statement to the fact how easily these creatures may mutate and adapt to local conditions. It may however validate the findings published in "Lab 257": Tuebingen, the place where German/US warfare spirochete expert Traub was continuing his

spirochete experiments in the early 50s, is situated in the Black Forest also. Were these spirochetes genuine or have they escaped from a university laboratory?

Making the diagnosis

It appears that many patients with MS, ALS, Parkinson's disease, autism, joint arthritis, chronic fatigue, sarcoidosis and even cancer are infected with Borrelia burgdorferi. But is the infection causing the illness or is it an opportunistic infection simply occurring in people weakened by other illnesses.

My experience is based on:

a) using direct microscopic proof of the presence of Borrelia burgdoferi (Bb) and other spirochetes (4, 5)

b) the information many affected clients have brought to me

c) my own clinical training and experience (30 years in Medical practice, 15 years Bb cognizant)

d) ART testing (autonomic response testing), which is the most advanced and scientifically validated method of muscle testing (6)

e) regular lab parameters affected by Lyme:

- Abnormal lipid profile (moderate cholesterol elevation with significant LDL elevation)
- insulin resistance
- borderline low wbc, normal SED rate and CRP
- normal thyroid hormone tests but positive Barnes test and excellent response to giving T3
- type 2 (high cortisol, low DHEA) or type 3 adrenal failure (low cortisol and DHEA)
- low testosterone and DHEA
- decreased urine concentration (low specific gravity)
- complex changes in cytokines, interferones, NK cells, white blood cell indicators, etc.

Bb tends to infect the B-lymphocytes and other components of the immune system which are responsible for creating the antibodies, which are then measured by an ELISA test or Western Blot test. Since antibody production is greatly compromised in infected individuals, it makes no sense to use these tests as the gold standard or benchmark for the presence of Bb (7). We also are aware that in endemic areas in the US up to 22% of stinging flies and mosquitoes (2, 8, 9, and 10) are carriers of Bb and co-infections. In South East Germany and Eastern Europe 12 % of mosquitoes have been shown to be infected. Also many spiders, flees, lice and other stinging insects carry spirochetes and co-infections.

Making the history of a tick bite a condition for a physician to be willing to even consider the possibility of a Bb infection seems cynical and cruel.

To use conventional diagnostic tests such as the Western Blot, one has to think in paradoxes: the patient has to be treated with an effective treatment modality first before the patient recovers enough to produce the antibodies,

which then are looked for in the test. A positive Western Blot proves that the treatment given worked to some degree. *A negative Western Blot does not and cannot prove the absence of the infection.*

Having taken another route altogether, we have recognized that today many if not most Americans are carriers of the infection. Most infected people are symptomatic, but the severity and type of the symptoms varies greatly. The microbes often invade tissues that had been injured: your chronic neck pain or sciatica really may be a Bb infection. The same may be true for your chronic TMJ problem, your adrenal fatigue, your thyroid dysfunction, your GERD and many other seemingly unrelated symptoms. Many Bb symptoms are mistaken for problems of natural or premature aging.

In most places the diagnosis of an active Bb infection is made only if the symptoms are severe, persistent, obvious, and many non-specific and fruitless avenues of treatment have been exhausted. Acute new “typical” cases of Bb infection are rare in my practice. Symptoms tend to get stranger and more obscure every year.

Frequently, if the patient is fortunate enough to see a practitioner who is “Lyme cognizant”, the diagnosis of a supposedly fresh case of symptomatic Lyme disease is made when a significant tissue toxin level has been reached (threshold phenomenon) or when a new co-infection has occurred recently. The symptoms can mimic any other existing medical, psychological or psychiatric condition. The list of significant co-infections is limited: roundworms, tapeworms, threadworms, toxoplasmosis, giardia and amoebas, clostridia, the herpes virus family, parvovirus B 19, active measles (in the small intestine), leptospirosis, chronic strep infections and their mutations, Babesia, Brucella, Ehrlichiosis, Bartonella, mycoplasma, Rickettsia, Bartonella and a few others. Molds and fungi are always part of the picture. The pattern of co-infections and the other preexisting conditions such as mercury toxicity determine the symptom-picture but not the severity.

The severity of symptoms correlates most closely with the overall summation or body burden of coexisting conditions and with the genetically determined ability to excrete neurotoxins. The genes coding for the glutathione S-transferase and for the different alleles of apolipoprotein E (E2, E3 and E4) play a major role. E2 can carry twice as much sulfhydrylaffinitive toxins (such as mercury and lead) out of the cell as the E3 subtype, E4 carries out none. Trouble in the methylation, acetylation and sulfation pathways is also common. Other factors, such as diet and food allergies, past toxic and electromagnetic exposures, emotional factors and unhealed ancestral trauma, scar interference fields and occlusal jaw and bite problems are also important (6). The severity of symptoms is not related to the number of spirochetes in the system but rather to the individual’s immune responses.

Taken all of the above into account, we do not distinguish between people who have the Bb infection and those who don't. We distinguish between people who have Lyme disease and those who don't.
a) patients who are infected with any type of Borrelia and are symptomatic have "Lyme" disease
b) healthy people who are not symptomatic often already have a spirochete infection as well. They may or may not be disasters waiting to happen. But they do not (yet) have Lyme "disease".
Most often several of the "co-infections" are already present prior to the infection with Bb or other spirochetes.
In treatment we focus on exploring the difference between symptomatic and asymptomatic carriers. We treat what the symptomatic person is missing (such as enough magnesium in the diet) or has extra (such as mercury) compared to the asymptomatic one.
The group suffering most is newborn babies and young children, who rarely are diagnosed correctly and therefore are not treated appropriately. They often carry the labels ADHD, autistic spectrum disorder (ASD), seizure disorder and others. Detoxifying these kids with transdermal DMPS and treating the chronic infections is often curative.

The 3 Components of Lyme disease

Lyme disease has three components, which should be recognized and addressed with treatment:
Component #1: The presence of spirochete **infection and co-infections**
The co-infections are bacterial, viral, fungal and parasitic. Since the spirochetes paralyze multiple aspects of the immune system, the organism is without defenses against many microbes. Many - if not most - of the coinfections are really a consequence of the spirochete infection and not truly a simultaneously occurring "co-infection".
For this aspect of treatment we use pulsed electromagnetic fields (KMTmicrobial inhibition frequencies), niacin in high doses (12) herbs, minerals, bee venom (6) and - sometimes - ant parasitic medication and antibiotics.
The KMT microcurrent technology is new and revolutionary (17). The instruments are FDA approved for pain control. Designed by Japanese engineers they use four different - but simultaneously applied - high frequency superimposed biological waveforms. The interference pattern is creating thousands of harmonics which are then manipulated into the specific published microbial inhibition frequencies (against Bb, mycoplasma etc.).
This stealthy microcurrent travels freely through the body reaching every tissue. The instrument measures the skin conductance over a 100 times/second adjusting the amperage constantly (so that the body never

creates habituation/resistance against it). The microbes are inhibited in their metabolic and sexual activity and gradually die out or disappear from the body
The instrument looks not much different then a TENS unit and is applied via four electrodes to the skin or used by translating the electric field into a vector force field using signal enhancer technology. The KMT frequencies are designed to not only interfere with the reproductive mechanism of the microbes and parasites, but also to awaken the immune system, entrain the white cells to recognize the invaders and at the same time help to absorb and shuttle the effective medication to the body compartment, where the infection actually is. Otherwise, most treatment substances given never reach the target in sufficient concentration.
Component #2: the illness producing effect of microbial exo- and endotoxins and toxins produced by the host in response to microbial trigger
Most of these are neurotoxins, some appear to be carcinogenic as well, others block the T3 receptor on the cell wall, etc. Decreased hormonal output of the gonads and adrenals is a commonly observed toxin mediated problem in Lyme patients. Central inhibition of the pineal gland, hypothalamus and pituitary gland is almost always an issue that has to be resolved somewhat independently from treating the infection. Furthermore, biotoxins from the infectious agents have a synergistic effect with heavy metals, xenobiotics and thioethers from cavitations and NICO lesions in the jaw and from root filled teeth. My published neurotoxin elimination protocol can be downloaded for free (6).
We use toxin binding agents such as fiber rich ground up raw vegetables, chlorella (14), cholestyramine (13), beta-Sitosterol, propolis powder, apple pectin and Mucuna bean powder (14). A solid heavy metal detoxification program should be used simultaneously with the first phases of the Lyme treatment. Safe toxic metal elimination is an art unto itself. However, the information is widely available now (15).
The more difficult objective is to choose agents and methods to trigger the release of neurotoxins from their respective binding sites. Only then can they be transported to the liver, processed and enter the small intestine from where they can be carried out by the binding agents.
The toxins occupying the T3 receptor are competitively displaced by oral T3 - cycled with the Wilson protocol (*available at most compounding pharmacies*). The toxins blocking the cortisol receptor are mobilized with the herb forskolin. CGF chlorella - a sophisticated mix of chlorella and chlorella growth factor (14) - and cilantro given together with a nonirradiated Mucuna bean powder mobilize most everything else. I also use alternate day dosing of an energetically enhanced

phospholipid/EDTA/Alpha-Lipoic acid mix ("PhosphoLipid Exchange") which is currently the most tolerated and effective form of phospholipids for the Lyme patient (14).
The KMT microcurrent frequencies dramatically increase the speed of toxin mobilization and access body compartments the biochemical compounds cannot (17). Psychotherapeutic intervention (15) to uncover and treat old trauma is most profoundly effective in triggering a neurotoxin release when none of the other methods appear to work anymore. After each APN session we pre-medicate the patient with CGF-chlorella. Sometimes the extraction of a devitalized tooth or the injection of one of the facial/cervical ganglia with glutathione or another detox agent can trigger a major neurotoxin release (16). Lymph drainage in combination with colon hydrotherapy accesses toxins stored in the lymphatic body-compartment. German practitioners have pioneered the combination of oral cilantro and the "Toxaway" microcurrent footbath.
Component #3: The **immune reactions** provoked by the presence of both toxins and microbes (there are three sub-possibilities, which have to be recognized and addressed)
The immune reactions are largely depending on host factors, such as genetics, prior illnesses, mental-emotional baggage, early childhood traumatization, current exposure to electromagnetic fields (sleeping location, use of cell phones, poor wiring in car or home, etc), food allergies and diet, socio-economic background, marital stress etc. A multitude of biochemical serum markers is used today to determine the status of the infection (see below). A subset of NK killer cells, CD 57+ is emerging as a valid marker for activity of the illness (lower counts indicate worsening).
1: Anergy - the absence of reaction due to the successful evasion of the host-defenses. One of the more known mechanisms the microbes use to create anergy is hyper coagulation. The microbes tend to live in the endothelium, where the food is most abundant. They trigger the host's coagulation mechanism to lay down a layer of fibrin on top of them to evade recognition by the immune system, etc. For this aspect we use three techniques:
a) the KMT-microcurrent technology and homeopathics to wake up and entrain the immune system
b) Rechtsregulat ("right rotatory fluid") which is an enzyme rich extract of fermented fruits and vegetables (14). It has outperformed the s.c. injection of heparin in our own trials and frequently leads to rapid subjedtive improvement. Lumbrokinase is far more effective then Nattokinase. Both appear weak when compared to Rechtsregulat. We also work on recognizing and eliminating those factors that block the

client's system (geopathic stress, EM stress, food allergies, emotional factors, interference fields such as scars and disturbed ganglia and we substitute vitamins and minerals based on ART testing).
c) the Enderlein remedies (especially the haptens) from Pleomorphic-Sanum
2: Allergy - appropriate or exaggerated immune reactions (both cellular TH1-reaction and TH2-cytokine activation). In Lyme disease often (not always)TH-1 is overly active early in the illness and can easily be downregulated by fluconazole, later TH2 becomes overly active. Nothing works better then the APN-desensitization procedure (15): while the patient is exposed to the allergen (we use a glass-carrier fixated culture of the offending microbes) the ANS is kept in a state of equilibrium, using tapping of acupuncture-points, hypnotherapeutic trauma-recall and intervention techniques and our proprietary psycho kinesiology (musclebiofeedback psychotherapy).
A very effective and yet simple technique to re-regulate TH1 and TH2 back is auto-urine therapy. The patient's urine concentrates the antigens (disposed cell walls and cell fragments of offending microbes which the immune system has successfully eliminated). By passing the client's urine through a micro pore filter and injecting it i.m., the lymphocytes on patrol in the connective tissue are brought in contact with the antigen and quickly mount a specific and appropriate immune response. We use 2 ml of filtered urine once weekly for 12 weeks. All other similar approaches (autohemotherapy, homeopathic autonosodes, manipulating the immune system with supplements) are far less effective.
3: Autoimmunity – the toxins and microbes often act as haptens – marking the cell, cell wall or tissue in which they are hiding as foreign and therefore for destruction . This happens especially against a back drop of pre existing heavy metal toxicity, which has to be addressed aggressively and prior to treating the microbes themselves. We use the MELISA test (memory lymphocyte immune-stimulation assay) to establish which metals the patient is reactive to. The same lab in Bremen, Germany also offers the most sensitive Bb test.
The KMT microcurrent technology is very effective in recognition entrainment, helping the immune cells to mount a specific and targeted attack on the invaders, sparing the body's own tissues. It breaks through one of the prime mechanisms the offending germs are using: molecular mimicry (the pathogens present antigens on their surface that are indistinguishable from a normal body tissue).
The technique also breaks another trick the spirochetes have developed: the molecular interaction that occurs between a specific Lyme virulence

factor (OspE) and a host protein fH (factor H). Some surface antigens in the spirochete are identical to myelin. This explains why anti-myelin antibodies are often present.

The novice in the field tends to treat component #1 only. We have only rarely observed lasting improvement when course after course of antibiotics was given. Because of the defense mechanisms inherent in the Bb and coinfections, current wisdom suggests that 18 months of antibiotics would be curative in many cases (25). We have observed severe, lasting and unacceptable side effects from this approach (such as tinnitus, kidney failure, intractable immune system breakdown and others).

By using the synergistic effect between treatment-modalities which simultaneously address the three issues outlined above, lasting improvements are the norm rather than the exception. By using the synergy principle and abandoning the arrogant idea of being able to eradicate all of the microbes in the system “for good”, chronic Lyme patients can often live a normal healthy life again. The use of herbs alone or in combination with antibiotics has emerged as the most important core strategy.

The Mineral Issue

To feed, fuel and perk up the cells of the immune system (especially NK cells and macrophages) numerous interventions have been attempted, mostly based on orthomolecular and herbal medicine principles. We found that amongst those approaches, abundant mineral substitution based on the red cell mineral analysis is most rewarding. Rarely should medical drugs be used.

Amazingly, the most depleted minerals in our Lyme patients are often copper, magnesium, manganese (in Lyme) and iron (in Babesiosis). Bb and Bartonella need magnesium to duplicate and deplete the host’s body rapidly. Copper and iron have all but disappeared from most of our supplements based on faulty interpretation of hair analysis. The immune system uses those two metals in the process of phagocytosis. They are the main constituent of the enzymes (or “bullets”) the immune cells use in the battle against the invaders.

Oxidized used-up iron and copper get displaced into the extracellular compartment and body fluids and appears in the hair and skin, as the body’s most efficient way of excreting toxins without hurting the kidneys. This has led to the dangerous and in its consequence catastrophic assumption, that these metals are the enemy and need to be restricted. It is true, that oxidized metals pose a danger and have to be reduced (=substitution of electrons) or eliminated. However, when copper and iron are needed and substituted appropriately, major improvements have been observed. Appropriate antioxidant treatment can reduce these metals. Homeopathic copper and iron

will lead to beneficial redistribution of these metals and makes them bioavailable again.

Lithium-orotate or aspartate in low doses (15 mg/day) has been shown to protect CNS structures from neurotoxin damage. Patients almost always benefit clinically from frequent treatment with parenteral magnesium. It is most meaningfully given in a modified Meyer's cocktail. We also use a 5:2 ratio of folic acid (not folinic) and hydroxycobolamine (not methyl- or cyano-) sublingually several times/day. In addition methyl-cobolamine is given i.m. twice weekly and is important in the methylation/restoration of reduced glutathione. Hydroxy-B12 protects the brain from nitric oxide induced damage.

Many Lyme patients suffer from Pyrroluria, a metabolic illness where abnormal porphyrins carry out significant amounts of needed zinc and vitamin B6. Diagnosis is made with the appropriate test at the Pfeiffer institute in Chicago. Even though it is assumed that this illness is hereditary I have my doubts, since most Lyme sufferers have a degree of it. I suspect that the appearance of kryptopyrroles in the urine is induced by the illness. However, I am careful with excessive substitution of zinc. Zinc has a synergistic effect with mercury in the brain and also promotes the growth of the herpes viruses.

If clients show abnormal high losses of sex steroid hormones in the urine, the patient may be cobalt deficient. The urine hormone test and cobalt drops are available at the *Tahoma Clinic* Renton, WA. For a while selenium should be given in high doses to suppress viral replication and render bioavailable mercury non-reactive.

The element most critical in the Lyme patient however is iodine. A two inch square of Lugol's iodine is painted on the patient's skin and should remain visible for 24 hours. The sooner it is absorbed the more deficient the patient. An oral form of Lugol's is available under the name *Iodoral (Optimox, Torrance, Ca).*

Filling up the body's mineral reserves has always been the most essential part of our heavy metal detox program. It is also the most essential part of our Lyme treatment.

Sequencing

There is an inherent order in which the microbes should be treated. If the order is correct, gentle methods work. Treatment should always combine electromagnetic interventions, using specific microbial inhibition frequencies (KMT technology) with the appropriate herb, antibiotic or other antimicrobial strategy. It should also always be combined with a toxin elimination program, good psychotherapy and general life style hygiene.

The Lyme ABC

A. We start with **deworming** our clients. We often use a simple yet aggressive seasalt/Vit C protocol (19) which has an independent effect against the spirochetes also. The high salt concentration kills large parasites by osmotically induced dehydration (osmotic shock). High salt levels also increase the enzyme elastase which has a strong antimicrobial/antispirochete effect (4).
Protocol: 1.5 grams of sea salt per 20 lbs of body weight in 4 divided doses per day for 3 weeks. With each dose also give 1-4 gms of Vit C (dose has to be just below bowel tolerance). Three 3-6-week cycles with a 2 week break in-between. The blood pressure should be monitored and not elevate outside acceptable levels. Five percent of the population are salt sensitive and react with a significantly increased blood pressure. In the off weeks we give 1/2 tsp of sea salt in a glass of water first thing in the morning.
Sometimes we enhance the program by using the “Arise-and-Shine” herbal program. Often I will add in a course of Albendazole (same family as metronidazole), Biltricide or ALinia in high doses and parasitic CDs for entrainment of the immune system. The frequencies of the CDs were developed by German physicists by taping the sounds of microbes in their respective live activity in an underground lab which was soundproof and electromagnetically completely shielded (6).
B. The next step is the treatment of **giardia, entamoeba histolytica and trichomonas**, which most often are overlooked. Lab detection of large parasites in most US labs is hopeless. Amoeba and giardia trophozoites can only be detected in a fresh stool for about 20 minutes. None of the labs available to us comply with this necessity. The detection rate is so substandard that only ART testing, a therapeutic trial or abdominal palpation by an experienced practitioner is capable of establishing the diagnosis.
Protocol: organic freeze dried garlic (14) treats all of the above astoundingly successfully. Sometimes we add Tinidazole 500 mg bid for 10 days always followed by long term garlic therapy (three caps tid after meals).
C. Next we attend to the chronic **strep infections**, which often coexist with the herpes viruses. No other treatment has been as successful as Pleo Not (penicillum notatum) from Pleomorphic-Sanum followed by a six month course of Pleo Sancom (antidotes for aspergillus niger and mucor racemosus).
We always look at the tonsils: if they are scarred with crypts, or lymph tissue has regrown since the tonsillectomy (“tonsillar tags”), surgical intervention is needed. Otherwise these patients (which are most of them) never get well. We recommend a procedure developed by Dr. Sergej Dorochov, MD, PhD called “regenerative cryotherapy” (20). It involves freezing the surfaces of all lymphatic tissue of the head/neck region which creates a barrage of

growth factor and cytokine responses, which often lead to dramatic improvements in our Lyme patients.
Lymph drainage using the KMT technology has been superb in speeding the healing of the sinus/head/neck/region.
D. The next step is the treatment of **Babesia**. There are now at least 17 subtypes of this intracellular Malaria-like organism. Eye, brain and dental symptoms are most often caused by this mean microbe.
Protocol: Frequency #2 in the KMT 23 TENS unit inhibits the metabolic activity of Babesia and is used 3 times weekly.
I also use PC-Noni extract and Artemisinin: see the protocol below. Watch iron levels! Artemisinin provokes the intestinal wall to secrete an enzyme which destroys the medication before it can be absorbed. This process builds up over 3 weeks. After a one week pause the enzyme has disappeared and takes another 3 weeks to reemerge. Grapefruit juice prevents formation of this enzyme.
Alternatives are the Swiss Malaria drug Riamet (1 course) which is very well tolerated but only seems to work short term, and Mepron, which is forbiddingly expensive
E. The next step is to start the client on a systemic **antiviral treatment**. I use the ayurvedic herb cocktail - Indian Gooseberry, Chebulic and Beleric myrobalan (14), which has given the most profound and lasting effect on the viruses of the herpes family, which flourish in the immune suppressed Lyme patient. I also use liquid olive leaf, which shows some effect. The Japanese mushroom extracts have also been helpful. I also like the North American product “Pro Boost” (thymus extract) to help awaken the cellular immune system. As long as clients use bee venom therapy, the symptoms of herpes viral infections (seizures, brain fog, emotional ups and downs, chronic pain sysndromes especially “discogenic” back pain and sciatica, fatigue) disappear.
Virox and other chaparral- derivatives have been disappointing. The insomnia of Lyme disease is often herpes viral in nature (EBV, VZ or HSV 1, HSV II). As a diagnostic trial I often use 1000 mg of the medical antiviral drug Valtrex at bedtime. If there is a dramatic improvement, herbal antiviral treatment has to be considered for a long time.
We have designed an antiviral program for the KMT instruments (frequency #4) and an anti viral CD, which is played through a walk man or regular sound system at low volume 3 times/week. This has been extremely effective. Zinc fosters the growth of HSV I and II, copper and selenium inhibit it.
F. I simultaneously address the **fungal/yeast** component which is most often present, especially if clients had prior antibiotic treatment. Fungi and viruses

seem to support each other in yet unknown ways. I use both the antifungal CD and the KMT TENS-frequencies in program #4 which contains all known anti-yeast and anti-mold frequencies (6).
With ART technology we could show that the most successful and well tolerated antifungal is either fluconazole (100 mg twice daily) which also has an anti Bb effect and seems to downregulate TH-1, the drug amphotericin B (250 mg bid) or the combination of organic freeze dried garlic (14) and oil of oregano. Substitution with microbes is important. We use “Matrix Microbes” (14) which contains over 80 lesser known beneficial microbes. Every patient is also on a more traditional acidophilus/bifidus/FOS product.
Eating a low carbohydrate diet is often a must. We monitor the fasting insulin level. If it is low, we are ok. If it is high, we restrict the carbohydrates. Do not restrict the carbohydrates if it is not necessary. We have seen dangerous mistakes in this field. In general, bacterial infections benefit from the acidic environment created by a high protein diet. Molds and fungi benefit from a high carb environment. Metabolic typing is a safeguard, but time consuming to do at home, especially if you are very ill. I use the “diet therapy software” (21) for a rapid and profound diet evaluation and recommendation. Most successful is the ART food sensitivity test for every single item in the client’s diet (6). It may take 15 minutes, is more sensitive then the ELISA, MELISA and other lab tests - and it does not incur lab fees (6). The rotation diet by Sally Rockwell prevents relapses.
G. Mycoplasma responds well to enzymes, when it is treated in sequence with the other microbes as outlined here. The most effective strategy is the German product Rechtsregulat (14). This simple drink has been extremely effective in eradicating mycoplasma and other cell wall deficient microbes. It also has a heparin like anti-fibrin effect that surpasses injected heparin by far. It has just like heparin, a strong biological effect against Babesia as well. Dosage: 1 tbs/2 times per day in a glass of water. The KMT program #4 is designed for treatment of mycoplasma (6).
H. The spirochetes and their close relatives (**Bartonella, Babesia**, **Rickettsia, Ehrlichiosis, and Brucella abortis**) are best treated last - with antimicrobial herbs or antibiotics. Many herbs have enormous potential in the treatment of chronic Lyme disease (see below).
Frequency #1 in the KMT TENS unit inhibits the microbial growth of spirochetes and Bartonella, # 2 is a series of anti-Babesia frequencies. This modulated microcurrent simultaneously activates specific immune responses and aids the uptake of antimicrobial herbs.
Injected bee venom has long been my favorite during this phase of the treatment (22, 23). The peptide mellitin has strong antibiotic activity against

all spirochetes (24). Bee venom also contains nerve growth factor, the very substance needed for healing, when everything else has been attended to.
For the psychiatric presentations of Lyme disease I use large doses of Niacin. (Niacinamide and no-flush Niacin do not work.) 3-6 gms in 3-4 divided doses often show amazing results. It appears that Niacin has tremendous antibiotic potential against all types of Borrelia (12). I suspect that our mentor and genius in orthomolecular psychiatry, Abraham Hoffer, MD discovered a treatment for Bb long before Lyme-disease was known.
The current antibiotic protocols are discussed and listed elsewhere (10).
Often patients develop sarcoidosis, which is rarely recognized (11). The Lyme infected lymph nodes produce abnormal amounts of 1.25 di-hydroxy vitamin D. The client often develops marked osteoporosis (most often in the spine) along with other more typical Lyme symptoms. The blood test (1.25 di-OH vit D) will usually reveal the pathology (levels over 45), necessitating therapy with the Trevor Marshall protocol (18). It uses antibiotics together with the angiotensin II receptor blocker olmesartan –medoxomil. By adding the KMT lymph drainage technology twice/week results are often rapid and miraculous. We hope to find alternatives to the antibiotic regimen in the near future.
When the sequence outlined here is observed, few people have severe Herxheimer reactions, which are the rule in other approaches.

Outlook

Most clients will need some support for several years, before they have found and adapted to a new life style in which the symptoms are absent. Lyme disease is marked by cyclic rhythms and unexpected returns of the symptom from time to time. Once a patient has figured out what works for him or her best, most of my patients learn how to manage the illness with very little help - on their own, living normal healthy lives worth living.
In the course of conquering the illness there has been a lot of personal growth and a lot of learning. Many treatment modalities have been surprisingly ineffective: ozone, hyperbaric oxygen, ICHT (intracellular hyperthermia). Some treatments have been unexpectedly effective: dental splints, color therapy, Tomatis therapy and neuro sensory stimulation, elevating the body temperature with T3 supplementation, regular bee venom injections, tonsillectomies and cryotherapy and many others.
After 15 years of dealing consciously with this illness, Lyme disease is still a mystery to me. Currently its impact outweighs other important issues like heavy metal toxicity, unresolved psychological issues and nutritional deficiencies.
There has been much speculation, why Lyme disease seems to be increasingly common. The book “Lab 257”is an investigative report on the

issues involved. The insects which are the vectors for these microbes thrive in warmer climates. I have no doubt, that to a large degree the greenhouse effect is responsible and will be confronting us with the onslaught of more and more aggressive microbes. The partial pressure of oxygen on the earth at sea level has decreased from 30% 150 years ago to 19% today. The oxygen producing algae in the oceans are dying.

The response of the public health system so far has been denial and anger towards those who try to uncover the puzzle and help the afflicted patients. This will certainly change in the near future. I expect that by the time the institutions discover Lyme disease as a far more important factor in chronic illness then is currently acknowledged, we will be confronted with new, far more dangerous microbes. Antibiotics have disappointed in the treatment of Lyme disease as a single modality. Antibiotics alone will not help us to cope with the coming plagues.

All of us as practitioners have to start looking beyond antibiotics for help and for hope. The microbes have always been with us. They are not the enemy. It is us who have altered the environment so severely and in a way which facilitates the growth of lower evolved species like cell wall deficient microbes and viruses - and ends the life for many more evolved species. Extinction may be forever.

Lyme disease is a messenger. If we don't change, we may be on the endangered species list someday not too far from now.

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Brief introduction to the cast of characters:

Borrelia:

One of eight genera of spirochetes. Hundreds of species in these eight genera. “Borrelia” is the genus, “Burgdorferi” the species. Other famous spirochets: treponema pallidum (syphilis), leptospira (leptopspirosos from animal feces contaminated drinking water, common in Maui, New Mexico, etc). Bb sensu lato includes B. Afzelii, B. garinii, B.lonstari, B.andersonii and many others. Bb sensu stricto refers only to Bb, but includes many species that cause identical symptoms. In Europe, five strains of Bb sensu lato, in Japan 61 strains.

Also be aware that microbes constantly exchange via plamids DNA with each other and we found Bb microbes with properties usually only found in Babesia or mycoplasma, etc. There are no fixed boundaries between many of these microbes.

6 major sites of infection:

1. Large joints (Bb sensu stricto) and connective tissue: onset 4.3 months after insect bite, often self limited (4 years). Flare ups during Herxheimer reactions very common.

Bb has recently been found by us as one of the causes of spinal osteoporosis, disc degeneration and many other “orthopedic” problems.

2. Skin and connective tissue (B. afzelii):

_ acrodermatitis chronica atrophicans
_ general collagen breakdown (premature aging)
_ collagen diseases
3. CNS (B.garinii), PHS and ANS: after insect bite it only takes a few hours before spirochetes are found in CNS even though it takes on average 2 years before symptoms are established. Most common symptom: brain fog and short term memory loss. Later stages demyelination. Severe early changes in SPECT scan (functional), MRI changes much later (physical)
CNS problems:
o Physical: epileptic seizures, insomnia, tremor, ataxia, movement disorders (torticollis, etc.)
o Emotional: irritability (key symptom in children), depression, biphasic behavior (manic
depression), bouts of anger, listlessness;
o Mental: confusion, **difficulty thinking**, poor short term **memory**, increasingly messy household and desk, difficulty finding the right word, feeling of information overload;
o Mixed pictures:
_ can resemble or imitate any known psychiatric illness.
_ Chronic Fatigue (more severe in the early afternoon);
_ Lack of endurance;
_ Non-healing infections in the jaw bone, devitalized teeth, dental pain;
_ Fibromyalgia;
_ Multiple Chemical Sensitivity;
_ loss of zest for life,
_ sensitivity to electric appliances.
Peripheral Nervous System problems:
_ Paraesthesia
_ Burning
_ vibration
_ numbness
_ shooting pains
Cranial Nerve Problems:
_ Facial nerve: Bell's palsy (60 % are caused by Lyme disease, 30 % by one of six common viruses from the herpes family, such as EBV, Herpes simplex type I, type II, type 6 etc);
_ Trigeminal nerve: sense of vibration in the face, TMJ and facial pain, headache, tension and cramps in the face/skull/jaw;
_ Ears (VII, VIII): tinnitus, vertigo, and hypersensitivity to noise;
_ Eyes (II, III, IV, VI): decreasing and changing eye sight (fluctuates

during the day), light sensitivity, floaters;
_ Vagus (X), Glossopharyngeal nerve (IX) and Hypoglossus (XII): difficulty swallowing, faulty swallowing, reflux, hiatus hernia, heart palpitations, supraventricular arrythmias.
.
4. Heart: Lyme carditis is difficult to diagnose with current methods (PET scan positive early on) and has multiple symptoms from arrhythmia to angina. Has to be taken serious with first symptoms.
5. Kidney/bladder: the highest concentration of tissue spirochetes has been found in kidney and bladder. Symptoms often include:
o interstitial cystitis
o prostatitis (Babesia often also involved)
o sexual dysfunction
o loss of libido
o pelvic pain
o menstrual disorders
o filtration problems in the kidney (low specific weight of urine)
o urethritis after intercourse (the spirochetes are attracted during intercourse to the urethra and cause acute inflammation).
6. Immune system infection (white blood cells, thymus, brain, lymphnodes, adrenals, etc)
o Non-healing infections in the jaw bone (also Babesia, Bartonella)
o devitalized teeth
o dental pain;
o Immune system failure: with all known secondary illnesses such as herpes virus infection, intestinal parasites, malaise, : hair loss
Babesia:
intra-cellular Malaria like protozoal organism. Infects red-cells. 2/3rds of Lyme clients also have Babesia, which is hard to diagnose: over 17 antigendifferent subspecies. Most common: B.microti, WA-1 strain in Western States and B. divergens and others in Europe
Diagnosis: best is long term observation of blood under darkfield microscope. Babesia tends to leave dying cells while under darkfield observation.
_ Vertigo
_ headache fatigue
_ dental problems: accelerated tooth decay and cavitation formation
_ TMJ problems
_ eye problems (floaters and blurry vision)
_ weight loss and abdominal problems (GERD)
_ fibromyalgia,

_ shortness of breath
_ malaise
_ drenching night sweats and fever/chills during Herxheimer reactions.
Therapy: think Mepron or Noni, Artemisinin and Oxo

Bartonella

B.henselae is the most commonly found intra-cellular co-infection today found in rbc's, endothelial cells, bone marrow and macrophages. 70% of the cats in Italy are infected with it (cat scratch disease), cat–to-human transfer is common. B.quintana brough down Napoleon's troups in Russia, the true cause for his defeat. The microbes are found today in his troup's teeth in the mass graves.
Other types are found on a regular basis.
Symptoms:
_ swollen lymph glands
_ endocardits
_ hepatitis
_ neovacularization
_ fatigue
_ low grade fever
_ jaw bone cavitations
_ devitalized teeth
_ often co-infection in ALS
_ fibromyalgia and joint pain
Therapy: think Zithro, Doxy and Rifampin together or: Polygonum and Stephania root

Ehrlichiosis

Human granulocytic Ehrlichiosis (HGE) is caused by Anaplasma phagocytophila. Human monocytic Ehrlichiosis (HME) is caused by Ehrlichia chaffeensis. Often found in clients that have contact with horses and farm animals.
Symptoms:
_ Fever (only after initial infection)
_ Myalgia and arthralgia
_ Headcache
_ Lycopenia and thrombocytopenia
_ Hyponatremia
_ Mental confusion
_ Skin rashes, genital and oral ulcers
_ Severe pain syndromes
_ Nausea and vomiting (acute flare-ups)
Therapy: think astragalus (elevates interferon gamma) and colchicine (read

papers by Michael Rask – not to be used during dental surgery or pregnancy)

General Guidelines for the Biological Treatment of Lyme Disease

Bee Venom Therapy

The most influential pioneer of this work was the beekeeper Charles Mraz from Middlebury, Vermont to whom I owe my health and understanding.

The pain relieving effect of bee venom in the treatment of clinical conditions similar to Lyme disease has been established a long time ago. Bee venom contains a number of potent peptides which are responsible for its healing effect ("Bee Venom Therapy for Chronic Pain, Dietrich Klinghardt, J. of Neurol and Orthop. Med and Surg., Vol. 11, Issue 9, Oct 1990, pp. 195-197).

Recent research proved that one of the peptides in bee venom, melittin, has a strong inhibitory effect on the Lyme spirochete at very low doses (“Bee Stings as Lyme Inhibitor” by L. L. Lubke and C. F. Garon, J. Clin. Infect. Diseases, July 1997, 25 Suppl. 1, pp. 48-51). When the spirochete is inhibited it does not multiply and is vulnerable to the host's own immune system and to medication.

The dosage and frequency of treatment is determined by the patient’s clinical response. Patients with Babesia or Mycoplasma infections require higher dosages then those with only B. burgdorferi infections.

Different bee venoms are on the market. I use the product VeneX, which comes in two different strengths: VeneX-10 and VeneX-20 (Table 1.). VeneX-20 is twice as concentrated as VeneX-10. VeneX-10 contains 1.0 mg of bee venom per 1.0 ml. A 0.1 ml of this solution delivers approximately the same amount of bee venom as a natural bee sting. The venom is harvested and purified by Michael Simics who is worldwide considered the genius and master of this process, in which the bees are not harmed. VeneX Forte has added homeopathic dilutions of bee venom which has been most helpful in preventing allergic reactions.

The content of melittin in bee venom is dependent on where it is collected on the hive; the season and the pollen source the bees have access to at the time. Generally between one third and one half of the venom is melittin. Because of these variables the symptoms seen on administration of the venom can also vary. Bee venom is used for desensitization and is approved with the FDA for this purpose. There is an official monograph in the Homeopathic Pharmacopoea of the United States (HPUS), also recognized by the FDA.

Product Vial Size
(ml)
DVSE* /
vial

DVSE* /
1.0 ml
DVSE* /
0.1 ml
DVSE* /
VeneX- 9.0 90 10 1 0.005.5 m l
VeneX- 12.5 250 20 2 1
* Dried Venom Sac Equivalent (DVSE): 0.1 mg bee venom
Table 1. Comparison of Venom Solutions.
The average maintenance dosage is 1.0 ml of VeneX-10 (or 0.5 ml of VeneX-20) mixed with 2.0 ml preservative free buffered procaine (available from ApotheCure in Dallas, TX) injected subcutaneously, given between one and three times weekly for 6-12 months. Even though much of the venom's effect is systemic, independent of the location where it is given, additional benefits are observed by injecting the venom in specific target areas.

These areas include:

1. All tender areas in the body, transition areas in the body, where soft tissue meets bone, the occipital nuchal line, above and below the zygoma, around the mastoid and jaw bone, the para-sternal area, the spinous processes of the vertebrae;
2. The kidney and adrenal area (often palpation reveals significantly tender areas); tends to lessen allergic reactions, if treatment is started in this area for first few sessions
3. The thymus (upper end of the sternum);
4. Painful joints (in the most tender areas);
5. Acupuncture points (Bladder 23 for stimulating the adrenals, Gallbladder 1 to improve Lyme related problems with vision, Bladder 10 and Gallbladder 20 to stimulate melittin uptake into the brainstem (cranial nerve problems), Kidney 3 to improve Lyme related kidney dysfunction, etc.);
6. Neural therapy points: over the mastoid to improve Lyme related hearing and balance problems
7. Over the vagus nerve: to treat Lyme related dental and jaw problems (infected jaw bone, cavitations, Lyme related chronic pulpitis/sensitive teeth);
8. Frankenhauser points: to treat Lyme related bladder problems, pelvic/prostate/sexual dysfunction.

Procedure:

Distribute the 2.5 -3.0 ml bee venom and procaine mix over 10 areas, using 0.25 ml to 0.3 ml per injection. The injection is given with a 30 g 1/2" needle.

The needle is advanced just deep enough for the needle tip to barely reach beyond the sensory skin nerves. Procaine does not lessen the bee venom effect as some practitioners falsely assume. However, lidocaine and marcaine disturb the sensitive peptides in bee venom. Bee venom should be kept in the fridge most the time but not frozen and protected from uv-rays and electromagnetic fields (like very living substance should).
If it burns, the needle is not deep enough. If it never burns, most likely the injections are given too deep, where the medication will be quickly flushed away by the blood stream and lymphatics, without having the much-desired local effect. For a 1/2”long needle this means that the needle is inserted into the skin less than half way.
These injections should be painless and well tolerated. There is a welling up, itchiness and aching after 10 minutes or so, which becomes less with an increasing number of treatments. The discomfort may increase during the first four or five treatments and then lessen over time. The initial response determines the treatment frequency. The first injection often triggers an increase in well-being and a decrease of pain levels after a few hours; sometimes as late as 24 hours after the injection.
It may take several weeks of treatment before the first positive results are observed. The initial improvement may last between 12 hours and several days. This determines if the patient needs to be treated once a day or as little as once/week. If the improvement is less than desired a higher dose of bee venom may be needed.
I start with a low initial dose of 0.1 ml VeneX-10 or 0.05 ml VeneX-20 to ride out the often strong initial reactions. Over the next treatments I increase the dose, depending on the response, rather rapidly to the full treatment dose (Table 2. and Table 3.). It is wise to wait with injecting around the head until the patient no longer has strong local reactions (redness, swelling).
For the first 4-6 months the injections have to be given every other day, after that time, when the client and symptoms are stabilized, twice weekly until the patient is lastingly stable and well. Bee venom has a positive synergistic effect with most herbs but seems weakened by the concomitant use of antibiotics. I stop bee venom during courses of antibiotics but resume immediately afterwards.

The Herbs

Always take the herbs together with **Matrix Electrolyte** or ME (BioPure) for better absorption and transport of the active ingredients through the matrix to the cell membrane. ME also activates all functions of the ANS and improves trans-cell-membrane communication. **Freeze dried garlic** has a profound stabilizing effect in most symptomatic patients. It should either be taken immediately after meals on a full stomach (2-3 cap 3-4 times/day) or 2

caps should be dissolved in 1-2oz of water and taken away from meals.

1. BioPure PC Samento

(pentacyclic TOA-reduced energetically modified, ethically wild-crafted Amazon Cat's Claw):

In my work this product has shown the most consistent action against Borrelia, Bartonella, Ehrlichiosis, Rickettsia, mycoplasma and other coinfefctions. Herxheimer reactions are expected and may occur at any stage of the treatment (on the first day of use or after many months) and repeatedly. During the "Herxes"I recommend colon hydrotherapy, KMT lymphatic drainage, raw food diet, moderate exercise, drinking more water then usual, a massage and a nurturing environment.

Dosage: start with 4 drops twice daily (or 8 dr/day). Wait one week before increasing. If condition worsens, reduce dose. Sometimes patients initially tolerate only 1 drop/day (rare). Final dose: 2 dropperful/day

Contraindication: organ transplant immunotherapy. Don't use if trying to become pregnant. May interfere with blood thinning treatment

2. PC-Noni

(a concentrated energy-enhanced extract of Noni where the ingredients are made bio-available with a unique proprietary process)

It is in our experience the most reliable remedy to treat and eliminate intracellular microbes over time. This process is slow (months) and very rewarding. Several German practitioners have found this amazing property mostly with darkfield microscopy. I am not aware of unbiased published studies to confirm this. However, it is consistent with our ART findings and clinical observation.

Dosage: start with 6 drops twice daily and increase to a total of 3 dropper full/day for 1 year.

I suggest the each person makes a 1 liter glass bottle of filtered water in the morning and add the herbs for the day one by one into the bottle. Since PC Samento and PC Noni are also carriers for sophisticated anti-Lyme frequencies, the bottle should be sucussed 50 times after introducing PC Samento and again after introducing PC Noni. If other herbs are added to the bottle both should be added last. The content should then be taken throughout the day and used up by bedtime. It is best to take our herbs away from food. PC Samento has to be activated by acid. Either add the daily dose of Rechtsregulat (acidic ph) into the bottle or take on empty stomach, when stomach-ph is low.

3. Artemisinin

has disappointed in our experience in the treatment of Babesia, unless given in very high doses: 1200-1500 mg/day given 3 days in a row, repeat after a 2 week break. This is the way the drug is used in China for treatment of

Malaria. After the initial 2 courses a 2-3 day course should be given once/month
Contraindication: early pregnancy
The expanded herbal Lyme PDR
4. Andrographis paniculata:
Science:
_ rapid excretion via kidneys
_ **anti-spirochetal**
_ crosses blood brain barrier
_ protects heart muscle
_ anti-inflammatory
_ calming
_ potent modulating effect on mast cell and neutrophil activity: turns off inappropriate mast-cell allergic reactions in tissue
_ enhances liver function
_ significant **protective** effects against inflammation-mediated **neurodegeneration** of brain, spinal chord and CSF
Other published positive effects:
_ filaria
_ leptospirosis
_ malaria (suggesting strong effect against Babesia)
_ decreases heart muscle damage after MI
_ Hepatitis A and B
_ tuberculosis
_ tonsillitis
_ pneumonia
_ snake bites
_ e.coli
_ **herpes viruses**
_ mumps
_ **periodontal bacteria** (gum disease)
_ AIDS
_ cancers: prostate breast colon anal stomach skin melanoma leukemia
Dosage: 400 mg capsules standardized to 10% androgrpholides
Start with 1 cap 4 times/day. Slowly increase to 3 caps 4 times/day. Stay on this dose till Lyme sx significantly decreased, then slowly decrease dose. Stop during severe Herxheimer reactions. 1 year
Contraindications: andrograpis lowers progesterone (natural contraceptive), pregnancy, acute gallbladder disease
5. Polygonum cuspidatum (Japanese Knotweed)

Peer review literature/Science
Effective against:
_ Leptospirosis
_ Treponema denticola (spirochets in oral flora)
_ Bartonella (Buhner)
_ Many gram neg and gram pos bacteria
_ Anti-viral
_ Hepatitis B (and C?)
Other published positive effects:
_ Crosses blood brain barrier: anti-inflammatory, antimicrobial, protects against microbial endotoxins
_ High content of resveratrol increases microcirculation (vasodilation and inhibits platelet aggregation: pos effect on eye, heart, skin (ideal synergist)
_ Lowers cholesterol and lipids
_ Increases wound healing
_ Angiogenesis modulator
_ Ischemic heart disease
_ Potent antioxidant
_ Inhibits lipoxygenase (anti-inflammatory)
_ Inhibition prostaglandin E
_ Inhibits nuclear factor kappa B (NF kB) which upregulated in Lyme causing a cascade of immune mediated cellular responses
_ Leukemia
_ Stimulates fibroblasts (proliferative effect)
_ Rheumatoid arthritis
_ Psoriasis
_ Increases bone mass
_ Anti-aging
_ Reduces auto-immunity
_ Strongly neuroprotective
_ Effects against: ALS, Alzheimer, Parkinson MS cerebral ischemia
_ Stimulates microcirculation in brain
Dosage: Whole herb (Hu Zhang) standardized to 8% total resveratrols and 10 mg resveratrol. Source Naturals 500 mg tablet.
Use 3-4 caps 3-4 times/day. Work up slowly to this dose
Contraindications: Pregnancy, Consider carefully when giving with blood thinners (synergistic effect)
Overdose: GI-symptoms
6. Smilax glabra (Sarsaparilla)
Peer review literature/Science:

effective against:
_ Leptospirosis
_ Treponema pallidum (syphilis)
_ liver flukes (clonorchis sinensis)
_ trypanosome
_ shigella and salmonella (common in chromnic Lyme)
_ leprosy and TB
_ fungal skin infections
Other published results:
_ Lyme endotoxin binding
_ Lessens Herxheimer reactions
_ Improvement in **mental and psychological** parameters in chronic syphilis
_ Modulates immune responses
_ **Arthritis** anti-inflammatory
_ **Psoriasis** and eczema
_ Neuroprotective (crosses blood brain barrier)
_ Reduces skin breakdown
_ Pain relief
_ Improves liver function
_ Lessens **fatigue**
_ Increases libido
_ Asthma, hay fever, rhinitis
_ **Cervical spondylosis** (Lyme related disc degeneration and facet joint arthritis)
_ Chronic liver disease (dramatic) including Hepatitis C
_ Reversal of cognitive impairment
_ Autoimmune dysregulation
_ Protects from anti-androgenic substances in Lyme (ie gossypol)
Dosage: 425-500 mg caps 1-3 caps 3-4 times/day. Increase slowly to full dosage, stay on it for 2 months, then slowly reduce to amintainance dose of 1 caps 3 times/day. At least 1 year
Contraindications: Increased digitalis and Bismuth absorbtion (careful with Am. Biologics Lyme protocol), increased elimination of hypnotic drugs
7. Stephania Root (Stephania tetrandra and S.cepharantha)
Peer review literature/Science:
effective against:
_ Potent anti-inflammatory
_ Alopecia
_ Radiation injury (leukemia)
_ Asthma

_ Induces IL-1 beta, IL-alpha, TNF-a, IL-6, IL-8 (especially in CNS and joints)
_ Reduces NF-kappa B and IL-6 during neuroborreliosis
_ Modulates HLA-DR expression (Lyme arthritis connected to CD3 generated HLA-DR alleles)
_ Treatment of silicosis (also breast implant immune complications)
_ Protects endothelium form endotoxin damage
_ Reduces vascular permeability
_ Bell's palsy
_ Free radical scavenger
_ Inhibits toxic glutamate levels in brain
_ Ca-channel blocker
_ Asthma and heart disease
_ Retinopathy (modulates formation of new blood vessels and improvement of vision)
_ Malaria (and Babesia)
_ Inhibits cancer cell proliferation
_ Anti-fibrotic/anti-scar formation
_ Blocks abnormal histamine release/stabilizes mast cells
Dosage: 1:5 tincture of both forms of Stepania, 1/2-1 tsp t.i.d.
Contraindication: use judgement when using together with Ca-channel blockers. Constipation. May potentiate the effect of other drugs.
8. Other important herbs:
Teasel Root: give high doses over 3 months (1-2 tsp 3-4 times/day)
_ Has been shown first by German ethno-botanist Stoerl to be highly effective against Bb.
_ Good for **arthritis** and Lyme related insomnia
Turmeric, nettle and devils claw also good for Lyme-arthritis
Poke Root and Red Root for lymphatic drainage
Colchicum autumnale: effective against Ehrlichiosis. Best used iv (Eli Lilly). Give 1 amp (=1 mg) twice weekly for 6 weeks. Has to be given strictly i.v. with 25 g butterfly, otherwise causes severe long lasting burn. Alternative: oral tincure: 15 -20 drops daily for 7 days. Repeat after 2 week pause. 4-6 courses
Astragalus: potent anti-viral. Good synergistic effects with the other herbs. Elevates interferon gamma which is depressed in Ehrlichiosis, MS and many of the more severe Lyme related illnesses
Practical Considerations and Recipes:
Neuroborreliosis:
o read and understand the "Klinghardt Neurotoxin Elimination Protocol"

o bee venom therapy is superior
o most patients have a degree of kryptopyrroluria (excretion of abnormal hemoglobin breakdown products) which leads to a loss of excessive amounts of zinc, B6, and Omega 6 fatty acids. It is recognized by either ART testing or by finding a low or low normal alkaline phosphatase (below 50). Replace zinc, copper, Magnesium, B6 (I ask the client to increase the amount until he/she has vivid dreams regularly) Niacin (work up to 3000 mg/day) and Udo's oil.
o Do not give zinc without copper in Lyme!
o Use KMT to vagus, spheno-palatine ganglion and superior cervical ganglion. Always use Rechtsregulat to reach microcirculation.
o Always use BioPure "Phospholipid Exchange".
o In Bell's palsy use Stephania root.1 tsp t.i.d.
o At least 2/3rds of clients with Bb also have a Babesia co-infection which has to be treated early.
o Use polygonum, smilax and andrographis early on
o Use neural therapy, especially in the ganglia together with glutathione or DMPS

Considerations in connective tissue/lyme **arthritis**:
o Bee venom therapy is most effective in the long run
o antioxidants (especially polygonum/resveratrol), cetyl-meristoleate, intra-articular ozone injections)
o para-joint neural therapy
o APN desensitization
o L-carnosine and growth hormone for premature tissue aging (collagen breakdown).
o KMT therapy directly to involved joint
o Consider high dose enzyme therapy
o Use Stephania root 1/2 tsp t.i.d.

Ocular borreliosis:
o Bee venom therapy very effective (sting Gb-1 and SI-3 regularly)
o high doses B2 (700-1000 mg/day) for a few months
o eyebright tincture 2 dropperfull 2-3 times/day
o gingko extract
o KMT microcurrent directly to the eye
o Manual lymph/fluid drainage to eye (Klinghardt method)
o Use Stephania root 1 tsp t.i.d.and Polygonum! Valuable especially for macular degeneration

Lyme **carditis**:
o Systemic bee venom therapy 3 times/week for 3 months, then 2 times/week till resolved (years)

o always use antibiotics early on in high doses
o Use KMT over stellate ganglia and right vagus
o APN desensitization
o Use neural therapy over the heart with Enderlein remedies (Pleo SanBruc, San Strep, Nig and Muc, Lat)
o Always use "Phospholipid Exchange" from BioPure
o Turn off excessive immune activity with auto-urine therapy
o Use the herbs: andrographis, polygonum and hawthorne.

Ehrlichiosis:

o include Colchicine injections (or Colchicum drops) and astragalus

Bartonella:

o include Polygonum/ Resveratrol

References in: "Healing Lyme – Naturtal Healing and Prevention of Lyme Borreliosis and its Co-Infections" Stephen Buhner Raven Press 2005

Treatment should always keep in mind that our immune-system is in a never- ending training and adaptation program. We are evolving. The same is true for the microbes. We are seeking a peaceful inner state - in which microbes are welcome as long as they contribute to the greater whole.
We do not yet understand Lyme disease in this way, but our unconscious and our immune-system does. Plant adaptogens have far greater potential in helping us in this necessary process of evolution then any man-made chemical compound. Plant medicines are intelligent, human medications are usually quite dumb. Antibiotics have their place, but it is limited.

Potential investment into research to discover unique local causative agents causing a growing number of Australians debilitating illness:

Stephen L. Doggett, Richard C. Russell, Richard Lawrence and David Dickeson

Originally appeared in: *Inoculum*, **4**: 1-4. Modified and updated; November, 1997.

LYME DISEASE IN AUSTRALIA

History - Australia

The first Australian cases of a syndrome consistent with Lyme disease (LD) were reported from the Hunter Valley region of New South Wales in 1982. Serology was initially negative on one of the 6 patients, but later reported as positive in low titre. Cases of EM with febrile illness were reported in 1986 from the south and central coasts of New South Wales. All had negative serology. In Queensland, from 1986 to 1989, the State Health Laboratories tested 1,247 patients for *B.burgdorferi* antibody using an IFAT and reported 186 (15%) positive (titre 64) titres. In none of these cases was confirmatory serology (WB) undertaken.

In 1988 at Westmead Hospital, a multidisciplinary investigation of putative LD in coastal New South Wales began, encompassing clinical, serological, vector and reservoir host studies.

Clinical investigations - Australia

Over the past 6 years, due principally to local publicity, there has been an increase in serological testing for LD. This is often initiated by patients, who believe that LD may be an explanation for an undiagnosed health problem. Thus, most patients seen by infectious diseases specialists are self selected and referred for assessment on the basis of tick exposure and reported positive screening serology.

Patients frequently have long-standing symptoms for which no other diagnosis has been established including myalgia, arthralgia without objective evidence of joint disease, neurological symptoms such as frequent headaches, inability to concentrate and impairment of memory, and syndromes resembling chronic fatigue syndrome. The late LD dermatological manifestation, ACA, has not been reported in Australia.

A few cases of EM have been reported from South-Eastern Australia. However, diagnosis can be confounded by a spectacular erythematous hypersensitivity reaction to the bite of *I. holocyclus*, the most common tick biting humans in New South Wales. Of eight skin biopsies submitted to Westmead Hospital for spirochaete isolation, one, from a patient returning from a LD endemic area in Europe, was culture positive for *B.burgdorferi*. There has been no isolation from local patients.

Serological Investigations - Australia

No significant difference was found in seroprevalence rates for *B.burgdorferi* infection in humans between high (rural residents) and low (urban residents) tick exposure groups, using an IgG ELISA. The overall seropositive rate was 2.2% (9/400). The seroprevalence in New South Wales is comparable with that in non-LD areas, where 1-3% of human sera are seropositive due to cross reacting antibodies and contrasts with reports from known endemic areas, outside Australia, where rural populations have considerably higher seropositive rates. A serosurvey of dogs in New South Wales showed a similar result with 2.5% (6/239) seropositive and another from Brisbane also showed no evidence of *B.burgdorferi* infection. These suggest that southeastern Australia is a non-endemic area.

From 1988 to 1994 at Westmead Hospital, 78 (1.8%) of 4,372 from local patients with suspected LD were positive for IgG by ELISA and IFAT. All 78 were tested by WB, using North American and European strains of *Borrelia*; 46 sera showed one or more bands. None, including those with putative late stage disease, showed more than 4 specific bands and thus were all negative by international criteria. Twenty-four patients with various bacterial, viral or autoimmune syndromes unrelated to LD were tested in parallel and 11/24 showed one or 2 indicative bands. Thus a high degree of cross reactivity was demonstrated with non-LD patients.

Recently, there have been reports from eastern Australia of LD-like illness associated with WB serology yielding bands at 31kDa (OspA) and the highly-cross reactive 41kDa band. None of these results conforms with internationally accepted criteria for a positive WB. Concomitant with this are results of WB analysis of sera from patients with syndromes unrelated to LD, >30% of which reacted with a 41kDa band and >10% with the OspA band.

The sensitivity of serological testing for LD sometimes depends on the strain of *Borrelia* used and could confound interpretation of results in Australia, where no local spirochaete has been isolated for use as a reference antigen.

Vector and Reservoir Host Investigations - Australia

To detect a possible causative agent, ticks were collected from areas associated with putative infections and examined for spirochaetes by dark field microscopy, culture of gut contents, and direct testing of ticks with PCR for the *Borrelia*-specific flagellin gene.

In total, over 12,000 ticks were tested including >1,000 by PCR. Spirochaete-like objects (SLOs), were observed in 92 cultures from bloodfed ticks but were not typical of *Borrelia* spp. They were found only found in cultures with bacterial contaminants, presumably from the bloodmeal. Electron micrographs were similar to those of SLOs recovered from contaminated cultures from ticks in Missouri, USA and were composed of aggregations of bacterial flagella, thought to originate from the contaminants. Molecular characterisation indicated that the SLOs shared some antigens with *B.burgdorferi*, but were not genetically related. Similar objects found in cultures from dissected bloodfed ticks taken from animals on the mid-north coast of NSW were purported to be related to *B.burgdorferi* and the probable cause of LD in Australia.

A small number (17) of native vertebrate animals were sampled by ear punch biopsy for culture and PCR investigation but there was no evidence of borreliae.

It is possible that the PCR primers used were unable to identify Australian spirochaetes. However, the tick gut contents were also negative by culturing and dark field microscopy.

Conclusions - Australia

There are some major differences between Australia and the endemic areas of the northern hemisphere with respect to the natural history of LD:

No ticks of the *I. persulcatus* complex, the principal vectors to humans in the northern hemisphere, occur in Australia. In eastern Australia, the logical candidate vector would be *I. holocyclus* which has a wide host range and is the most common tick biting humans. It was unable to transmit a North American strain of *B.burgdorferi* but an association with a so far undiscovered Australian spirochaete can not be excluded.

None of the mammal species identified as reservoir hosts in the northern hemisphere are present in Australia. There are reports of spirochaetes in Australian native animals, and a local mammal could be a reservoir host for an indigenous spirochaete that occasionally infects humans through a tick vector and produces a clinical syndrome similar to LD; however, no spirochaete was detected in the 12,000 ticks or animals processed.

Summary

The diagnosis of LD outside known endemic areas cannot be based solely on serological tests especially if they fail to conform with internationally accepted criteria, because of the high incidence of false positive results.

A clinical diagnosis in a non-endemic disease area (especially of Stage II or III disease), is difficult to support without isolation of the causative agent from the patient, from other patients with similar illness or from a known vector in the region.

The existence of LD in Australia will remain controversial until an organism is isolated from a local patient and fully characterised, or until a tick-borne organism can be shown to be responsible for the human infection. If it exists it shares few of the epidemiological or clinical characteristics of US or European patterns of LD.

Research into signs and symptoms of Australian patients suffering from multiple systemic infections: (MSIDS, Horowitz, Multiple systemic infection disease syndrome):

Suggested Australian Research:

Horowitz (p18 of this submission), believes that his sixteen-point differential diagnostic map, with his thirty eight item questionnaire, can be best applied to solve the mystery for those individuals with a number of complex chronic medical illnesses and provide treatment options and relief not previously available.

Three I's – infection, immunity, and inflammation with associated cytokines, are likely to be responsible for many of the clinical manifestations of chronic disease.

A valuable research project would be to set up the Horowitz questionnaire online so that all practitioners, seeing these patients with chronic illness, could be requested to have their patient's complete the questionnaire, to benefit the practitioner, and the author's research project.

Lyme disease is a clinical diagnosis. The doctor will need to assess the signs of the patients disease, their symptoms, and their laboratory findings in order to arrive at a proper diagnosis.

The biggest difference between the MSIDS model and the standard medical approach to illness is a new emphasis on several distinct aspects of treatment. detoxification

improving functional medicine pathways

a. resolving chronic infections with associated immune dysfunction and inflammation

b. food allergies
c. vitamin and mineral deficiencies
d. environmental toxiciy
e. mitochondrial dysfunction
f. undiagnosed hormonal abnormalities
g. while having been exposed to multiple infections

It would be valuable to have these features shown in a patient diagnostic/treatment sheet. This should be available online for future analysis.

An excellent research project would be to refine the format on paper/computer until it is acceptable to a representative group of practitioners. This would avoid the poor acceptance of the current patient computer system being offered by the Australian Government to GP's.

Biochemical testing lacks specificity and sensitivity for Borrelia burgdorferi and it co-infections probably due to changing phenotypes.

Ried K, at NIIM in Melbourne, is researching diagnosis of Lyme patients using PCR and DNA testing.

Another Melbourne group is researching a bio-resonance approach to testing and treatment of Lyme/MSIDS patients.

Further Reading

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Stephen L. Doggett, Richard C. Russell, Richard Lawrence and David Dickeson

<http://www.uri.edu/artsci/zool/ticklab/HomePg.html> (Information from America for ticks, Lyme disease, Ehrlichiosis, Human Babesiosis).<http://www.dis.strath.ac.uk/vie/LymeEU/index.htm> (Lyme disease from Europe, tick biology, control and images).

<http://www.lymenet.org/> (Lyme disease network from America).

Australian doctors divided over Lyme disease diagnoses

Harriet Alexander

What is Lyme disease?

Initially spread by ticks and hard to diagnose Lyme disease presents a wide and changing set of symptoms. as Dr Ann Mitrovic explains.

Lewis Newstead's agony came on suddenly, when at the age of seven he woke up one morning with muscle pain so severe he was unable to get out of bed.

The cause was a mystery. Over a period of four months, doctors investigated flu, glandular fever, chronic fatigue, Ross River fever, meningococcal disease and cancer.

"Eventually they said he didn't have cancer but there was

nothing more they could do," his mother, Tina Newstead, said.

Lewis Newstead, 10, with his mother Tina. Lewis was initially treated for Lyme disease.*Photo: Peter Stoop*

But there was one possibility that no doctor wanted to discuss: Lyme disease.

Lewis had been bitten by an insect before his illness and one test for the disease had come back positive, although

doctors had dismissed it.

So Mrs Newstead went hunting: "I went from one doctor to another doctor for someone, anyone, to look outside the square. I was looking for someone to understand this whole Lyme thing."

Lyme disease sufferers in Australia are struggling to convince medical experts the disease exists in this country.

For many patients suffering from unexplained illnesses, Lyme disease is a diagnosis of last resort.

They may endure muscle pains, fatigue and neurological symptoms for years before finding succour with a Lyme specialist doctor, who is often the first medical professional to give their condition a name and the means to treat it.

However, Lyme disease is controversial in Australia because no large, peer-reviewed studies have been able to prove the local existence of Borrelia bacteria, which causes the disease in patients bitten by carrier ticks in the United States and Europe.

Mainstream doctors are concerned that while the Lyme lobby has a noisy social presence, many patients are spending thousands of dollars on consultation fees, tests and treatment that might harm their health and won't address the real cause of their illness.

Mrs Newstead consulted a Lyme doctor who was featured on a current affairs program. He promptly diagnosed Lewis with the condition and put him on a long course of antibiotics.

However, after 10 weeks, Lewis' symptoms were getting worse.

"[The doctor] said that's just what the bacteria did," Mrs Newstead said.

"He kept assuring us that we had to keep on with it because [Lewis] had to have enough antibiotics to kill the bacteria."

Unconvinced, they changed tack and took Lewis to see paediatric rheumatologist Jeffrey Chaitow,from the Children's Hospital at Westmead, who started treating Lewis for juvenile arthritis. He was cured within six months.

Dr Chaitow said he had seen several children who had

been given a Lyme diagnosis but actually had juvenile arthritis or systemic lupus and needed immune suppressing drugs and steroids rather than antibiotics.

"It delays instituting the correct treatment and that's a big concern to me," Dr Chaitow said.

He was aware of one 15-year-old girl who was admitted to hospital after travelling to a Lyme clinic in Germany for a radical treatment that involved bringing the body temperature up to 42 degrees.

She was suffering clostridium difficile colitis, a severe complication of high-dose broad-spectrum antibiotics, and was severely dehydrated and in early renal failure.

Children's Hospital at Westmead paediatric infectious disease specialist David Isaacs was concerned that doctors would diagnose Lyme in children who had not left Australia, though he did not doubt they were ill.

"Some patients have a proven infection ... some have a suspected infection and some patients have psychological problems that can be a cause of their problem or a result of their disease," Dr Isaacs said.

A clinical advisory committee led by the national Chief Medical Officer, Chris Baggoley , last year pronounced that a conclusive finding of a bacterial species that could cause Lyme disease was yet to be made, although the government is continuing to monitor the disease.

Infectious disease experts think it is possible that Australian ticks do carry bacteria that causes illness in humans, but no researcher has yet been able to identify what it is, so it is impossible to test for it.

ANU infectious diseases professor Peter Collignon said it was likely that ticks caused diseases that had not yet been discovered, but that prescribing long courses of antibiotics was not circumspect, especially when Borrelia bacteria had not been proved to exist here.

Overuse of antibiotics encourages the development of drug-resistant strains of deadly infections such as golden staph, he said.

"There's no convincing evidence that giving prolonged intravenous or oral therapy makes any difference for Lyme disease, even in the US,"
Professor Collignon said. "There's a lot of dispute about how much benefit it gives and no doubt that it causes harm."

But Pymble Grove Health Centre GP Richard Schloeffel, who is treating 400 patients for Lyme disease, said antibiotics were the lesser of two evils and the only cure.

Dr Schloeffel is part of a University of Sydney study that is attempting to identify a strain of borreliosis that might cause Lyme disease in Australia and establish a test for it.

"I've never seen anything like these patients," he said.

"The illness is far more dangerous than the administration of antibiotics. It can be life-threatening."

One of his patients, 21-year-old Tahlia Smith, is raising money to undergo the German treatment to cure her agonising symptoms, which include seizures every 40 minutes, nausea, leg tremors and joint pain.

It is thought she was bitten by a tick while visiting the US in 2010, and she is currently receiving intravenous antibiotics at the Pymble Grove Health Centre.

Her mother, Lee Smith, said she visited 27 doctors before finding one who was prepared to treat Tahlia.

"You're watching our daughter die in front of your own eyes and you do turn to someone willing to help," Ms Smith said. "I have no other option. What would you do?"

Royal College of Pathologists spokesman Stephen Grave said laboratories that have been accredited by the government regulator have only identified Borrelia in patients who have travelled overseas.

However, one laboratory in Australia - Australian Biologics - routinely gives out positive test results for Lyme disease among patients who have not been out of Australia.

Director Jennie Burke estimated 20 per cent of her tests returned a positive finding of Borrelia, and this was because her tests were sensitive to all strains of the bacteria, including those that are carried by Australian ticks, and not

just the variety endemic to Connecticut where Lyme was first identified.

Other laboratories also used tests that did not pick up the infection in people who had long-term infections and were no longer producing antibodies, she said.

"We've got patients who have been sick for 20 years," she said. "There's a lot of it around and it's getting worse."

Peter Mayne, a general practitioner specialising in Lyme disease, said the far greater harm to patients was caused by "Lyme denialism" among conventional doctors, who were ignoring the evidence.

"Doctors should be reprimanded," he said.

About 90 per cent of his patients were infected by the local strain of Borrelia, but accredited laboratories were not using the right primers to identify it, he said. These patients needed antibiotics.

Dr Mayne said he was forced to charge $800 an hour due to the high cost of his indemnity insurance, which spiked after a complaint was made about him a year ago.

"As soon as Lyme disease is recognised in Australia, my medical indemnity insurance will fall again," he said.

These days Mrs Newstead is still not convinced that Lewis never had Lyme, but accepts it was the arthritis treatment that cured him.

"I was sick of seeing doctors who had no answers and [the Lyme doctor] seemed to have an answer for us," she said.

LYME RESEARCH ALLIANCE:

OVERVIEW: A HUGE NEED

It is remarkable that given the advances made by modern medicine there is still no reliable diagnosis for Lyme disease, nor an effective treatment for long-term Lyme disease. Lyme Research Alliance is dedicated to the discovery of a reliable diagnostic test and a cure for long-term, or persistent Lyme disease, to improve the lives of the millions of Americans living in tick-endemic areas.

The funds we raise are deployed with great deliberation and precision to support researchers at accredited universities, conducting evidence-based research. Our grant-making criteria give precedence to those programs and researchers whose work we judge most likely to deliver publication-worthy results and thereby contribute to the body of scientific knowledge describing the causes and mechanisms of tick-borne diseases.

For information on our grant application and review process click here.

Lyme Research Alliance's impact on the field of research into Lyme disease and its associated complications already includes two notable "firsts":

- Lyme Research Alliance co-funded America's first dedicated center for research into Lyme and tick-borne diseases at Columbia University Medical Center in New York (2007)
- LRA funded the research team that recently announced the first-ever drug to treat long-term Lyme disease, securing FDA pre-approval to enter clinical trials, anticipated to begin in October, 2014.

With the work under way on projects currently funded by Lyme Research Alliance we anticipate the addition of at least one new "First" within the next year.

The signs and symptoms Australians with Lyme-like illness are enduring, and the treatment they receive from medical professions:

Questionnaire for Lyme Disease from Dr Horowitz

You can use the following questionnaires to determine the probability of a Lyme – MSIDS diagnosis for yourself.

Dr Horowitz also highly recommends that physicians use this as a screening tool to determine if their patients might have Lyme disease.

Answer the following questions as honestly as possible. Think about how you

have been feeling over the previous month and how often you have been bothered by any of the following problems. Score the occurence of each symptom on the following scale: none, mild, moderate, severe.

Section 1: Symptom frequency score

0 None 1 mild 2 moderate 3 severe

( ) unexplained fevers sweats chills or flushing

( ) unexplained weight change; loss or gain

( ) fatigue tiredness

( ) unexplained here loss

( ) sore throat

( ) testicular or pelvic pain

( ) unexplained menstrual irregularity

( ) unexplained breast milk production, breast pain

( ) irritable bladder or bladder dysfunction

( ) sexual dysfunction or loss of libido

( ) upset stomach

( ) change in bowel function (constipation or diarrhoea)

( ) chest pain or group soreness

( ) shortness of breath or cough

( ) heart palpitations, pulse skips, heart block

( ) history of a heart murmur or valve prolapse

( ) joint pain or swelling

( ) Stiffness of the neck or back

( ) muscle pain or cramps

( ) twitching of the face or other muscles

( ) headaches

( ) Cracks or neck stiffness

( ) tingling, numbness, burning, or staring sensations

( ) facial paralysis (Bell's palsy)

( ) eyes /vision: double, blurry

( ) he is hearing buzzing ringing ear pain

( ) increased motion sickness vertigo

( ) lightheadedness poor balance difficulty walking

( ) tremors

( ) confusion difficulty thinking

( ) difficulty with concentration all reading

( ) forgetfulness poor short-term memory

( ) disorientation getting lost going to wrong places

( ) difficulty with speech or writing

( ) mood swings irritability depression

( ) disturb sleep too much too little early awakening

( ) exaggerated symptoms or worse hangover from alcohol

( ) add up your totals from each of the four columns this is your first score

score ( )

Section 2: Most common Lyme Symptoms Score:

if you rated 3 for each of the following in section 1 give yourself five additional points

( ) fatigue

( ) forgetfullness, poor short-term memory

( ) joint pain or swelling

( ) tingling numbness burning or stabbing sensations

( ) disturbed sleep too much too little early awakening

score ( )

Section 3: Lyme Incidence Score:

now please circle points for each of the following statements you can agree with

you have had a tick bite with no rash or flulike symptoms (three points)

you have had a tick bite an erythema migrans or undefined rash followed by flulike symptoms(five points)

you live in what is considered a Lyme endemic area (two points)

you have a family member who has been diagnosed with Lyme and/or other tickborne infections (one point)

you experience migratory muscle pain (four points)

you experience migratory joint pain (four points)

you experienced tingling burning numbness that migrates and/or comes and goes (four points)

you have received prior diagnosis of chronic fatigue syndrome or fibromyalgia (three points)

you have received prior diagnosis of a specific autoimmune disorder lupus MS or rheumatoid arthritis or of a non-specific autoimmune disorder (three points)

you have had a positive Lyme test i.e.IFA, ELISA, Western blot, PCR, and/or borrelia culture (5 points)

Total score ( )

Section 4:Overall Health Score:

Thinking about your overall physical health for how many days of the past 30 days was your physical health not good award yourself the following points based on the total number of days

0 to 5 days equals one point

6 to 12 days equals two points

13 to 20 days equals three points

21 to 30 days equals four points

Total ( )

thinking about your overall mental health for how many days during the past 30 days was your mental health not good

award yourself the following points based on the total number of days 0 to 5 days one point

6 to 12 days two points

13 to 20 days three points

21 to 30 days equals four points

Total ( )

Scoring

record your total scores for each section below and add them together to achieve your final score

section 1 total ( )

section 2 total ( )

section 3 total ( )

section 4 total ( )

Final score ____

if you scored 46 or more you have a high probability of a tick-borne disorder and should see a healthcare provider for further evaluation.

if you scored between 21 and 45 you possibly have a tickborne disorder and should see a healthcare provider for further evaluation.

if you scored under 21 you are not likely to have a tickborne disorder

Interpreting the results:

Dr Horowitz sees a high frequency of section 1 symptoms in his patients, including fatigue, joint and muscle pain that often migrates, sleep disorders,

as well as memory and concentration problems, and a high frequency of section 3 symptoms, especially neuropathic pain that comes and goes and migrates (tingling, numbness, burning, etc). These form a cluster of presenting symptoms that are characteristic of those with a high probability of having lyme – MSIDS.

In one recent study conducted in Dr Horowitz's office of 100 consecutive patients, he found that more than 25% reported that the following symptoms were present most or all of the time in the month preceding the office visit. Many of these patients reported that these symptoms affected their quality-of-life: 71% reported that they physical health was not good and 47% reported that their mental health was not good on at least 15 days in the previous month. The most common symptoms related to line and MSIDS are

fatigue and tiredness

headaches

stiffness of the neck or back

joint pain or swelling

tingling numbness and or burning of the extremities

confusion and difficulty thinking

difficulty with concentration or reading

forgetfulness poor short-term memory

disturbed sleep too much too little early awakening

difficulty with speech or writing

Dr Horowitz believes it is prudent that patients with these presenting symptoms be tested for tickborne disorders.

Treatment Protocols

Last Updated: January 21 2014

This is intended to provide a reference to protocols that I have found to contain valuable information on the treatment of Lyme Disease. It is not intended to replace the advice of your own physician.

Some of my favorite Lyme disease protocols are those from Dr. Klinghardt, Dr. Cowden, Master Herbalist Stephen Buhner, and Dr. Burrascano. Each approaches Lyme Disease in different ways and I find that each protocol contains significant information of value. I highly recommend reading these protocols and then making a decision on which approach feels more appropriate for your individual situation. For me, it has been an integration of different approaches which has made me feel more empowered to beat this disease and return to full health. I thank these practitioners

openly for sharing with us all so freely.

- tDr. Dietrich Klinghard's A Look Beyond Antibiotics and Lyme Protocol 2008 and Treating Lyme 2010
- Dr. Lee Cowden's Lyme Protocol (uses NutraMedix products)
- Stephen Buhner's Healing Lyme Protocol from an article in Public Health Alert
- Dr. Joseph Burrascano's Diagnostic Hints and Treatment Guidelines (2008 Revision shared with permission)
- Dr. Marty Ross MD and Dr. Tara Brooke ND - The Successful Treatment Recipe
- Dr. Garth Nicolson's Diagnosis and Therapy of Chronic Systemic Co-infections in Lyme Disease and Other Tick-Borne Infectious Diseases
- ILADS Treatment Guidelines

Mold and Biotoxin Illness Protocols

I continue to be amazed by how many people with chronic Lyme that don't seem to be getting better also have a mold issue. Living or working in an environment where one is exposed to mold and their mycotoxins on a regular basis will negate just about every other beneficial thing we do to recover our health. After years of struggle, I found that I was living in an apartment that was full of black mold. Thanks to Dr. Ritchie Shoemaker, the topic of mold was something I was able to explore and take action to address. His approach often works for people without mold exposure that have other sources of biotoxins as well.

- Dr. Ritchie Shoemaker's Biotoxin Illness Approach

General Chronic Illness Protocols It is my opinion that often times people hyper-focus on Lyme disease and do not consider the many other factors that must be evaluated and addressed in order to regain wellness. Some protocols that I think are excellent in considering these broader factors include:

Dr. Garry Gordon's F.I.G.H.T. ProtocolDr. Jacob Teitelbaum's SHINE Protocol

Articles An article was published in the July 2006 edition of Explore! magazine

which summarized the January 2006 conference on Lyme Disease and Other Neurotoxin-Mediated Illnesses by Dr. Dietrich Klinghardt. My notes from that conference can also be found here. The article was a good summary of various approaches that Dr. Klinghardt uses to address Lyme Disease. Subsequent to the publication, I contacted Chrystyne Jackson from Explore! and asked for permission to scan and reprint the article here. She kindly agreed to allow me to share this article further. I **urge** anyone with a serious interest in resolving their own health conditions to consider subscribing to the publication. It is a very significant source of information which I always find enlightening. To read the conference summary, click here. Subsequent to that article on Dr. Klinghardt, an article was written which discusses the detoxification benefits that might be received through the use of Laser Energetic Detoxification (LED). For those with an interest in detoxification (which should be all of us), don't miss this one. To read the LED article, click here.

BetterHealthGuy.com is intended to share my personal experience in recovering from my own chronic illness. Information presented is based on my journey working with my doctors and other practitioners as well as things I have learned from conferences and other helpful resources. As always, any medical decisions should be made only with the guidance of your own personal medical authority. Everyone is unique and what may be right for me may not be right for others.

See more at: http://www.betterhealthguy.com/lyme/protocols#sthash.BEIO573f.dpuf

ADVANCED TOPICS IN LYME DISEASE

DIAGNOSTIC HINTS AND TREATMENT GUIDELINES FOR LYME AND OTHER TICK BORNE ILLNESSES

Sixteenth Edition

JOSEPH J. BURRASCANO JR., M.D.

Board Member,
International Lyme and Associated
Diseases Society

DISCLAIMER: The information contained in this monograph is meant for informational purposes only. The management of tick-borne illnesses in any given patient must be approached on an individual basis using the practitioner's best judgment.

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WELCOME!

Welcome to the sixteenth edition of the "Guidelines".

Amazingly, this edition is not only the sixteenth in the series, but as the first edition appeared in 1984, this reflects *twenty four years of effort!*

Since the last edition, enough new information has become available to justify this revision. New insights regarding co-infections, tests and treatment regimens are included. Nearly every item has been revised, but despite great effort to condense the information, the huge amount of new information included here has resulted in more pages than ever. Information included here is based on the literature, presentations at scientific meetings, the many valuable observations noted by my colleagues, plus experience from caring for my own patients. I have tried to make this information as up-to-date as possible and as inclusive as is practical. Please use the information presented in this document as an information resource and guide. It can never replace your own experience and clinical judgment.

I once again extend my best wishes to the many Lyme patients and their caregivers whose wisdom I deeply appreciate, and a sincere thank you to my colleagues whose endless contributions have helped me shape my approach to tick borne illnesses. I hope that this new edition proves to be useful. Happy reading!

BACKGROUND INFORMATION

WHAT IS LYME DISEASE?

I take a broad view of what Lyme Disease actually is. Traditionally, Lyme is defined an infectious illness caused by the spirochete, *Borrelia burgdorferi* (Bb). While this is certainly technically correct, clinically the illness often is much more than that, especially in the disseminated and chronic forms.

Instead, I think of Lyme as the illness that results from the bite of an infected tick. This includes infection not only with *B. burgdorferi*, but the many co-infections that may also result. Furthermore, in the chronic form of Lyme, other factors can take on an ever more significant role- immune dysfunction, opportunistic infections, coinfections,

biological toxins, metabolic and hormonal imbalances, deconditioning, etc. I will refer to infection with *B. burgdorferi* as "Lyme Borreliosis" (LB), and use the designation "Lyme" and "Lyme Disease" to refer to the more broad definition I described above.

GENERAL PRINCIPLES

In general, you can think of LB as having three categories: acute, early disseminated, and chronic. The sooner treatment is begun after the start of the infection, the higher the success rate. However, since it is easiest to cure early disease, this category of LB must be taken VERY seriously. Undertreated infections will inevitably resurface, usually as chronic Lyme, with its tremendous problems of morbidity and difficulty with diagnosis and treatment and high cost in every sense of the word. So, while the bulk of this document focuses of the more problematic chronic patient, strong emphasis is also placed on earlier stages of this illness where closest attention and care must be made.

A very important issue is the definition of **"Chronic Lyme Disease".** Based on my clinical data and the latest published information, I offer the following definition. To be said to have chronic LB, these three criteria must be present:

1. Illness present for at least one year (this is approximately when immune breakdown attains clinically

significant levels).
2. Have persistent major neurologic involvement (such as encephalitis/encephalopathy, meningitis, etc.) or active arthritic manifestations (active synovitis).
3. Still have active infection with B. burgdorferi (Bb), regardless of prior antibiotic therapy (if any).
Chronic Lyme is an altogether different illness than earlier stages, mainly because of the inhibitory effect on the immune system (Bb has been demonstrated *in vitro* to both inhibit and kill B- and T-cells, and will decrease the

count of the CD-57 subset of the natural killer cells). As a result, not only is the infection with Bb perpetuated and allowed to advance, but the entire issue of co-infections arises. Ticks may contain and transmit to the host a multitude of potential pathogens. The clinical presentation of Lyme therefore reflects which pathogens are present and in what proportion. Apparently, in early infections, before extensive damage to the immune system has occurred, if the germ load of the co-infectors is low, and the Lyme is treated, many of the other ticktransmitted microbes can be contained and eliminated by the immune system. However, in the chronic patient, because of the inhibited defenses, the individual components of the co-infection are now active enough so that they too add to features of the illness and must be treated. In addition, many latent infections which may have pre-dated the tick bite, for example herpes viruses, can reactivate, thus adding to the illness.
An unfortunate corollary is that serologic tests can become *less* sensitive as the infections progress, obviously because of the decreased immune response upon which these tests are based. In addition, immune complexes form, trapping Bb antibodies. These complexed antibodies are not detected by serologic testing. Not surprisingly the seronegative patient will convert to seropositive 36% of the time after antibiotic treatment has begun and a recovery is underway. Similarly, the antibody titer may rise, and the number of bands on the western blot may increase as treatment progresses and the patient recovers. Only years after a successfully treated infection will the serologic response begin to diminish.
The severity of the clinical illness is directly proportional to the spirochete load, the duration of infection, and the presence of co-infections. These factors also are proportional to the intensity and duration of treatment needed for recovery. More severe illness also results from other causes of weakened defenses, such as from severe stress, immunosuppressant medications, and severe intercurrent illnesses. **This is why steroids and other immunosuppressive medications are absolutely contraindicated in Lyme. This also includes intraarticular steroids.**
Many collateral conditions result in those who have been chronically ill so it is not surprising that damage to virtually all bodily systems can result. Therefore to fully recover not only do all of the active infections have to be treated, but all of these other issues must be addressed in a thorough and systematic manner. ***No single treatment or medication will result in full recovery of the more ill patient. Only by addressing all of these issues and engineering treatments and solutions for all of them will we be able to restore full health to our patients.*** Likewise, a patient will not recover unless they are completely compliant with every single aspect of the treatment plan. This must be emphasized to the patient, often on repeated occasions.
It is clear that in the great majority of patients, chronic Lyme is a disease affecting predominantly the nervous system. Thus, careful evaluation may include neuropsychiatric testing, SPECT and MRI brain scans, CSF analysis when appropriate, regular input from Lyme-aware neurologists and psychiatrists, pain clinics, and occasionally specialists in psychopharmacology.

HYPOTHALAMIC-PITUITARY AXIS

As an extension of the effect of chronic Lyme Disease on the central nervous system, there often is a deleterious effect on the hypothalamic-pituitary axis. Varying degrees of pituitary insufficiency are being seen in these patients, the correction of which has resulted in restoration of energy, stamina and libido, and resolution of persistent hypotension. Unfortunately, not all specialists recognize pituitary insufficiency, partly because of the difficulty in making the laboratory diagnosis. However, the potential benefits of diagnosing and treating this justify the effort needed for full evaluation. Interestingly, in a significant number of these patients, successful treatment of the infections can result in a reversal of the hormonal dysfunction, and hormone replacement therapies can be tapered off!

CO-INFECTION

A huge body of research and clinical experience has demonstrated the nearly universal phenomenon in chronic Lyme patients of co-infection with multiple tick-borne pathogens. These patients have been shown to potentially

carry Babesia species, Bartonella-like organisms, Ehrlichia, Anaplasma, Mycoplasma, and viruses. Rarely, yeast forms have been detected in peripheral blood. At one point even nematodes were said to be a tick-borne

pathogen. Studies have shown that co-infection results in a more severe clinical presentation, with more organ damage, and the pathogens become more difficult to eradicate. In addition, it is known that Babesia infections, like Lyme Borreliosis, are immunosuppressive.

There are changes in the clinical presentation of the co-infected patient as compared to when each infection is present individually. There may be different symptoms and atypical signs. There may be decreased reliability of standard diagnostic tests, and most importantly, there is recognition that chronic, persistent forms of each of these infections do indeed exist. As time goes by, I am convinced that even more pathogens will be found. Therefore, real, clinical Lyme as we have come to know it, especially the later and more severe presentations, probably represents a mixed infection with many complicating factors. I will leave to the reader the implications of how this may explain the discrepancy between laboratory study of pure Borrelia infections, and what front line physicians have been seeing for years in real patients.

I must very strongly emphasize that all diagnoses of tick-borne infections remains a clinical one.

Clinical clues will be presented later in this monograph, but testing information is briefly summarized below.

In **Lyme Borreliosis**, western blot is the preferred serologic test. Antigen detection tests (antigen capture and PCR), although insensitive, are very specific and are especially helpful in evaluating the seronegative patient and those still ill or relapsing after therapy. Often, these antigen detection tests are the only positive markers of Bb infection, as seronegativity has been reported to occur in as many as 30% to 50% of cases. Nevertheless, active LB can be present even if all of these tests are non-reactive! Clinical diagnosis is therefore required.

In **Babesiosis**, no single test is reliable enough to be used alone. Only in early infections (less than two weeks duration) can the standard blood smear be helpful. In later stages, one can use serology, PCR, and fluorescent in-situ hybridization ("FISH") assay. Unfortunately, many other protozoans can be found in ticks, most likely representing species other than *B. microti*, yet commercial tests for only B. microti and B duncani (Formerly known as WA-1) are available at this time! In other words, the patient may have an infection that cannot be tested for. Here, as in Borrelia, clinical assessment is the primary diagnostic tool.

In **Ehrlichiosis and Anaplasmosis**, by definition you must test for both the monocytic and granulocytic forms. This may be accomplished by blood smear, PCR and serology. Many presently uncharacterized Ehrlichia-like organisms can be found in ticks and may not be picked up by currently available assays, so in this illness too, these tests are only an adjunct in making the diagnosis. Rarely, Rocky Mountain Spotted Fever can coexist, and even be chronic. Fortunately, treatment regimens are similar for all agents in this group.

In **Bartonella**, use both serology and PCR. PCR can be performed not only on blood and CSF, but as in LB, can be performed on biopsy specimens. Unfortunately, in my experience, these tests, even when both types are done, will presently miss over half the cases diagnosed clinically.

Frequent exposures to **Mycoplasmas** are common, resulting in a high prevalence of seropositivity, so the best way to confirm active infection is by PCR.

Chronic viral infections may be active in the chronic patient, due to their weakened immune response. PCR testing, and not serologies, should be used for diagnosis. Commonly seen viruses include HHV-6, CMV, and EBV.

COLLATERAL CONDITIONS

Experience has shown that collateral conditions exist in those who have been ill a long time. The evaluation should include testing both for differential diagnosis and for uncovering other subtle abnormalities that may coexist.

Test **B12 levels**, and be prepared to aggressively treat with parenteral formulations. If neurologic involvement is

severe, then consideration should be given to treatment with methylcobalamin (as outlined below in the section on nutritional support).

Magnesium deficiency is very often present and quite severe. Hyperreflexia, muscle twitches, myocardial irritability, poor stamina and recurrent tight muscle spasms are clues to this deficiency. Magnesium is

predominantly an intracellular ion, so blood level testing is of little value. Oral preparations are acceptable for maintenance, but those with severe deficiencies need additional, parenteral dosing: 1 gram IV or IM at least once a week until neuromuscular irritability has cleared.

Pituitary and other endocrine abnormalities are far more common than generally realized. Evaluate fully, including growth hormone levels. Quite often, a full battery of provocative tests is in order to fully define the problem. When testing the thyroid, measure free T3 and free T4 levels and TSH, and nuclear scanning and testing for autoantibodies may be necessary.

Activation of the **inflammatory cascade** has been implicated in blockade of cellular hormone receptors. One example of this is insulin resistance; clinical hypothyroidism can result from receptor blockade and thus hypothyroidism can exist despite normal serum hormone levels. These may partly account for the dyslipidemia and weight gain that is noted in 80% of chronic Lyme patients. In addition to measuring free T3 and T4 levels, check basal A.M. body temperatures. If hypothyroidism is found, you may need to treat with both T3 and T4 preparations until blood levels of both are normalized. To ensure sustained levels, when T3 is prescribed, have it compounded in a time-release form.

Neurally mediated hypotension (NMH) is not uncommon. Symptoms can include palpitations, lightheadedness and shakiness especially after exertion and prolonged standing, heat intolerance, dizziness, fainting (or near fainting), *and an unavoidable need to sit or lie down*. It is often confused with hypoglycemia, which it mimics. NMH can result from autonomic neuropathy and endocrine dyscrasias. If NMH is present, treatment can dramatically lessen fatigue, palpitations and wooziness, and increase stamina. NMH is diagnosed by tilt table testing. This test should be done by a cardiologist and include Isuprel challenge. This will demonstrate not only if NMH is present, but also the relative contributions of hypovolemia and sympathetic dysfunction. Immediate supportive therapy is based on blood volume expansion (increased sodium and fluid intake and possibly Florinef plus potassium). If not sufficient, beta blockade may be added based on response to the Isuprel challenge. The long term solution involves restoring proper hormone levels and treating the Lyme to address this and the autonomic dysfunction.

SPECT scanning of the brain- Unlike MRI and CT scans, which show structure, SPECT scans show function. Therefore SPECT scans give us information unattainable through X-rays, CT scans, MRI's, or even spinal taps. In the majority of chronic Lyme Borreliosis patients, these scans are abnormal. Although not diagnostic of Lyme specifically, if the scan is abnormal, the scan can not only quantify the abnormalities, but the pattern can help to differentiate medical from psychiatric causes of these changes. Furthermore, repeat scans after a course of treatment can be used to assess treatment efficacy. Note that improvement in scans lag behind clinical improvement by many months.

If done by knowledgeable radiologists using high-resolution equipment, scanning will show characteristic abnormalities in Lyme encephalopathy- global hypoperfusion (may be homogenous or heterogeneous). What these scans demonstrate is neuronal dysfunction and/or varying degrees of cerebrolvascular insufficiency. If necessary, to assess the relative contributions of these two processes, the SPECT scan can be done before and after acetazolamide. If the post acetazolamide scan shows significant reversibility of the abnormalities, then vasoconstriction is present, and can be treated with vasodilators, which may clear some cognitive symptoms. Therapy can include acetazolamide, serotonin agonists and even Ginkgo biloba, provided it is of pharmaceutical quality. Therapeutic trials of these may be needed.

Acetazolamide should not be given if there is severe kidney/liver disease, electrolyte abnormalities, pregnancy, sulfa allergy, recent stroke, or if the patient is taking high dose aspirin treatment

LYME BORRELIOSIS

DIAGNOSTIC HINTS

Lyme Borreliosis (LB) is diagnosed clinically, as no currently available test, no matter the source or type, is definitive in ruling in or ruling out infection with these pathogens, or whether these infections are responsible for the patient's symptoms. The entire clinical picture must be taken into account, including a search for concurrent conditions and alternate diagnoses, and other reasons for some of the presenting complaints. Often, much of the diagnostic process in late, disseminated Lyme involves ruling out other illnesses and defining the extent of damage that might require separate evaluation and treatment.

Consideration should be given to tick exposure, rashes (even atypical ones), evolution of typical symptoms in a

previously asymptomatic individual, and results of tests for tick-borne pathogens. Another very important factor is response to treatment- presence or absence of Jarisch Herxheimer-like reactions, the classic four-week cycle of waxing and waning of symptoms, and improvement with therapy.

ERYTHEMA MIGRANS

Erythema migrans (EM) is diagnostic of Bb infection, but is present in *fewer than half*. Even if present, it may go unnoticed by the patient. It is an erythematous, centrifugally expanding lesion that is raised and may be warm. Rarely there is mild stinging or pruritus. The EM rash will begin four days to several weeks after the bite, and may be associated with constitutional symptoms. Multiple lesions are present less than 10% of the time, but do represent disseminated disease. Some lesions have an atypical appearance and skin biopsy specimens may be helpful. When an ulcerated or vesicular center is seen, this may represent a mixed infection, involving other organisms besides B. burgdorferi.

After a tick bite, serologic tests (ELISA. IFA, western blots, etc.) are not expected to become positive until several weeks have passed. Therefore, if EM is present, treatment must begin immediately, and one should not wait for results of Borrelia tests. You should not miss the chance to treat early disease, for this is when the success rate is the highest. Indeed, many knowledgeable clinicians will not even order a Borrelia test in this circumstance.

DIAGNOSING LATER DISEASE

When reactive, serologies indicate exposure only and do not directly indicate whether the spirochete is now currently present. Because Bb serologies often give inconsistent results, test at well-known reference laboratories. The suggestion that two-tiered testing, utilizing an ELISA as a screening tool, followed, if positive, by a confirmatory western blot, is illogical in this illness. The ELISA is not sensitive enough to serve as an adequate screen, and there are many patients with Lyme who test negative by ELISA yet have fully diagnostic western blots. I therefore recommend against using the ELISA. Order IgM and IgG western blots- but be aware that in late disease there may be repeatedly peaking IgM's and therefore a reactive IgM may not differentiate early from late disease, but it does suggest an active infection. When late cases of LB are seronegative, 36% will transiently become seropositive at the completion of successful therapy. In chronic Lyme Borreliosis, the CD-57 count is both useful and important (see below).

Western blots are reported by showing which bands are reactive. 41KD bands appear the earliest but can cross react with other spirochetes. The 18KD, 23-25KD (Osp C), 31KD (Osp A), 34KD (Osp B), 37KD, 39KD, 83KD and the 93KD bands are the species-specific ones, but appear later or may not appear at all. You should see at least the 41KD and one of the specific bands. 55KD, 60KD, 66KD, and 73KD are nonspecific and nondiagnostic.

PCR tests are now available, and although they are very specific, sensitivity remains poor, possibly less than 30%. This is because Bb causes a deep tissue infection and is only transiently found in body humors. Therefore, just as in routine blood culturing, multiple specimens must be collected to increase yield; a negative

result does not rule out infection, but a positive one is significant. You can test whole blood, buffy coat, serum, urine, spinal and other body fluids, and tissue biopsies. Several blood PCRs can be done, or you can run PCRs on whole blood, serum and urine simultaneously at a time of active symptoms. The patient should be antibioticfree for at least six weeks before testing to obtain the highest yield.

Antigen capture is becoming more widely available, and can be done on urine, CSF, and synovial fluid. Sensitivity is still low (on the order of 30%), but specificity is high (greater than 90%).

Spinal taps are not routinely recommended, as a negative tap does not rule out Lyme. Antibodies to Bb are mostly found in Lyme meningitis, and are rarely seen in non-meningitic CNS infection, including advanced encephalopathy. Even in meningitis, antibodies are detected in the CSF in less than 13% of patients with late disease! Therefore, spinal taps are only performed on patients with pronounced neurological manifestations in whom the diagnosis is uncertain, if they are seronegative, or are still significantly symptomatic after completion of treatment. When done, the goal is to rule out other conditions, and to determine if Bb (and Bartonella) antigens or nucleic acids are present. It is especially important to look for elevated protein and white cells, which would dictate the need for more aggressive therapy, as well as the opening pressure, which can be elevated and add to headaches, especially in children.

I strongly urge you to **biopsy** all unexplained skin lesions/rashes and perform PCR and careful histology. You

will need to alert the pathologist to look for spirochetes.

THE CD-57 TEST

Our ability to measure CD-57 counts represents a breakthrough in LB diagnosis and treatment.

Chronic LB infections are known to suppress the immune system and can decrease the quantity of the CD-57 subset of the natural killer cells. As in HIV infection, where abnormally low T-cell counts are routinely used as a marker of how active that infection is, in LB we can use the degree of decrease of the CD-57 count to indicate how active the Lyme infection is and whether, after treatment ends, a relapse is likely to occur. It can even be used as a simple, inexpensive screening test, because at this point we believe that only Borrelia will depress the CD-57. Thus, a sick patient with a high CD-57 is probably ill with something other than Lyme, such as a coinfection.

When this test is run by LabCorp (the currently preferred lab, as published studies were based on their assays), we want our Lyme patients to measure above 60; a normal count is above 200. There generally is some degree of fluctuation of this count over time, and the number does not progressively increase as treatment proceeds. Instead, it remains low until the LB infection is controlled, and then it will jump. If the CD-57 count is not in the normal range when a course of antibiotics is ended, then a relapse will almost certainly occur.

CHECK LIST OF ***CURRENT*** SYMPTOMS: This is not meant to be used as a diagnostic scheme, but is provided to streamline the office interview. Note the format- complaints referable to specific organ systems and specific co-infections are clustered to clarify diagnoses and to better display multisystem involvement.

Have you had any of the following in relation to this illness? (CIRCLE "NO" OR "YES")

Tick bite N Y "EM" rash (discrete circle) N Y

Spotted rash over large area N Y Linear, red streaks N Y

CURRENT SEVERITY CURRENT FREQUENCY

SYMPTOM OR SIGN NONE MILD MODERATE SEVERE NA NEVER OCCASIONAL OFTEN CONSTANT

Persistent swollen glands
Sore throat
Fevers
Sore soles, esp. in the AM
Joint pain
Fingers, toes
Ankles, wrists
Knees, elbows
Hips, shoulders
Joint swelling
Fingers, toes
Ankles, wrists
Knees, elbows
Hips, shoulders
Unexplained back pain
Stiffness of the joints or back
Muscle pain or cramps
Obvious muscle weakness
Twitching of the face or other muscles
Confusion, difficulty thinking
Difficulty with concentration, reading, problem absorbing new information
Word search, name block
Forgetfulness, poor short term memory, poor attention

Disorientation: getting lost, going to wrong places
Speech errors- wrong word, misspeaking
Mood swings, irritability, depression
Anxiety, panic attacks
Psychosis (hallucinations, delusions, paranoia, bipolar)
Tremor
Seizures
Headache
Light sensitivity
Sound sensitivity

Vision: double, blurry, floaters
Ear pain

CURRENT SEVERITY CURRENT FREQUENCY

SYMPTOM OR SIGN NONE MILD MODERATE SEVERE NA NEVER OCCASIONAL OFTEN CONSTANT

Hearing: buzzing, ringing, decreased hearing
Increased motion sickness, vertigo, spinning
Off balance, “tippy” feeling
Lightheadedness, wooziness, unavoidable need to sit or lie
Tingling, numbness, burning or stabbing sensations, shooting pains, skin hypersensitivity
Facial paralysis-Bell's Palsy
Dental pain
Neck creaks and cracks, stiffness, neck pain
Fatigue, tired, poor stamina
Insomnia, fractionated sleep, early awakening
Excessive night time sleep
Napping during the day
Unexplained weight gain
Unexplained weight loss
Unexplained hair loss
Pain in genital area
Unexplained menstrual irregularity
Unexplained milk production; breast pain
Irritable bladder or bladder dysfunction
Erectile dysfunction
Loss of libido
Queasy stomach or nausea

Heartburn, stomach pain
Constipation
Diarrhea
Low abdominal pain, cramps
Heart murmur or valve
prolapse?
Heart palpitations or skips
"Heart block" on EKG
Chest wall pain or ribs sore
Head congestion
Breathlessness, "air hunger",
unexplained chronic cough
Night sweats

Exaggerated symptoms or
worse hangover from alcohol
Symptom flares every 4 wks.
Degree of disability

DIAGNOSTIC CHECKLIST

To aid the clinician, a workable set of diagnostic criteria were developed with the input of dozens of front line physicians. The resultant document, refined over the years, has proven to be extremely useful not only to the clinician, but it also can help clarify the diagnosis for third party payers and utilization review committees.

It is important to note that the CDC's published reporting criteria are for surveillance only, not for diagnosis. They should not be misused in an effort to diagnose Lyme or set guidelines for insurance company acceptance of the diagnosis, nor be used to determine eligibility for coverage.

LYME BORRELIOSIS DIAGNOSTIC CRITERIA RELATIVE VALUE

Tick exposure in an endemic region..... 1
Historical facts and evolution of symptoms over time consistent with Lyme 2
Systemic signs & symptoms consistent with Bb infection (other potential diagnoses excluded):
Single system, e.g., monoarthritis 1
Two or more systems, e.g., monoarthritis and facial palsy 2
Erythema migrans, physician confirmed.. 7
Acrodermatitis Chronica Atrophicans, biopsy confirmed.... 7
Seropositivity.... 3
Seroconversion on paired sera.... 4
Tissue microscopy, silver stain... 3
Tissue microscopy, monoclonal immunofluorescence..... 4
Culture positivity 4
B. burgdorferi antigen recovery 4
B. burgdorferi DNA/RNA recovery 4

DIAGNOSIS

Lyme Borreliosis Highly Likely..... .... 7 or above
Lyme Borreliosis Possible..... 5-6
Lyme Borreliosis Unlikely..... ... . 4 or below

I suggest that when using these criteria, you state Lyme Borreliosis is "unlikely", "possible", or "highly likely" based upon the following criteria"- then list the criteria.

LYME DISEASE TREATMENT GUIDELINES

LYME BORRELIOSIS:
GENERAL INFORMATION

After a tick bite, Bb undergoes rapid hematogenous dissemination, and for example, can be found within the central nervous system as soon as *twelve hours* after entering the bloodstream. This is why even early infections require full dose antibiotic therapy with an agent able to penetrate all tissues in concentrations known to be bactericidal to the organism.

It has been shown that the longer a patient had been ill with LB prior to first definitive therapy, the longer the duration of treatment must be, and the need for more aggressive treatment increases.

More evidence has accumulated indicating the severe detrimental effects of the concurrent use of immunosuppressants including steroids in the patient with active B. burgdorferi infection. **Never give steroids or any other immunosuppressant to any patient who may even remotely be suffering from Lyme, or serious, permanent damage may result, especially if given for anything greater than a short course.** If immunosuppressive therapy is absolutely necessary, then potent antibiotic treatment should begin at least 48 hours prior to the immunosuppressants.

TREATMENT RESISTANCE

Bb contains beta lactamases and cephalosporinases, which, with some strains, may confer resistance to cephalosporins and penicillins. This is apparently a slowly acting enzyme system, and may be overcome by higher or more continuous drug levels especially when maintained by continuous infusions (cefotaxime) and by depot preparations (benzathine penicillin). Nevertheless, some penicillin and cephalosporin treatment failures do occur and have responded to sulbactam/ampicillin, imipenem, and vancomycin, which act through different cell wall mechanisms than the penicillins and the cephalosporins.

Vegetative endocarditis has been associated with Borrelia burgdorferi, but the vegetations may be too small to detect with echocardiography. Keep this in mind when evaluating patients with murmurs, as this may explain why some patients seem to continually relapse after even long courses of antibiotics.

COMBINATION THERAPY

Treatment of chronic Lyme usually requires combinations of antibiotics. There are four reasons for this:

1. TWO COMPARTMENTS- Bb can be found in both the fluid and the tissue compartments, yet no single antibiotic currently used to treat Bb infections will be effective in both compartments. This is one reason for the need to use combination therapy in the more ill patient. A logical combination might use, for example, azithromycin plus a penicillin.
2. INTRACELLULAR NICHE- Another reason, discussed below, is the fact that Bb can penetrate and remain viable within cells and evade the effects of extracellular agents. Typical combinations include an extracellular antibiotic, plus an intracellular agent such as an erythromycin derivative or metronidazole. Note that some experts discourage the co-administration of bactericidal plus bacteriostatic agents, thus the recommendation to avoid a cell wall drug combined with a tetracycline.
3. L-FORMS (SPHEROPLAST)- It has been recognized that B. burgdorferi can exist in at least two, and possibly three different morphologic forms: spirochete, spheroplast (or l-form), and the recently discovered cystic form (presently, there is controversy whether the cyst is different from the l-form). L-forms and cystic forms do not contain cell walls, and thus beta lactam antibiotics will not affect them. Spheroplasts seem to be susceptible to tetracyclines and the advanced erythromycin derivatives. Apparently, Bb can shift among the three forms during the course of the infection. Because of this, it may be necessary to cycle different classes of antibiotics and/or prescribe a combination of dissimilar agents.

4. CYSTIC FORM- When present in a hostile environment, such as growth medium lacking some nutrients, spinal fluid, or serum with certain antibiotics added, Bb can change from the spiral form ("spirochete") into a cyst form. This cyst seems to be able to remain dormant, but when placed into an environment more favorable to its growth, Bb can revert into the spirochete form. The antibiotics commonly used for Lyme do not kill the cystic form of Bb. However, there is laboratory evidence that metronidazole and tinidazole will disrupt it. Therefore, the chronically infected patient who has resistant disease may need to have metronidazole (or tinidazole) added to the regimen. More details are provided in the section on treatment options.

BORRELIA NEUROTOXIN (With thanks to Dr. Shoemaker)

Two groups have reported evidence that Borrelia, like several other bacteria, produce neurotoxins. These compounds reportedly can cause many of the symptoms of encephalopathy, cause an ongoing inflammatory reaction manifested as some of the virus-like symptoms common in late Lyme, and also potentially interfere with hormone action by blocking hormone receptors. At this time, there is no assay available to detect whether this compound is present, nor can the amount of toxin be quantified. Indirect measures are currently employed, such as measures of cytokine activation and hormone resistance. A visual contrast sensitivity test (VCS test) reportedly is quite useful in documenting CNS effects of the neurotoxin, and to follow effects of treatment. This test is available at some centers and on the internet.

It has been said that the longer one is ill with Lyme, the more neurotoxin is present in the body. It probably is stored in fatty tissues, and once present, persists for a very long time. This may be because of enterohepatic circulation, where the toxin is excreted via the bile into the intestinal tract, but then is reabsorbed from the intestinal tract back into the blood stream. This forms the basis for treatment.

Two prescription medications that can bind these toxins include cholestyramine resin and Welchol pills. When take orally in generous amounts, the neurotoxin present in the intestinal tract binds to the resin, is trapped, and then excreted. Thus, over several weeks, the level of neurotoxin is depleted and clinical improvement can be seen. Current experience is that improvement is first seen in three weeks, and treatment can continue for a month or more. Retreatment is always possible.

These medications may bind not only toxins but also many drugs and vitamin supplements. Therefore no other oral medications or supplements should be taken from a half hour before, to two hours after a dose of one of these fiber agents.

Cholestyramine should be taken two to four times daily, and Welchol is prescribed at three pills twice daily. While the latter is obviously much simpler to use, it is less effective than cholestyramine. The main side effects are bloating and constipation, best handled with increased fluid intake and gentle laxatives.

TREATING LYME BORRELIOSIS

LYME DISEASE TREATMENT INFORMATION

There is no universally effective antibiotic for treating LB. The choice of medication used and the dosage prescribed will vary for different people based on multiple factors. These include duration and severity of illness, presence of co-infections, immune deficiencies, prior significant immunosuppressant use while infected, age, weight, gastrointestinal function, blood levels achieved, and patient tolerance. Doses found to be effective clinically are often higher than those recommended in older texts. This is due to deep tissue penetration by Bb, its presence in the CNS including the eye, within cells, within tendons, and because very few of the many strains of this organism now known to exist have been studied for antibiotic susceptibility. In addition, all animal studies of susceptibility to date have only addressed early disease in models that behave differently than human hosts. Therefore, begin with a regimen appropriate to the setting, and if necessary, modify it over time based upon antibiotic blood level measurements and clinical response.

ANTIBIOTICS

There are four types of antibiotics in general use for Bb treatment. The TETRACYCLINES, including doxycycline and minocycline, are bacteriostatic unless given in high doses. If high blood levels are not attained, treatment failures in early and late disease are common. However, these high doses can be difficult to tolerate. For example, doxycycline can be very effective but only if adequate blood levels are achieved either by high oral doses (300 to 600 mg daily) or by parenteral administration. Kill kinetics indicate that a large spike in blood and tissue levels is more effective than sustained levels, which is why with doxycycline, oral doses of 200 mg bid is more effective than 100 mg qid. Likewise, this is why IV doses of 400 mg once a day is more effective than any oral regimen.

PENICILLINS are bactericidal. As would be expected in managing an infection with a gram negative organism such as Bb, amoxicillin has been shown to be more effective than oral penicillin V. With cell wall agents such as the penicillins, kill kinetics indicate that sustained bactericidal levels are needed for 72 hours to be effective. Thus the goal is to try to achieve sustained blood and tissue levels. However, since blood levels are extremely variable among patients, peak and trough levels should be measured (for details, refer to the antibiotic dosage table). Because of its short half-life and need for high levels, amoxicillin is usually administered along with probenecid. An extended release formulation of amoxicillin+clavulanate (“Augmentin XR”) may also be

considered if adequate trough levels are difficult to attain. An attractive alternative is benzathine penicillin ("Bicillin-LA"- see below). This is an intramuscular depot injection, and although doses are relatively small, the sustained blood and tissue levels are what make this preparation so effective.
CEPHALOSPORINS must be of advanced generation: first generation drugs are rarely effective and second generation drugs are comparable to amoxicillin and doxycycline both in-vitro and in-vivo. Third generation agents are currently the most effective of the cephalosporins because of their very low MBC's (0.06 for ceftriaxone), and relatively long half-life. Cephalosporins have been shown to be effective in penicillin and tetracycline failures. Cefuroxime axetil (Ceftin), a second generation agent, is also effective against staph and thus is useful in treating atypical erythema migrans that may represent a mixed infection that contains some of the more common skin pathogens in addition to Bb. Because this agent's G.I. side effects and high cost, it is not often used as first line drug. As with the penicillins, try to achieve high, sustained blood and tissue levels by frequent dosing and/or the use of probenecid. Measure peak and trough blood levels when possible.
When choosing a third generation cephalosporin, there are several points to remember: Ceftriaxone is administered twice daily (an advantage for home therapy), but has 95% biliary excretion and can crystallize in the biliary tree with resultant colic and possible cholecystitis. GI excretion results in a large impact on gut flora. Biliary and superinfection problems with ceftriaxone can be lessened if this drug is given in interrupted courses (known commonly as "pulse therapy"- refer to chapter on this on page 20), so the current recommendation is to administer it four days in a row each week. Cefotaxime, which must be given at least every eight hours or as a continuous infusion, is less convenient, but as it has only 5% biliary excretion, it never causes biliary concretions, and may have less impact on gut flora.
ERYTHROMYCIN has been shown to be almost ineffective as monotherapy. The azalide azithromycin is somewhat more effective but only minimally so when given orally. As an IV drug, much better results are seen. Clarithromycin is more effective as an oral agent than azithromycin, but can be difficult to tolerate due to its tendency to promote yeast overgrowth, bad aftertaste, and poor GI tolerance at the high doses needed. These problems are much less severe with the ketolide telithromycin, which is generally well tolerated.
Erythromycins (and the advanced generation derivatives mentioned above) have impressively low MBCs and they do concentrate in tissues and penetrate cells, so they theoretically should be ideal agents. So why is it that erythromycin ineffective, and why have initial clinical results with azithromycin (and to a lesser degree, clarithromycin) have been disappointing? It has been suggested that when Bb is within a cell, it is held within a vacuole and bathed in fluid of low pH, and this acidity may inactivate azithromycin and clarithromycin. Therefore, they are administered concurrently with hydroxychloroquine or amantadine, which raise vacuolar pH,

rendering these antibiotics more effective. It is not known whether this same technique will make erythromycin a more effective antibiotic in LB. Another alternative is to administer azithromycin parenterally. Results are excellent, but expect to see abrupt Jarisch-Herxheimer reactions.
Telithromycin, on the other hand, is stable in the intracellular acid environment, which may be why this is currently by far the most effective drug of this class, and may replace the others in the majority of patients with LB. Likewise, there is no need to co-administer amantadine or hydroxychloroquine. This antibiotic has other advantages- it has been engineered to prevent drug resistance, has almost no negative impact on E. coli in the intestinal tract (hopefully minimizing the risk for diarrhea), and it can be taken with or without food.
However, there are disadvantages:
1. May interact with a wide variety of medications because it is an inhibitor of the cytochrome CYP3A4. It is vital that this be taken into account as many Lyme patients take a variety of medications concurrently, and often from several practitioners.
2. May lengthen the QT interval. This should be measured prior to prescribing this drug, and if borderline, rechecked after it is begun.
3. Can transiently cause blurry vision, delayed accommodation, and even double vision.
4. Liver enzymes may become elevated. Blood tests should be done regularly to monitor this.
5. The usual precautions of any antibiotic also still apply- risk for allergy, stomach upset, Herxheimer reactions, etc.
QTc INTERVAL
· QTc is the QT corrected for heart rate

· Measure the precordial lead that has the best T wave (usually V-2 or V-5)
· Measure from the start of the Q wave to the end of the T wave
· QT interval is inversely related to the heart rate (slow pulse results in a longer QT)
· QTc = QT ¸ Ö RR interval
· Normals: Females <450 ms, Males < 470 ms
· Want K+ > 4.0, Mg++ > 2.0; avoid hypocalcemia

METRONIDAZOLE (Flagyl) When present in a hostile environment, such as growth medium lacking some nutrients, spinal fluid, or serum with certain antibiotics added, Bb can change into a cyst form. This cyst seems to be able to remain dormant, but when placed into an environment more favorable to its growth, the cyst can revert into the spirochete form. The conventional antibiotics used for Lyme, such as the penicillins, cephalosporins, etc do not kill the cystic form of Bb, yet there is laboratory evidence that metronidazole will kill it. Therefore, the trend now is to treat the chronically infected patient who has resistant disease by combining metronidazole with one or two other antibiotics to target all forms of Bb. Because there is laboratory evidence that tetracyclines may inhibit the effect of Flagyl, this class of medication should not be used in these two- and three-drug regimens. Some clinicians favor tinidazole as this may be equally effective but result in fewer side effects. However, this has yet to be documented.

Important precautions:

1. Pregnancy while on Flagyl is not advised, as there is a risk of birth defects.
2. No alcohol consumption! A severe, "Antabuse" reaction will occur, consisting of severe nausea, flushing, headache, and other symptoms.
3. Yeast overgrowth is especially common. A strict anti-yeast regimen must be followed.
4. Flagyl can be irritative to the nervous system- in the short term, it may cause irritability, "spacey" feelings, etc. Longer term, it can affect the peripheral nerves, causing tingles, numbness, etc. If mild, a change in dose may be required. Often, extra vitamin B can clear these symptoms. If the nerve symptoms persist or are strong, then metronidazole must be discontinued or these symptoms may become very long lasting.
5. Strong Herxheimer-like reactions are seen in almost everyone.

RIFAMPIN is a well-known antibiotic that has been in use for many decades. It is primarily used to treat tuberculosis, but also has been used in other conditions, such as prevention of meningitis in those exposed, for treating resistant Staph, etc. Potentially, rifampin may be effective in treating Bartonella, Ehrlichia, Mycoplasma, and Borrelia. There are as yet no formal clinical studies on the use of this medication in these illnesses, but many patients have been treated with rifampin and have had favorable results. When used, regular blood tests (CBC, liver enzymes) are usually performed to monitor for side effects. Rifampin can also discolor urine, tears and sweat (brownish-orange). It may also stain some types of water-permeable contact lenses. Taking rifampin during pregnancy is not advised. Finally, because this drug is an inducer of cytochromes (CYP3A4), co-administration with other medications may result in lower and more brief blood levels of the coadministered
drug. Thus, be aware of these potential drug interactions.

BENZATHINE PENICILLIN Comparative studies published by Fallon et. al. at Columbia University have shown that parenteral therapy is superior to oral therapy in chronic patients. Options include intramuscular long acting penicillin G (benzathine penicillin, or “Bicillin-LA”) or intravenous antibiotics.

For an antibiotic in the penicillin class to be effective, time-killing curves show that significant levels of antibiotic must be sustained for 72 hours. Bicillin LA is a sustained release formulation that meets these criteria.

Published studies in children and adults, combined with over a decade of experience with this therapy by front line, Lyme-treating physicians have established the efficacy, safety and usefulness of this medication. In many patients it is more effective than oral antibiotics for treating Lyme, and compares closely to intravenous therapy in terms of efficacy if the dose is high enough.

It is usually administered three or four times weekly for six to twelve months. It has the advantage of being relatively inexpensive, free of gastrointestinal side effects, unlikely to promote the overgrowth of yeast, and has an excellent safety record spanning many decades.

Finally, an added plus is that family members can be trained to administer this treatment at home.

CEFTRIAXONE TREATMENT A subset of patients who have severe, longstanding illness due to Borrelia burgdorferi carry persistent infection despite having previously received antibiotic treatments which have eliminated the disease in less ill individuals. The mechanism for such persistence has been the subject of many peer reviewed articles. They include persistence of B. burgdorferi in protective niches, inhibition and lysis of lymphocytes, survival in phagocytic vacuoles, antigenic shifts, slow growth, shifting into alternate forms, and dormancy and latency.

One successful approach in the more ill patient, published in the early 1990s, is to use higher doses of ceftriaxone in a pulsed-dose regimen. Since then, clinical experience has expanded upon this concept, and at the MLDA Lyme Congress in September, 2002, Cichon presented data on a pulsed, high dose regimen which supports and refines this concept. This regimen is now considered the current standard of care in the use of ceftriaxone.

Treatment with ceftriaxone is dosed at 4 grams daily- given either as 2 grams IV twice daily, or 4 grams slowly once a day, four days in a row each week, usually for 14 or more weeks. Such a regimen is not only more effective in the Chronic Lyme patient, but regular interruptions in treatment lessen the potential complications of intensive antibiotic therapy with ceftriaxone, such as biliary sludging and colitis. Hence a more effective, safer regimen that by virtue of the treatment breaks, is less costly and affords the patient a more acceptable lifestyle. IV access with a heparin lock becomes possible (and preferred).

COURSE DURING THERAPY

As the spirochete has a very long generation time (12 to 24 hours *in vitro* and possibly much longer in living systems) and may have periods of dormancy, during which time antibiotics will not kill the organism, treatment has to be continued for a long period of time to eradicate all the active symptoms and prevent a relapse,

especially in late infections. If treatment is discontinued before all symptoms of active infection have cleared, the patient will remain ill and possibly relapse further. In general, early LB is treated for four to six weeks, and late LB usually requires a minimum of four to six months of continuous treatment. All patients respond differently and therapy must be individualized. It is not uncommon for a patient who has been ill for many years to require open ended treatment regimens; indeed, some patients will require ongoing maintenance therapy for years to remain well.

Several days after the onset of appropriate antibiotic therapy, symptoms often flare due to lysis of the spirochetes with release of increased amount of antigenic material and possibly bacterial toxins. This is referred to as a Jarisch Herxheimer-like reaction. Because it takes 48 to 72 hours of therapy to initiate bacterial killing, the Herxheimer reaction is therefore delayed. This is unlike syphilis, in which these reactions can occur within hours.

It has been observed that symptoms will flare in cycles every four weeks. It is thought that this reflects the organism's cell cycle, with the growth phase occurring once per month (intermittent growth is common in Borrelia species). As antibiotics will only kill bacteria during their growth phase, therapy is designed to bracket at least one whole generation cycle. This is why the minimum treatment duration should be at least four weeks. If the antibiotics are working, over time these flares will lessen in severity and duration. The very occurrence of ongoing monthly cycles indicates that living organisms are still present and that antibiotics should be continued.

With treatment, these monthly symptom flares are exaggerated and presumably represent recurrent Herxheimer-like reactions as Bb enters its vulnerable growth phase and then are lysed. For unknown reasons, the worst occurs at the fourth week of treatment. Observation suggest that the more severe this reaction, the higher the germ load, and the more ill the patient. In those with long-standing highly symptomatic disease who are on I.V. therapy, the week-four flare can be very severe, similar to a serum sickness reaction, and be associated with transient leucopenia and/or elevations in liver enzymes. If this happens, decrease the dose temporarily, or interrupt treatment for several days, then resume with a lower dose. If you are able to continue or resume therapy, then patients continue to improve. Those whose treatment is stopped and not restarted at this point usually will need retreatment in the future due to ongoing or recurrent symptoms because the infection was not eradicated. Patients on I.V. therapy who have a strong reaction at the fourth week will need to continue parenteral antibiotics for several months, for when this monthly reaction finally lessens in severity, then oral or IM medications can be substituted. Indeed, it is just this observation that guides the clinician in

determining the endpoint of I.V. treatment. In general, I.V. therapy is given until there is a clear positive response, and then treatment is changed to IM or po until free of signs of active infection for 4 to 8 weeks. Some patients, however, will not respond to IM or po treatment and I.V. therapy will have to be used throughout. As mentioned earlier, leucopenia may be a sign of persistent Ehrlichiosis, so be sure to look into this. Repeated treatment failures should alert the clinician to the possibility of an otherwise inapparent immune deficiency, and a workup for this may be advised. Obviously, evaluation for co-infection should be performed, and a search for other or concurrent diagnoses needs to be entertained.

There are three things that will predict treatment failure regardless of which regimen is chosen: Noncompliance, alcohol use, and sleep deprivation. Advise them to take a break when (or ideally before) the inevitable mid afternoon fatigue sets in (napping is encouraged).

All patients must keep a carefully detailed daily diary of their symptoms to help us document the presence of the classic four week cycle, judge the effects of treatment, and determine treatment endpoint. One must follow such diaries, temperature readings in late afternoon, physical findings, notes from physical therapists, and cognitive testing to best judge when to change or end antibiotics.

Remember- there currently is no test for cure, so this clinical follow-up assumes a major role in Lyme Disease care.

ANTIBIOTIC CHOICES AND DOSES

ORAL THERAPY: Always check blood levels when using agents marked with an *, and adjust dose to achieve a peak level above ten and a trough greater than three. Because of this, the doses listed below may have to be raised. Consider Doxycycline first in early Lyme due to concern for Ehrlichia co-infections.

*Amoxicillin- Adults: 1g q8h plus probenecid 500mg q8h; doses up to 6 grams daily are often needed

Pregnancy: 1g q6h and adjust.

Children: 50 mg/kg/day divided into q8h doses.

*Doxycycline- Adults: 200 mg bid with food; doses of up to 600 mg daily are often needed, as doxycycline is only effective at high blood levels. Not for children or in pregnancy.

If levels are too low at tolerated doses, give parenterally or change to another drug.

*Cefuroxime axetil- Oral alternative that may be effective in amoxicillin and doxycycline failures. Useful in EM rashes co-infected with common skin pathogens.

Adults and pregnancy: 1g q12h and adjust. Children: 125 to 500 mg q12h based on weight.

Tetracycline- Adults only, and not in pregnancy. 500 mg tid to qid

Erythromycin- Poor response and not recommended.

Azithromycin- Adults: 500 to 1200 mg/d. Adolescents: 250 to 500 mg/d

Add hydroxychloroquine, 200-400 mg/d, or amantadine 100-200 mg/d

Cannot be used in pregnancy or in younger children.

Overall, poor results when administered orally

Clarithromycin- Adults: 250 to 500 mg q6h plus hydroxychloroquine, 200-400 mg/d, or amantadine 100-200 mg/d. Cannot be used in pregnancy or in younger children.

Clinically more effective than azithromycin

Telithromycin- Adolescents and adults: 800 mg once daily

Do not need to use amantadine or hydroxychloroquine

So far, the most effective drug of this class, and possibly the best oral agent if tolerated. Expect strong and quite prolonged Herxheimer reactions.

Must watch for drug interactions (CYP3A-4 inhibitor), check the QTc interval, and monitor liver enzymes.

Not to be used in pregnancy.

*Augmentin- Standard Augmentin cannot exceed three tablets daily due to the

clavulanate, thus is given with amoxicillin, so that the total dose of the amoxicillin component is as listed above for amoxicillin. This combination can be effective when Bb beta lactamase is felt to be significant.
*Augmentin XR 1000- This is a time-release formulation and thus is a better choice than standard Augmentin.
Dose- 1000 mg q 8 h, to 2000 mg q 12 h based on blood levels.
Chloramphenicol- Not recommended as not proven and potentially toxic.
Metronidazole: 500 to 1500 mg daily in divided doses. Non-pregnant adults only.

PARENTERAL THERAPY

Ceftriaxone- Risk of biliary sludging (therefore often Actigall is co-administered- one to three tablets daily).
Adults and pregnancy: 2g q12 h, 4 days in a row each week

Children: 75 mg/kg/day up to 2g/day
Cefotaxime- Comparable efficacy to ceftriaxone; no biliary complications.
Adults and pregnancy: 6g to 12g daily. Can be given q 8 h as divided doses, but a continuous infusion may be more efficacious. When exceeding 6 g daily, use pulsed-dose schedule
Children: 90 to 180 mg/kg/day dosed q6h (preferred) or q8h, not to exceed 12 g daily.
*Doxycycline- Requires central line as is caustic.
Surprisingly effective, probably because blood levels are higher when given parenterally and single large daily doses optimize kinetics of killing with this drug.
Always measure blood levels.
Adults: Start at 400 mg q24h and adjust based on levels.
Cannot be used in pregnancy or in younger children.
Azithromycin- Requires central line as is caustic.
Dose: 500 to 1000 mg daily in adolescents and adults.
Penicillin G- IV penicillin G is minimally effective and not recommended.
Benzathine penicillin- Surprisingly effective IM alternative to oral therapy. May need to begin at lower doses as strong, prolonged (6 or more week) Herxheimer-like reactions have been observed.
Adults: 1.2 million U- three to four doses weekly.
Adolescents: 1.2 to 3.6 million U weekly.
May be used in pregnancy.
Vancomycin- observed to be one of the best drugs in treating Lyme, but potential toxicity limits its use. It is a perfect candidate for pulse therapy to minimize these concerns. Use standard doses and confirm levels.
Primaxin and Unisyn- similar in efficacy to cefotaxime, but often work when cephalosporins have failed.
Must be given q6 to q8 hours.
Cefuroxime- useful but not demonstrably better than ceftriaxone or cefotaxime.
*Ampicillin IV- more effective than penicillin G. Must be given q6 hours.

TREATMENT CATEGORIES

PROPHYLAXIS of high risk groups- education and preventive measures. Antibiotics are not given.
TICK BITES - Embedded Deer Tick With No Signs or Symptoms of Lyme (see appendix):
Decide to treat based on the type of tick, whether it came from an endemic area, how it was removed, and length of attachment (anecdotally, as little as four hours of attachment can transmit pathogens). The risk of transmission is greater if the tick is engorged, or of it was removed improperly allowing the tick's contents to spill into the bite wound. High-risk bites are treated as follows (remember the possibility of co-infection!):
1) Adults: Oral therapy for 28 days.
2) Pregnancy: Amoxicillin 1000 mg q6h for 6 weeks. Test for Babesia, Bartonella and Ehrlichia.

Alternative: Cefuroxime axetil 1000 mg q12h for 6 weeks.
3) Young Children: Oral therapy for 28 days.
EARLY LOCALIZED - Single erythema migrans with no constitutional symptoms:
1) Adults: oral therapy- must continue until symptom and sign free for at least one month, with a 6 week minimum.
2) Pregnancy: 1st and 2nd trimesters: I.V. X 30 days then oral X 6 weeks
3rd trimester: Oral therapy X 6+ weeks as above.
Any trimester- test for Babesia and Ehrlichia
3) Children: oral therapy for 6+ weeks.
DISSEMINATED DISEASE - Multiple lesions, constitutional symptoms, lymphadenopathy, or any other

manifestations of dissemination.
EARLY DISSEMINATED: Milder symptoms present for less than one year and not complicated by immune deficiency or prior steroid treatment:
1) Adults: oral therapy until no active disease for 4 to 8 weeks (4-6 months typical)
2) Pregnancy: As in localized disease, but treat throughout pregnancy.
3) Children: Oral therapy with duration based upon clinical response.
PARENTERAL ALTERNATIVES for more ill patients and those unresponsive to or intolerant of oral medications:
1) Adults and children: I.V. therapy until clearly improved, with a 6 week minimum. Follow with oral therapy or IM benzathine penicillin until no active disease for 6-8 weeks. I.V. may have to be resumed if oral or IM therapy fails.
2) Pregnancy: IV then oral therapy as above.
LATE DISSEMINATED: present greater than one year, more severely ill patients, and those with prior significant steroid therapy or any other cause of impaired immunity:
1) Adults and pregnancy: extended I.V. therapy (14 or more weeks), then oral or IM, if effective, to same endpoint. Combination therapy with at least two dissimilar antibiotics almost always needed.
2) Children: IV therapy for 6 or more weeks, then oral or IM follow up as above. Combination therapy usually needed.
CHRONIC LYME DISEASE (PERSISTENT/RECURRENT INFECTION)
By definition, this category consists of patients with active infection, of a more prolonged duration, who are more likely have higher spirochete loads, weaker defense mechanisms, possibly more virulent or resistant strains, and probably are significantly co-infected. Neurotoxins may also be significant in these patients. Search for and treat for all of these, and search for concurrent infections including viruses, chlamydias, and mycoplasmas. Be sure to do an endocrine workup if indicated. These patients require a full evaluation for all of these problems, and each abnormality must be addressed.
This group will most likely need parenteral therapy, especially high dose, pulsed therapy, and antibiotic combinations, including metronidazole. Antibiotic therapy will need to continue for many months, and the antibiotics may have to be changed periodically to break plateaus in recovery. Be vigilant for treatment-related problems such as antibiotic-associated colitis, yeast overgrowth, intravenous catheter complications, and abnormalities in blood counts and chemistries.
If treatment can be continued long term, then a remarkable degree of recovery is possible. However, attention must be paid to all treatment modalities for such a recovery- not only antibiotics, but rehab and exercise programs, nutritional supplements, enforced rest, low carbohydrate, high fiber diets, attention to food sensitivities, avoidance of stress, abstinence from caffeine and alcohol, and absolutely no immunosuppressants, even local doses of steroids (intra-articular injections, for example).
Unfortunately, not all patients with chronic Lyme disease will fully recover and treatment may not eradicate the active Borrelia infection. Such individuals may have to be maintained on open-ended, ongoing antibiotic therapy, for they repeatedly relapse after antibiotics are stopped. Maintenance antibiotic therapy in this select group is thus mandatory.
In patients who have chronic Lyme, who do not fully respond to antibiotics, one must search for an explanation.

In many cases, these patients are found to have pituitary insufficiency of varying degrees. The abnormalities may be extremely subtle, and provocative testing must be done for full diagnosis. Persistent fatigue, limited stamina, hypotension, and loss of libido suggest this possibility.

Similarly, a small but significant number of these patients harbor toxic levels of heavy metals. Challenge testing by knowledgeable, experienced clinicians is necessary for evaluation. Treatment must be directed toward correcting the specific abnormalities found, and post-treatment retesting to assess efficacy of treatment and endpoint of therapy should be done. Suspect this when poor immune responsiveness and persistent neuropathic signs and symptoms are present.

INDICATORS FOR PARENTERAL THERAPY

(The following are guidelines only and are not meant to be absolute. It is based on retrospective study of over 600 patients with late Lyme disease.)

· Illness for greater than one year
· Prior immunosuppressive therapy while infected with Bb.
· Major neurological involvement
· Active synovitis with high sedimentation rate
· Elevated protein or cells in the CSF

ADVANCED TREATMENT OPTIONS

PULSE THERAPY consists of administering antibiotics (usually parenteral ones) two to four days in a row per week. This allows for several advantages:

* Dosages are doubled (ie: cefotaxime, 12 g daily), increasing efficacy
* More toxic medications can be used with increased safety (ie: vancomycin)
* May be effective when conventional, daily regimens have failed.
* IV access may be easier or more tolerable
* More agreeable lifestyle for the patient
* Often less costly than daily regimens

Note that this type of treatment is expected to continue for a minimum of ten weeks, and often must continue beyond twenty weeks. The efficacy of this regimen is based on the fact that it takes 48 to 72 hours of continuous bactericidal antibiotic levels to kill the spirochete, yet it will take longer than the four to five days between pulses for the spirochetes to recover. As with all Lyme treatments, specific dosing and scheduling must be tailored to the individual patient's clinical picture based upon the treating physician's best clinical judgment.

COMBINATION THERAPY (see page 12)

This consists of using two or more dissimilar antibiotics simultaneously for antibiotic synergism, to better compensate for differing killing profiles and sites of action of the individual medications, and to cover the three known forms of Bb. A typical combination is the use of a cell wall agent plus a protein inhibitor (ie: amoxicillin plus clarithromycin). Note that GI intolerance and yeast superinfections are the biggest drawbacks to this type of treatment. However, these complications can often be prevented or easily treated, and the clinically observed benefits of this type of regimen clearly have outweighed these problems in selected patients.

LYME DISEASE AND PREGNANCY

It is well known that B. burgdorferi can cross the placenta and infect the fetus. In addition, breast milk from infected mothers has been shown to harbor spirochetes that can be detected by PCR and grown in culture. The Lyme Disease Foundation in Hartford, CT had kept a pregnancy registry for eleven years beginning in the late 1980s. They found that if patients were maintained on adequate doses of antibiotic therapy during gestation, then no babies were born with Lyme. My own experience over the last twenty years agrees with this. The options for treating the mother include oral, intramuscular, and intravenous therapy as outlined above. It is vital that peak and trough antibiotic levels be measured if possible at the start of gestation and at least once more during treatment.

During pregnancy, symptoms generally are mild as the hormonal changes seem to mask many symptoms.

However, post-partum, mothers have a rough time, with a sudden return of all their Lyme symptoms including profound fatigue. Post partum depression can be particularly severe. I always advise help in the home for at least the first month, so adequate rest and time for needed treatments are assured.
I also advise against breast feeding for obvious reasons as mentioned above.

MONITORING THERAPY

Drug levels are measured, where possible, to confirm adequate dosing. Often, the regimen may have to be modified to optimize the dose. This may have to be repeated again at any time major changes in the treatment regimen occur, and serially during pregnancy. With parenteral therapy, CBC and chem/liver panels are done at least twice each month, especially during symptom flares, with urinalysis and pro-time monitored less frequently.

SAFETY

Over two decades of experience in treating thousands of patients with Lyme has proven that therapy as described above, although intense, is generally well tolerated. The most common adverse reaction seen is allergy to probenecid. In addition, yeast superinfections are seen, but these are generally easily recognized and managed. The induction of Clostridium difficile toxin production is seen most commonly with ceftriaxone, but can occur with any of the antibiotic regimens mentioned in this document. However, pulsed dose therapy and regular use of the lactobacillus preparations seems to be helpful in controlling yeast and antibiotic related colitis, as the number of cases of C. difficile in Lyme patients is low when these guidelines are followed. Be sure to test stool for both toxin A and toxin B when evaluating for C. difficile colitis.
When using central intravenous lines including PICC lines (peripherally inserted central catheters), if ANY line problems arise, it is recommended that the line be pulled for patient safety. Salvage attempts (urokinase, repairing holes) are often ineffective and may not be safe.
Please advise all patients who take the tetracyclines of skin and eye sensitivity to sunlight and the proper precautions, and advise birth control if appropriate. When doxycycline is given parenterally, do not refreeze the solution prior to use!
Remember, years of experience with chronic antibiotic therapy in other conditions, including rheumatic fever, acne, gingivitis, recurrent otitis, recurrent cystitis, COPD, bronchiectasis, and others have not revealed any consistent dire consequences as a result of such medication use. Indeed, the very real consequences of untreated, chronic persistent infection by B. burgdorferi can be far worse than the potential consequences of this treatment.

CO-INFECTIONS IN LYME

PIROPLASMOSIS (Babesiosis)

GENERAL INFORMATION

It had been thought that Babesia microti is the only significant piroplasm affecting humans. Now it is believed that many of the over two dozen known species of piroplasms can be carried by ticks and potentially be transmitted to the human. Unfortunately, we have no widely available tests for these non-microti species. That is why, again, a clinical diagnosis is required.
Piroplasms are not bacteria, they are protozoans. Therefore, they will not be eradicated by any of the currently used Lyme treatment regimens. Therein lies the significance of co-infections- if a Lyme patient has been extensively treated yet is still ill, and especially if they are experiencing atypical symptoms, suspect a coinfection.
From the literature:

· "Co-infection generally results in more intense acute illness, a greater array of symptoms, and a more prolonged convalescence than accompany either infection alone."
· "Spirochete DNA was evident more often and remained in the circulation longer in co-infected subjects than in those experiencing either infection alone."
· "Co-infection might also synergize spirochete-induced lesions in human joints, heart and nerves."
· "Babesia infections may impair human host defense mechanisms…"
· "The possibility of concomitant Babesia infection should be considered when moderate to severe Lyme Disease has been diagnosed."

Babesia infection is becoming more commonly recognized, especially in patients who already have Lyme Disease. It has been published that as many as 66% of Lyme patients show serologic evidence of co-infection

with Babesia microti. It has also been reported that Babesia infections can range in severity from mild, subclinical infection, to fulminant, potentially life threatening illness. Subclinical infection is often missed because the symptoms are incorrectly ascribed to Lyme. Babesia infections, even mild ones, may recur even after treatment and cause severe illness. This phenomenon has been reported to occur at any time, including up to several years after the initial infection! Furthermore, such Babesia carriers pose a risk to the blood supply as this infection has been reported to be passed on by blood transfusion.

SYMPTOMS

Clues to the presence of Babesiosis include a more acute initial illness- patients often recall a high fever and chills at the onset of their Lyme. Over time, they can note night sweats, air hunger, an occasional cough, persistent migraine-like headache, a vague sense of imbalance without true vertigo, encephalopathy and fatigue. The fulminant presentations are seen in those who are immunosuppressed, especially if asplenic, and in advanced ages. They include high fevers, shaking chills and hemolysis, and can be fatal.

DIAGNOSTIC TESTS

Diagnostic tests are insensitive and problematic. There are at least thirteen, and possibly as many as two dozen Babesia forms found in ticks, yet we can currently only test for B. microti and WA-1 with our serologic and nuclear tests. Standard blood smears reportedly are reliable for only the first two weeks of infection, thus are not useful for diagnosing later infections and milder ones including carrier states where the germ load is too low to be detected. Therefore, multiple diagnostic test methods are available and each have their own benefits and limitations and often several tests must be done. Be prepared to treat based on clinical presentation, even with negative tests.

· SEROLOGY- Unlike Lyme, Babesia titers can reflect infection status. Thus, persistently positive titers or western blots suggest persistent infection.

· PCR- This is more sensitive than smears for B. microti, but will not detect other species.

· ENHANCED SMEAR- This utilizes buffy coat, prolonged scanning (up to three hours per sample!) and digital photography through custom-made microscopes. Although more sensitive than standard smears, infections can still be missed. The big advantage is that it will display multiple species, not just B. microti.

· FLUORESCENT IN-SITU HYBRIDIZATION ASSAY (FISH)- This technique is also a form of blood smear. It is said to be 100-fold more sensitive than standard smears for B. microti, because instead of utilizing standard, ink-based stains, it uses a fluorescent-linked RNA probe and ultraviolet light. The Babesia organisms are then much easier to spot when the slides are scanned. The disadvantage is that currently only B. microti is detected.

TREATMENT

Treating Babesia infections had always been difficult, because the therapy that had been recommended until 1998 consisted of a combination of clindamycin plus quinine. Published reports and clinical experience have shown this regimen to be unacceptable, as nearly half of patients so treated have had to abandon treatment due to serious side effects, many of which were disabling. Furthermore, even in patients who could tolerate these drugs, there was a failure rate approaching 50%.

Because of these dismal statistics, the current regimen of choice for Babesiosis is the combination of atovaquone (Mepron, Malarone), 750 mg bid, plus an erythromycin-type drug, such as azithromycin (Zithromax), clarithromycin (Biaxin), or telithromycin (Ketek) in standard doses. This combination was initially studied in animals, and then applied to Humans with good success. Fewer than 5% of patients have to halt treatment due to side effects, and the success rate is clearly better than that of clindamycin plus quinine.

The duration of treatment with atovaquone combinations for Babesiosis varies depending on the degree of infection, duration of illness before diagnosis, the health and immune status of the patient, and whether the patient is co-infected with Borrelia burgdorferi. Typically, a three-week course is prescribed for acute cases, while chronic, longstanding infections with significant morbidity and co-infection will require a minimum of four months of therapy. Relapses have occurred, and retreatment is occasionally needed.

Problems during therapy include diarrhea, mild nausea, the expense of atovaquone (over $600.00 per bottleenough for three weeks of treatment), and rarely, a temporary yellowish discoloration of the vision. Blood counts, liver panels and amylase levels are recommended every three weeks during any prolonged course of

therapy as liver enzymes may elevate. Treatment failures usually are related to inadequate atovaquone levels. Therefore, patients who are not cured with this regimen can be retreated with higher doses (and atovaquone blood levels can be checked), as this has proven effective in many of my patients. Artemesia (a nonprescription herb) should be added in all cases. Metronidazole or Bactrim can also be added to increase efficacy, but there is minimal clinical data on how much more effective this will be.

BARTONELLA-LIKE ORGANISMS

It has been said that Bartonella is the most common of all tick-borne pathogens. Indeed, there seems to be a fairly distinct clinical syndrome when this type of organism is present in the chronic Lyme patient. However, several aspects of this infection seem to indicate that this tick-associated strain of Bartonella is different from that described as "cat scratch disease". For example, in patients who fit the clinical picture, standard Bartonella blood testing is commonly non-reactive. Furthermore, the usual Bartonella medications do not work for this- they suppress the symptoms but do not permanently clear them. For these reasons I like to refer to this as a "Bartonella-like organism" (BLO), rather than assume it is a more common species.

Indicators of BLO infection include CNS symptoms out of proportion to the other systemic symptoms of chronic Lyme. There seems to be an increased irritability to the CNS, with agitation, anxiety, insomnia, and even seizures, in addition to other unusually strong symptoms of encephalitis, such as cognitive deficits and confusion. Other key symptoms may include gastritis, lower abdominal pain (mesenteric adenitis), sore soles, especially in the AM, tender subcutaneous nodules along the extremities, and red rashes. These rashes may have the appearance of red streaks like stretch marks that do not follow skin planes, spider veins, or red papular eruptions. Lymph nodes may be enlarged and the throat can be sore.

Because standard Bartonella testing, either by serology or PCR, may not pick up this BLO, the blood test is very insensitive. Therefore, the diagnosis is a clinical one, based on the above points. Also, suspect infection with BLO in extensively treated Lyme patients who still are encephalitic, and who never had been treated with a significant course of specific treatment.

The drug of choice to treat BLO is levofloxacin. Levofloxacin is usually never used for Lyme or Babesia, so many patients who have tick-borne diseases, and who have been treated for them but remain ill, may in fact be infected with BLO. Treatment consist of 500 mg daily (may be adjusted based on body weight) for at least one month. Treat for three months or longer in the more ill patient. It has been suggested that levofloxacin may be more effective in treating this infection if a proton pump inhibitor is added in standard doses.

Another subtlety is that certain antibiotic combinations seem to inhibit the action of levofloxacin, while others seem to be neutral. I advise against using an erythromycin-like drug, as clinically such patients do poorly. On the other hand, combinations with cephalosporins, penicillins and tetracyclines are okay. Alternatives to

levofloxacin include rifampin, gentamicin and possibly streptomycin. A very recent article suggests that prior use of quinine-like drugs including atovaquone (Mepron, Malarone) may render Levaquin less effective. Therefore, in a co-infected patient, treat the BLO before you address Babesia species.

Levofloxacin is generally well tolerated, with almost no stomach upset. Very rarely, it can cause confusion- this is temporary (clears in a few days) and may be relieved by lowering the dose. There is, however, one side effect that would require it to be stopped- it may cause a painful tendonitis, usually of the largest tendons. If this happens, then the levofloxacin must be stopped or tendon rupture may occur. It has been suggested that loading the patient with magnesium may prevent this problem, and if the tendons do become affected, parenteral high dose vitamin C (plus parenteral magnesium) may afford rapid relief.

Unfortunately, levofloxacin and drugs in this family cannot be given to those under the age of 18, so other alternatives, such as azithromycin, are used in children.

Incidentally, animal studies show that Bartonella may be transmitted across the placenta. No human studies have been done.

EHRLICHIA (AND ANAPLASMA)

GENERAL INFORMATION

While it is true that this illness can have a fulminant presentation, and may even become fatal if not treated, milder forms do exist, as does chronic low-grade infection, especially when other tick-borne organisms are present. The potential transmission of Ehrlichia during tick bites is the main reason why doxycycline is now the first choice in treating tick bites and early Lyme, before serologies can become positive. When present alone or

co-infecting with B. burgdorferi, persistent leukopenia is an important clue. Thrombocytopenia and elevated liver enzymes, common in acute infection, are less often seen in those who are chronically infected, but likewise should not be ignored. Headaches, myalgias, and ongoing fatigue suggest this illness, but are extremely difficult to separate from symptoms caused by Bb.

DIAGNOSTIC TESTING

Testing is problematic with Ehrlichia, similar to the situation with Babesiosis. More species are known to be present in ticks than can be tested for with clinically available serologies and PCRs. In addition, serologies and PCRs are of unknown sensitivity and specificity. Standard blood smears for direct visualization of organisms in leukocytes are of low yield. Enhanced smears using buffy coats significantly raise sensitivity and can detect a wider variety of species. Despite this, infection can be missed, so clinical diagnosis remains the primary diagnostic tool. Again, consider this diagnosis in a Lyme Borreliosis (LB) patient not responding well to Lyme therapy who has symptoms suggestive of Ehrlichia.

TREATMENT

Standard treatment consists of Doxycycline, 200 mg daily for two to four weeks. Higher doses, parenteral therapy, and longer treatment durations may be needed based on the duration and severity of illness, and whether immune defects or extreme age is present. However, there are reports of treatment failure even when higher doses and long duration treatment with doxycycline is given. In such cases, consideration may be given for adding rifampin, 600 mg daily, to the regimen.

SORTING OUT THE CO-INFECTIONS

In addition to *Borrelia burgdorferi* (Bb), ticks may carry and transmit other infections. Furthermore, patients with disseminated Lyme complicated by these co-infections are usually immunocompromized and may also manifest signs and symptoms of reactivated latent infections and opportunists. All can add to morbidity and may need to be treated.

Because of the large number of these other infections, the cost of reliably testing for all of them as a matter of routine is prohibitive. Also, as in the case with Bb infection, laboratory tests for them are often insensitive. Thus there is a need to sort it all out clinically to provide guidance in testing and treatment. Here are some clues:

CLASSIC LYME (Bb infection)-

· Gradual onset of initial (viral-like) symptoms- this often makes it difficult to pinpoint when the infection began.

· Multisystem- almost always, in disseminated stages, involves more than one part or system (i.e. joint pain plus cognitive dysfunction).

· Migratory- first a knee will hurt, then over time this may lessen and the elbow or shoulder acts up, and later the joints calm down but headaches worsen.

· Stiff joints and loud joint crepitus, especially the neck (“Lyme shrug”).

· Headaches are often nuchal and associated with stiff, painful and crepitant neck.

· Afternoon fevers, often unnoticed- most Lyme patients have subnormal temperatures in the AM but rise to 99+ by early to mid-afternoon. No obvious sweats.

· Tiredness and limited stamina- often is a strong need to rest or even nap in the afternoon, especially when the flushed face and elevated temperature appears.

· 4-week cycles- Bb activity, and thus symptoms, wax and wane in a cycle that repeats roughly every four weeks. This cycle, if clear, can guide your treatments.

· Slow response to treatment, with an initial symptom flare in most (“Herxheimer-like reaction”) then improvement over weeks, punctuated by the monthly symptom flares. Likewise, if treatment is ended too soon, an initial period of well-being will gradually, over a few weeks, be replaced by a return of symptoms.

· EM rash in 25% to 50%

BARTONELLA & ”BARTONELLA-LIKE ORGANISMS”-

· Gradual onset of initial illness.

· CNS symptoms are out of proportion to the musculoskeletal ones- if a patient has no or minimal joint complaints but is severely encephalopathic (see below), then think of Bartonella/BLO.

· Obvious signs of CNS irritability can include muscle twitches, tremors, insomnia, seizures, agitation,

anxiety, severe mood swings, outbursts and antisocial behavior.
· GI involvement may present as gastritis or abdominal pain (mesenteric adenitis).
· Sore soles, especially in the morning.
· Tender sub-cutaneous nodules along the extremities, especially outer thigh, shins, and occasionally along the triceps.
· Occasional lymphadenopathy.
· Morning fevers, usually around 99. Occasionally light sweats are noted.
· Elevated vascular endothelial growth factor (VEGF) occurs in a minority, but the degree of elevation correlates with activity of the infection and may be used to monitor treatment.
· Rapid response to treatment changes- often symptoms improve within days after antibiotics are begun, but relapses occur also within days if medication is withdrawn early.
· May have papular or linear red rashes (like stretch marks that do not always follow skin planes), especially in those with GI involvement.

BABESIA SPECIES-

· Rapid onset of initial illness, often with sudden onset of high fever, severe headaches, sweats and fatigue, thus it is easy to know when infection began.
· Obvious sweats, usually at night, but can be day sweats as well.
· Air hunger, need to sigh and take a deep breath; dry cough without apparent reason.
· Headaches can be severe - dull, global (involves the whole head, described like the head is in a vise).
· Fatigue is prominent, does not clear with rest, and is made worse with exercise.
· Mental dullness and slowing of reactions and responses.
· Dizziness- more like a tippy feeling, and not vertigo or purely orthostasis.
· Symptoms cycle rapidly, with flares every four to six days.
· Hypercoaguable states are often associated with *Babesia* infections.
· Rarely, splenomegaly

· Very severe Lyme Disease can be a clue to *Babesia* infection, as it will make Lyme symptoms worse and Lyme treatments less effective.

EHRLICHIA/ANAPLASMA-

· Rapid onset of initial illness with fever, headache, prostration.
· Headaches are sharp, knife-like, and often behind the eyes.
· Muscle pain, not joint pain, and can be mild or severe.
· Low WBC count, elevated liver enzymes, and (rarely) inclusions seen in the WBCs.
· Rarely see diffuse vasculitic rash, including palms and soles (less than 10%).
· Rapid response to treatment.

DNA VIRUSES (HHV-6, EBV, CMV)

· Persistent fatigue, made worse with exercise.
· Sore throat, lymphadenopathy, and other viral-like complaints.
· May see elevated liver enzymes and low WBC counts.
· Autonomic dysfunction.

SUPPORTIVE THERAPY

CERTAIN **ABSOLUTE RULES** MUST BE FOLLOWED IF LYME SYMPTOMS ARE TO BE PERMANENTLY CLEARED:

1. Not allowed to get behind in sleep, or become overtired.
2. No caffeine or other stimulants that may affect depth or duration of sleep, or reduce or eliminate naps.
3. Absolutely no alcohol!
4. No smoking at all.
5. Aggressive exercises are required and should be initiated as soon as possible.
6. Diet must contain generous quantities of high quality protein and be high in fiber and low in fat and carbohydrates- no simple carbohydrates are allowed. Instead, use those with low glycemic index.
7. Certain key nutritional supplements should be added.
8. COMPLIANCE!

NUTRITIONAL SUPPLEMENTS IN DISSEMINATED LYME DISEASE
BACKGROUND INFORMATION
Studies on patients with chronic illnesses such as Lyme and Chronic Fatigue have demonstrated that some of the late symptoms are related to cellular damage and deficiencies in certain essential nutrients. Double blinded, placebo controlled studies, and in one case direct assay of biopsy specimens have proven the value of some of the supplements listed. Some are required, while others are optional -see below. They are listed in order of importance.
I suggest patients use a pill organizer. These are multi-compartment boxes that you pre-fill with your pills once a week. This makes the task of taking a large number of tablets much, much simpler and can markedly minimize missed doses. The Vitamin Shop sells a variety of good organizers.
I have found that the quality of supplements used is often more important than the dose. In fact, I do not recommend "mega doses". Instead, seek out, if possible, pharmaceutical grade products, especially if USP certified. I recommend, among others, Pharmanex, Researched Nutritionals and Nature Made products because they fit these criteria. In the list below, it is indicated whether the products should be gotten from Pharmanex, Researched Nutritionals, a different specific manufacturer, or even if a generic substitute is OK.

To order products from Pharmanex, users need to register as a customer with a referral from another registered customer. You may use my referral number (US9256681) to get started. Call 1-800-487-1000.
To order products from Researched Nutritionals, patients will need a physician's referral. If you do not have your own account, you may use my name when ordering. Call 1-800-755-3402.
Nature Made products are widely available in vitamin stores and pharmacies.
BASIC DAILY REGIMEN (in order of importance)
1. PROBIOTICS (required when on antibiotics)
Kefir: This is a yogurt-like drink that is said to more permanently replenish beneficial flora. It is only necessary to drink 2 to 4 ounces a day.
Acidophilus: the best kinds are frozen or refrigerated to ensure potency. Usual dose is two with each meal. Plan to mix together several different brands to broaden the spectrum. Acidophilus can be gotten from most vitamin stores but some generic brands are of unknown freshness and potency. An alternative that does not need refrigeration and can be taken only once a day is a high potency, patented product called "**Pro Bio**" from Pharmanex. The ultimate mix of pre- and probiotics with soil based organisms is a product called "**Prescript-Assist Pro**" from Researched Nutritionals. This too does not need refrigeration.
In addition, have 4 ounces of sugar-free **yogurt** on occasion.
2. MULTI-VITAMIN (required)
I recommend the **Life Pack** family of multivitamins available through Pharmanex. These are unique supplements- pharmaceutical grade and USP certified, they are the only products clinically proven in double blinded, placebo controlled crossover studies to quench free radicals and raise antioxidant levels in the blood and lipids. Choose LifePak for males under 40, LifePak Women for hormonally active women, LifePak Prenatal when pregnant, and LifePak Prime for postmenopausal women and for men over 40. LifePak Teen is also available. Continue long term.
3. CO-Q10- ***required, but do not use while taking the prescription drug atovaquone (Mepron, Malarone).***
Deficiencies have been related to poor function of the heart, limitations of stamina, gum disease, and poor resistance to infections. Heart biopsy studies in Lyme patients indicated that they should take between 300 and 400mg daily. I recommend the Co Q-10 from Researched Nutritionals. One caplet contains 400 mg, so the dose is one a day with food.
4. ALPHA LIPOIC ACID (required)
This facilitates entry of CoQ-10 into mitochondria. Dose is 300 mg twice daily. Generic is OK.
5. VITAMIN B (required).
Clinical studies demonstrated the need for supplemental vitamin B in infections with Borrelia, to help clear neurological symptoms. Take one 50 mg B-complex capsule daily. If neuropathy is severe, an additional 50 mg of B-6 can be added. Generics are OK.
6. MAGNESIUM (required)
Magnesium supplementation is very helpful for the tremors, twitches, cramps, muscle soreness, heart skips

and weakness. It may also help in energy level and cognition. The best source is magnesium L-lactate dehydrate ("**Mag-tab SR**", sold by Niche Pharmaceuticals: 1-800-677-0355, and available at Wal-Mart). DO NOT rely on "cal-mag", calcium plus magnesium combination tablets, as they are not well absorbed. Take at least one tablet twice daily. Higher doses increase the benefit and should be tried, but may cause diarrhea. In some cases, intramuscular or intravenous doses may be necessary.

7. ESSENTIAL FATTY ACIDS: (required)

Studies show that when EFAs are taken regularly, statistically significant improvements in fatigue, aches weakness, vertigo, dizziness, memory, concentration and depression are likely. There are two broad classes: GLA (omega-6 oils) and EPA (omega-3 oils), derived respectively from plant and fish oils. This is what to take:

Plant Oil: Use a refrigerated liquid product of mixed omega oils obtained from the local health food store (always avoid capsules as the plant oils within may be rancid and you would never know). Take one to two tablespoons of the liquid oil daily. May be mixed with food, put on salads, etc.

Fish Oil: Use "**Marine Omega**" by Pharmanex. Use four daily, taken on a full stomach (this brand is required because it is made not from fish, but from Krill and is certified to be free of any measurable amounts of heavy metals and organic toxins).

8. NT-FACTOR

This product addresses the mitochondrial damage thought to underlie the metabolic dysfunction associated with chronic diseases which, in patients with tick-borne illnesses, is manifest by fatigue and neurologic dysfunction. *It is the single most reliable agent I have found that can give noticeably increased energy levels.* When supplements known to support neurological function are added (see below), improved cognition and memory often result. Effects will be noted in two to three weeks. It also contains high quality prebiotics and probiotics. Available from Researched Nutritionals.

OPTIONAL SUPPLEMENTS FOR SPECIAL CIRCUMSTANCES

FOR NEUROLOGIC SYMPTOMS- here, the goal is three-fold- supply the metabolic needs, replenish what has become depleted, and protect the neurons and their supportive cells. The "required" supplements, above, must be taken, and the items that follow below are considered "add-ons".

ACETYL-L-CARNITINE- this is taken along with **SAM-e**. This combination can result in noticeable gains in short term memory, mood and cognition. The Acetyl Carnitine also is said to help heart and muscle function. Doses: Acetyl-L-carnitine- 1500-2000 mg daily on empty stomach; SAM-e- 400 mg daily with the acetyl carnitine. Positive results may appear as early as 3 weeks; use for 2 to 3 months, but repeat or extend this course if needed. Available in most vitamin stores; Generic acetyl carnitine is okay, but I recommend "Nature Made" brand SAM-e (also available at most vitamin stores).

METHYLCOBALAMIN (Methyl B12)

Methylcobalamin is a prescription drug derived from vitamin B12. This can help to heal problems with the central and peripheral nervous system, improve depressed immune function, and help to restore more normal sleeping patterns. Many patients note improved energy as well. Because the oral form is not absorbed when swallowed or dissolved under the tongue, Methyl B12 must be taken by injection. Dose is generally 25 mg. (1 c.c.) daily for 3 to 6 months. Long term studies have never demonstrated any side effects from this drug. However, *the urine is expected to turn red shortly after each dose*- if the urine is not red, a higher dose may be needed or the present supply may have lost potency. The injectable form of this is not available in regular drug stores. It must be manufactured (compounded) by specialty pharmacies on order.

GREEN TEA

Green, but not black or white tea contains some of the most potent antioxidants around (80-100 times more effective than vitamin C). I strongly recommend this to any patient with degenerative changes to the central nervous system. At least four cups daily are needed to reap this benefit, and the tea must be decaffeinated. A nice alternative is **"TeGreen"** capsules by Pharmanex. They contain 97% pure tea polyphenols and each capsule is the equivalent of four cups of decaffeinated green tea. Take one to three daily.

CORDYMAX

Cordyceps is a well-known herb from Tibet that has been shown in clinical studies to improve stamina, fatigue, and enhance lung and antioxidant function. It also raises superoxide dismutase levels, important to prevent

lesions in the central nervous system, which is why *this (along with green tea) is essential if neurodegeneration is part of your illness.* The positive effects can be dramatic; should be used long term. USP- certified cordyceps is available from Pharmanex as "**CordyMax**".

CITICHOLINE

Many studies have shown benefits to cognition, *especially memory*. Benefits are slow to notice, so plan to use this long-term. Dose is 500 to 1000 mg twice a day.

FOR IMMUNE SUPPORT

"**REISHI MAX** "

This enhanced extract from cracked spores of the Reishi mushroom has been shown in clinical studies to augment function of the Natural Killer Cells as well as macrophages. Recommended in all patients who have a CD-57 count below 60. Take four a day. Available only from Pharmanex.

TRANSFER FACTORS are the body's natural signals meant to activate the pathogen-killing effects of the cellular immune system. Therapy with these agents consists of taking both a general stimulator, plus specific transfer factors for the infection you have. Personal experience made me a believer in transfer factor therapy. For Lyme patients, use **Transfer Factor Multi-Immune** as the general stimulant, and **Transfer Factor Lyme-Plus** as the specific agent. Both are exclusives from Researched Nutritionals, and I have found them to be surprisingly effective in making the very ill respond better to treatment. Take as directed on the label.

FOR JOINT SYMPTOMS

GLUCOSAMINE

Glucosamine can be of long term benefit to the joints. Do not be misled into buying a product that also contains chondroitin, as this chemical does not add anything, but it can make the product more expensive. Look for a product that contains the herb Boswellia serrata- this is a non-irritative anti-inflammatory. Although many generics exist, the Pharmanex product, "**Cartilage Formula**" has the right ingredients and is of proven efficacy. Expect improvement only over time (several weeks), but plan to use this indefinitely to maintain joint health.

VITAMIN C

Vitamin C is important to aid in maintaining healthy connective tissues. High doses are recommended- 1000 to 6000 mg a day as tolerated (if the dose is too high for you it may cause acid stomach, gas and loose stools, so therefore dose titration is necessary). Consider using **"Ester-C"** (non-acid and longer acting), or **"C-Salts"** (very well tolerated). Start with a low dose and increase slowly to find your tolerance level.

FLEX CREAM

This is an amazing liniment-like product that really works and has a money back guarantee. Use for any type of body pain- spread on a thick layer and do not rub in. It takes 30 to 60 minutes to work, then lasts many hours. A Pharmanex exclusive.

OTHER OPTIONAL SUPPLEMENTS

VITAMIN D

Surprisingly, most people in America are vitamin D deficient. In the Lyme patient, low vitamin D levels can cause diffuse body aches and cramps that are not responsive to magnesium or calcium supplements. Some also believe that vitamin D is essential for normal immune and hormone function. I strongly urge you to have a fasting blood level drawn. It is recommended that the blood levels be in the upper half or the normal range. If it is not, then 2000 to 4000 units daily are needed for several weeks to make up for the deficit, and then a lower maintenance dose may be necessary, based on results from repeated blood level monitoring. If vitamin D is needed, improvements take 2 to 3 weeks to note, but are well worth the wait.

CREATINE

Creatine has been shown to be of benefit in neuromuscular degenerative diseases such as Lou Gherig's Disease (ALS) and can be very helpful in supporting low blood pressure, as in NMH. It may also benefit strength, stamina, and heart function. Important: To use this safely, you must have an adequate fluid intake.

The creatine product should contain taurine, an amino acid needed to enhance creatine absorption, plus some

carbohydrate to aid creatine entry into muscle. You will need a 20 gram daily loading dose for the first five days, then 4 to 10 grams daily maintenance. Try "**Cell Tech**" from the Vitamin Shop, and follow label directions.

MILK THISTLE

Useful to support liver function. Take 175 mg daily- use an 80% Silymarin extract. Available from many vitamin stores.

LYME DISEASE REHABILITATION

Despite antibiotic treatments, patients will NOT return to normal unless they exercise, so therefore an aggressive rehab program is absolutely necessary. It is a fact that a properly executed exercise program can actually go beyond the antibiotics in helping to clear the symptoms and to maintain a remission.

Although the scientific basis for the benefits of exercises is not known, there are several reasonable theories. It is known that Bb will die if exposed to all but the tiniest oxygen concentrations. If an aggressive exercise program can increase tissue perfusion and oxygen levels, then this may play a role in what is being seen. Also, during aggressive exercise, the core body temperature can rise above 102 degrees; it is known that B. burgdorferi is very heat sensitive. Perhaps it is the added tissue oxygenation, or higher body temperature, or the combination that weakens the Lyme Borrelia, and allows the antibiotics and our defenses to be more effective. Regular exercise-related movements can help mobilize lymph and enhance circulation. In addition, there is now evidence that a carefully structured exercise program may benefit T-cell function: this function will depress for 12 to 24+ hours after exercise, but then rebound. This T-cell depression is more pronounced after aerobics which is why aerobics are not allowed. The goal is to exercise intermittently, with exercise days separated by days of total rest, including an effort to have plenty of quality sleep. The trick is to time the exercise days to take advantage of these rebounds. For an example, begin with an exercise day followed by 3 to 5 rest days; as stamina improves, then fewer rest days will be needed in between workouts. However, because T-cell functions do fall for at least one day after aggressive exercises, be sure to never exercise two days in a row. Finally, an in intermittent exercise program, properly executed, may help to reset the HPA axis more towards normal. On the following page is an exercise prescription that details these recommendations.

This program may begin with classical physical therapy if necessary. The physical therapy should involve massage, heat, ultrasound and simple range of motion exercises to relieve discomfort and promote better sleep and flexibility. Ice (vasoconstriction) and electrical stimulation (muscle spasm and trauma) should not be used!

The program must evolve into a graded, ultimately strenuous exercise program that consists of a specific regimen of *non-aerobic* conditioning- see below. Have the patient complete a gentle hour of prescribed exercise, then go home, have a hot bath or shower, than try to take a nap. Initially, patients will need this sleep, but as they recover, the exercise will energize them and then a nap will no longer be needed.

NOTE: a cardiac stress test may be necessary prior to exercising to ensure safety.

LYME REHAB-PHYSICAL THERAPY PRESCRIPTION

NAME_____

D.O.B._____ DATE_____

Please enroll this patient in a program of therapy to rehabilitate him/her from the effects of chronic tick-borne diseases. If necessary, begin with classic physical therapy, then progress when appropriate to a **whole body conditioning program.**

THERAPEUTIC GOALS (to be achieved in order as the patient's ability allows):

PHYSICAL THERAPY (if needed):

1. **The role of physical therapy here is to prepare the patient for the required, preferably gym-based exercise program outlined below. Plan on several weeks of classic PT then transition to the gym.**
2. Relieve pain and muscle spasms utilizing multiple modalities as available and as indicated: massage, heat, ultrasound, and passive and active range of motion. DO NOT use ice or electrical stim unless specifically ordered by our office. Paraffin baths can be quite useful.
3. Increase mobility, tone and strength while protecting damaged and weakened joints, tendons, and ligaments, and teach these techniques to the patient. Use minimal resistance but a lot of repetitions in any

exercises prescribed. At the start of the exercise program, especially if the patient is weak, avoid free weights, bands and large exercise balls, and favor machines (especially hydraulics) that can guide limbs through a prescribed arc; free weights, etc. can risk hyperextension and uncontrolled movements that may cause or add to injuries. Transition the patient slowly to the gym-based program outlined below. **Noteaerobics are not permitted.**

4. Please see the patient two or three days per week- but do not schedule two days in a row!

EXERCISE Begin with a private trainer for careful direction and education.

PATIENT EDUCATION AND MANAGEMENT (to be done during the initial one-on-one sessions and reinforced at all visits thereafter):

1. Instruct patients on **correct exercise technique**, including proper warm-up, breathing, joint protection, proper body positioning during the exercise, and how to cool-down and stretch afterwards.
2. Please work one muscle group at a time and perform extensive and extended **stretching** to each muscle group immediately after each one is exercised, before moving on to the next muscle group.
3. A careful **interview** should be performed at the start of each session to make apparent the effects, both good and bad, from the prior visit's therapy, and adjust therapy accordingly.

PROGRAM:

1. **Aerobic exercises are NOT allowed**, not even low impact variety, until the patient has recovered.
2. **Conditioning**: work to improve strength and reverse the poor conditioning that results from Lyme, through a whole-body exercise program, consisting of light calisthenics and/or resistance training, using light resistance and many repetitions. This can be accomplished in exercise classes called "stretch and tone", or "body sculpture", or can be achieved in the gym with exercise machines or carefully with free weights (see cautions above).
3. **Each session should last one hour**. A gentle hour is preferable to a strenuous half-hour. If the patient is unable to continue for the whole hour, then decrease the intensity to allow him/her to do so.
4. **Exercise no more often than every other day**. The patient may need to start by exercising every 4th or 5th day initially, and as abilities improve, work out more often, but NEVER two days in a row. The nonexercise days should be spent resting.
5. This whole-body conditioning program is what is required to achieve wellness. A simple walking program will not work, and simply placing the patient on a treadmill or an exercise bike is not acceptable (except very briefly, as part of a warm-up), as aerobics can be damaging and must be avoided.

PHYSICIAN'S SIGNATURE_____

MANAGING YEAST OVERGROWTH

Many patients with weakened defenses, such as from chronic illnesses, including Lyme Disease, develop an overgrowth of yeast. This begins in the mouth and then spreads to the intestinal tract. Therefore the primary line of defense is careful oral hygiene, replenishing the beneficial bacteria by daily intake of yogurt, Kefir, and/or acidophilus, and by following a strict low carbohydrate diet.

ORAL HYGEINE:

CLEANSING

Brush the teeth, tongue, gums, inner cheeks and palate first with toothpaste, then again for 30 seconds while holding an antiseptic mouthwash in the mouth Then, rinse by scrubbing while holding plain water in the mouth.

TOOTHPASTE

Use “AP-24” toothpaste, sold by NuSkin Enterprises. Unlike conventional toothpastes that may contain alcohols, formaldehydes and abrasives, this product cleans in a unique way. It contains two “surfactants” (detergent-like cleansers) that are very effective without being harsh. This product is available in two forms- regular and whitening (both contain fluoride). Choose either one.

In addition, get from them their patented toothbrush that is designed to work with this toothpaste. It cleans better and is far gentler than regular or electric toothbrushes.

Order AP-24 products by calling 1-800-487-1000. The U.S. reference # is 9256681-R
MOUTHWASHES
Use an antiseptic mouthwash (Scope, Listerine, etc.), and brush the teeth, tongue, gums, cheeks and the roof of the mouth while holding the mouthwash in the mouth. Do this for 30 seconds, then rinse repeatedly with water.
For especially thick or resistant thrush, the most effective (and drastic) treatment, employed as a last resort, consists of using "Dakin's Solution" as a mouth rinse. Make this by mixing one teaspoon of household liquid bleach (Clorox) in four ounces of water. A small amount is held in the mouth while brushing, then spit out, and repeated until the thrush has cleared. This is usually a one-time treatment, but may have to be repeated every few weeks.
After using an antiseptic, it is necessary to immediately eat yogurt or chew an acidophilus capsule to replenish the beneficial flora in the mouth. Because the germ count, both harmful and beneficial, will be artificially reduced after such a cleaning, and because yeasts are opportunists, the yeast infection can come back. By having the yogurt or acidophilus then, the yeast will be crowded out and a more normal oral flora will result.
INTESTINAL TRACT: An overgrowth of yeast here will ferment dietary sugars and starches, forming acids, gas, alcohols and a variety of organic chemicals. Symptoms include gas, bloat, heartburn and/or pain in the stomach area, and because of the organic chemicals, there can be headaches, dizziness, lightheadedness, wooziness and post-meal fatigue. To clear intestinal yeast, first the tongue and mouth must be cleansed so yeast does not reenter the system with every swallow. Next, since yeast germs feed on sugars and starches, follow the low carbohydrate diet outlined below. Finally, to replenish the normal, beneficial microbes, eat PLAIN yogurt daily, drink Kefir, 4 ounces daily, and/or take acidophilus, 2 capsules three times daily after meals.
YEAST CONTROL DIET- restricted carbohydrate regimen
UNRESTRICTED FOODS
All protein foods, such as meat, fish, fowl, cheese, eggs, dairy, tofu
RESTRICTED FOODS
FRUITS
Fruits may be a problem because they contain a large amount of sugars. However, if the fruit contains a lot of fiber, this may make up for the sugars to some degree. Thus:
· Fruits are only allowed at the end of a meal, and never on an empty stomach
· Only high fiber fruits are allowed
· Only very small amounts!
EXAMPLES:

ALLOWED IN GENEROUS AMOUNTS
Grapefruit, lemons, limes, tomatoes, avocado
ALLOWED IN SMALL AMOUNTS ONLY! (The high fiber content in these hard, crunchy fruits partially makes up for the carbohydrates)
Pears, apples, strawberries, cantaloupe, etc.
NOT ALLOWED (These soft fruits do not have enough fiber)
Oranges, watermelons, bananas, grapes, etc.
No fruit juices either!
VEGETABLES
Green vegetables and salads are O.K. Avoid or limit starchy vegetables (potato, rice, beans, etc.) and avoid pasta.
STARCHES
None!! If it is made from flour- any kind of flour- it is not allowed. (No breads, cereals, cake, etc.)
SWEETENERS
NOT ALLOWED
No sugars at all, and no fructose or corn syrup
ALLOWED (if tolerated)
Stevia (safest), honey, and Splenda,

Aspartame (NutraSweet, Equal) may not be tolerated by some patients
Saccharin products are not recommended

DRINKS

ALLOWED
Water, seltzer, caffeine-free diet sodas, coffee and tea without sugar or caffeine, vegetable juices
NOT ALLOWED
Fruit juices, regular sodas, and any drinks sweetened with sugars or syrups
No Alcohol at all

OTHERS

Do not skip any meals. At least three regular meals daily are needed; a better option is to eat very small portions but have between meal snacks to maintain blood sugar and insulin levels. Bedtime snacks, if taken, must be totally carbohydrate free!

MANAGING LYME DISEASE, 16h edition, October, 2008
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PATIENT INSTRUCTIONS ON TICK BITE PREVENTION AND TICK REMOVAL

HOW TO PROTECT YOURSELF FROM TICK BITES

PROPERTY Remove wood piles, rock walls, and bird feeders as these attract tick-carrying small animals and can increase the risk of acquiring Lyme.

INSECTICIDES: Property should be treated with a product designed to target the rodents that carry ticks- bait boxes and a product called "Damminix" can be used. Use these products in conjunction with liquid or granular insecticides.

LIQUID & GRANULAR PESTICIDES: Products meant for widespread application such as permethrin and its derivatives are preferred. They are available as a liquid concentrate and as granules. If liquid insecticides are used, application should be by fogging, not by coarse sprays. Apply these products in a strip a few feet wide at the perimeter of the lawn at any areas adjacent to woods and underbrush. Also treat any ornamental shrubs near the house that may serve as a habitat for small animals. The best time to apply these products is in late Spring and early Fall. In every case, professional application is recommended.

CLOTHING When wearing long pants, tuck the cuffs into the socks so any ticks that get on shoes or socks will crawl on the outside of the pants and be less likely to bite. Also, light colored clothing should be worn so the ticks will be easier to spot. Smooth materials such as windbreakers are harder for ticks to grab onto and are preferable to knits, etc.

Tick repellents that contain "permethrin" (Permanone, Permakill) are meant to be sprayed onto clothing. Spray the clothes before they're put on, and let them dry first. Do not apply this chemical directly to the skin.

Ticks are very intolerant of being dried out. After being outdoors in an infested area, place clothes in the dryer for a few minutes to kill any ticks that may still be present.

SKIN: Insect repellents that contain "DEET" are somewhat effective when applied to the arms, legs, and around the neck. Do not use any repellent over wide areas of the body as they can be absorbed causing toxicity. Also, it is inadvisable to use a product that contains more than 50% DEET, and 25% concentrations are preferred. Use repellents cautiously on small children, as they are more susceptible to their toxic effects. Be aware that this repellent evaporates quickly and must be reapplied frequently.

Check carefully for ticks not only when you get home but frequently while still outside!

HOW TO REMOVE AN ATTACHED TICK

Using a tweezer (not fingers!), grasp the tick as close to the skin as possible and pull straight out. Then apply an antiseptic. Do not try to irritate them with heat or chemicals, or grasp them by the body, as this may cause the tick to inject more germs into your skin. Tape the tick to a card and record the date and location of the bite. Remember, the sooner the tick is removed, the less likely an infection will result.

APPENDIX

RATIONALE FOR TREATING TICK BITES

Prophylactic antibiotic treatment upon a known tick bite is recommended for those who fit the following categories:

1. People at higher health risk bitten by an unknown type of tick or tick capable of transmitting Borrelia burgdorferi, e.g., pregnant women, babies and young children, people with serious health problems, and those who are immunodeficient.

2. Persons bitten in an area highly endemic for Lyme Borreliosis by an unidentified tick or tick capable of transmitting B. burgdorferi.
3. Persons bitten by a tick capable of transmitting B. burgdorferi, where the tick is engorged, or the attachment duration of the tick is greater than four hours, and/or the tick was improperly removed. This

means when the body of the tick is squeezed upon removal, irritated with toxic chemicals in an effort to get it to back out, or disrupted in such a way that its contents were allowed to contact the bite wound. Such practices increase the risk of disease transmission.
4. A patient, when bitten by a known tick, clearly requests oral prophylaxis and understands the risks. This is a case-by-case decision.
The physician cannot rely on a laboratory test or clinical finding at the time of the bite to definitely rule in or rule out Lyme Disease infection, so must use clinical judgment as to whether to use antibiotic prophylaxis. Testing the tick itself for the presence of the spirochete, even with PCR technology, is helpful but not 100% reliable. An established infection by B. burgdorferi can have serious, long-standing or permanent, and painful medical consequences, and be expensive to treat. Since the likelihood of harm arising from prophylactically applied anti-spirochetal antibiotics is low, and since treatment is inexpensive and painless, it follows that the riskbenefit ratio favors tick bite prophylaxis.

SUGGESTED ADDITIONAL READING

Evidence Based Guidelines for the Management of Lyme Disease. The International Lyme and Associated Diseases Society. Expert Rev. Anti-infect. Ther.2(1), Suppl. (2004)
Lyme Disease: Point/Counterpoint. Stricker, Raphael B. Lautin, Andrew. Burrascano, Joseph J. Expert Rev. Anti-infect. Ther, April 2005. 3(2), 155-165
An Understanding of Laboratory Testing for Lyme Disease. Harris, Nick S. J. Spiro. and Tick-Borne Dis. Vol 5, 1998. 16-26
Gestational Lyme Borreliosis. MacDonald, Alan B. Rheumatic Diseases Clinics of North America 15 (4), Nov. 1989. 657-678
Cerebral Malaria. Newton, Charles R.et al. J. Neurol. Neurosurg. Psychiatry. 2000. Vol 69, 433-441.

RESOURCES

International Lyme and Associated Diseases Society
www.ILADS.org
P.O. Box 341461
Bethesda, MD 20827-1461
Lyme Disease Association, Inc.
P.O. Box 1438, Jackson, NJ 08527
(888) 366-6611
www.lymediseaseassociation.org

What is Lyme Carditis

Grain-Bujadoux-Bannwarth syndrome (Grain-Bujadoux-Bannwarthsyndrome) synonyms: Lyme heart inflammation, lymphocytic meningeal radiculitis, the meninges multi radiculitis, Lyme disease. The disease is an infection caused by the spirochete Borrelia natural foci of tick-communicable diseases.

The disease in at least 22 countries throughout the world, the United States, Canada, United Kingdom, France, Australia, Japan, South Africa, Egypt and other distributed or popular reports of the disease is more common, particularly in the

U.S.. After investigation in China and confirmed that the infected area is mainly distributed in Xinjiang, Anhui, Henan, Inner Mongolia, Ningxia, Guangxi and Fujian provinces.

Symptoms of Lyme Carditis

Clinical manifestations:

The average incubation period of the disease on the 9th. After by course of the disease can be divided into early infection and late infection. Early infection, including a local erythema migrans and two systemic disseminated infections and a few weeks or a few months, intermittent symptoms, mainly for the performance of the early nervous system and heart damage; typical performance of the nervous system as meningitis, cranial neuritis and radiculitis pain, known as the triad of the nervous system. More common temporomandibular joint pain. Advanced infection that is three persistent infection, arthritis, late nervous system performance. Complex and diverse clinical manifestations of this disease, only one or two overlap, but also showed the typical three phase After.

The incidence of heart damage is less than 10%, may be subclinical process, mainly as: ⊖ acute Carditis: visible enlargement of the heart, myopericarditis examination could be heard and pericardial friction rub, heart failure can have difficulty breathing, gallop, and pulmonary rales. ⊜ arrhythmia: oriented prominent clinical manifestations of disease, the disease can affect the sinus node, the atrioventricular junction Purkinje system, and even the entire conduction system, atrioventricular block on clinical most common, a small number can be expressed as bundle branch The block can occur even sinoatrial block.

What Causes Lyme Carditis

Pathology:

Bo's sparse spiral body infection cause, damage to the invasion of the spiral body's direct role and blood, synovial fluid, and cerebrospinal fluid antigen-antibody complex nature, the pituitary gland in the prostate prime, leukocyte mediated hormone 1 (IL -1) active secretion increase and other many other factors induced

human multi-system inflammatory lesions. The clinical features of early extensive skin damage, nerve, heart or joint damage can occur after several weeks or months. Heart attack due to the disease known as Lyme carditis, is one of the serious complications of Lyme disease.

Tests and Diagnosis for Lyme Carditis

Diagnostic tests:

The cardiac electrophysiology check visible the block occurred above the His bundle, often temporary and reversible, multi-l ~ 2 weeks progressive improvement, a few may be extended to a few weeks or a few months.

ECG: ST-segment depression in T wave flat or inverted, block, and a variety of arrhythmias.

Treatments of Lyme Carditis

Treatment:

General treatment of a variety of support and symptomatic treatment, and other reasons carditis.

Application of antibiotics early application to quickly kill the spirochete in the body. Erythromycin, penicillin, third-generation cephalosporins, and tetracycline is effective.

3. Glucocorticoid glucocorticoids applies to antibiotics short-term poor outcome.

4. The above other combined second degree atrioventricular block should be hospitalized custody. Significantly reduced the ventricular rate or accompanied by a long interval, should be held in a temporary cardiac pacing therapy.

Lyme Disease Co-Infections

Is it normal to be losing your memory as you get older? Is it normal to have an Alzheimer's epidemic affecting not only the United States and also the rest of the world? Or is it possible that

there are multiple etiologies at the root of these conditions? We find that the majority of lyme patients with co- infections have severe memory and concentration problems.

Evidence of the connection between infection and dementia can be found in a report from pathologist Dr Alan MacDonald who examined brain biopsies from the McLean hospital (an affiliate of Harvard University) data bank from patients with confirmed Alzheimer's disease.

His PCR analysis show that 7/10 of these patients had the DNA of Borrelia Bergdorferi in their brain, the ETO logic agent of lyme disease. We also fine at The majority of our chronically ill patients with lyme disease and co- infections have been exposed to high levels of heavy metals, such as Mercury and lead, and occasionally to aluminium. These also can cause memory and concentration problems, and can cause the production of elevated levels of free radicals, which can increase inflammation.

Similarly we are exposed to hundreds of environmental chemicals every day that are fat-soluble and therefore are deposited in brain. These can and do affect cognitive processing. We have enough causes for an epidemic of dementia in the general population. Drugs prescribed for Alzheimer's only slow down the cognitive decline. Horowitz has seen the improvements in cognitive functioning after treating these patients for chronic tick borne infections, by detoxifying them of fat-soluble toxins with glutathione, by using oral chelation agents to remove mercury lead and aluminium, and by identifying and treating B12 deficiencies and/or hypothyroidism.

Horowitz screened 50 lyme patients for co- are infections with Ehrlichia, Babesia microti, Mycoplasma, and Bartonella henselae. He reported that treatment with two drugs was better than treatment with one drug, especially where intracellular bugs are concerned.

Horowitz tests for a broad range of co-infections including different strains of babesiosis, Ehrlichia, Anaplasma, Bartonella, Rickettsial infections such as Rocky Mountain spotted fever, Q fever, and typhus, tularemia, Brucella, chlamydia pneumonia, Mycoplasma species, viruses such as EBV, CMV, HHV 6, and parasites such as toxoplasmosis.

An increase in neuropsychiatric symptoms also takes place when a patient has contracted co-infections, such as a babesiosis, where Babesia can exacerbate underlying lyme disease symptoms including depression.

Other co- infections also can influence psychiatric symptoms. Ehrlichiosis can cause central nervous system symptoms, as can viruses and intracellular infections with Mycoplasma spp. And Chlamydia pneumonia, which are frequently found in MSIDS patients.

Often the patients with the worst neurological symptoms have lyme disease, mycoplasma and Bartonella simultaneously, with or without the other co- infections . Bartonella henselae, organism that causes cat Scratch fever, exacerbates many of the neurological and neuropsychiatric symptoms we see with lyme disease, and has been linked to anxiety disorders and depression, as well as various central nervous system abnormalities.

These include encephalomyelitis (involving inflammation in both the brain and spinal cord, leading to difficulties with cognition and motor function) ; transverse myelitis(inflammation and demyelination of the spinal cord, leaving to difficulty walking); spastic para paresis(stiffness and spasm in the lower extremities, affecting walking); seizures with hemiparesis (Weakness on one side of the body); cerebellar syndromes (Primarily defined by symptoms of dizziness and poor balance) and movement disorders (which can cause a variety of symptoms, including spasms twitching and involuntary movements).

Bartonella can also be transmitted to the foetus. Therefore resistant neuropsychiatric symptoms in children might be linked to maternal transmission of the organism, and should be suspected of the patient is living in a lyme endemic area or has cats at home.

Some patients with Bartonella have other severe neurologic manifestations with a ophthalmologic involvement i.e. inflammation VI manifesting as optic neuritis, episcleritis, conjunctivitis, uveitis r iritis.

Bartonella can also cause an oculoglandular syndrome with preauricular adenopathy and conjunctivitis, neuroretinitis, branch retinal artery occlusion and vision loss. Bartonella should therefore be considered when patients present with particularly severe opthalmological symptoms.

If we look at the symptom complex used to define CFS, we see that the symptoms overlap those seeing in lyme disease and associated co-infections and it explains why lyme disease is frequently misdiagnosed. Apart from the results of a spinal tap, one of the primary differences is the symptoms of persistent lyme disease tend to come and go, fluctuating with good and bad days, and the symptoms tend to migrate.

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• Lyme Disease:

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By Dr. James Howenstine, MD.

Lyme Disease was initially regarded as an uncommon illness caused by the spirochete Borrelia burgdorferi (Bb). The disease transmission was thought to be solely by the bite from a tick infected with this spirochete. The Bb spirochete is able to burrow into tendons, muscle cells, ligaments, and directly into organs. A classic bulls-eye rash is often visible in the early stage of the illness. Later in the illness the disease can afflict the heart, nervous system, joints and other organs. It is now realized that the disease can mimic amyotrophic lateral sclerosis, Parkinson's disease, multiple sclerosis, Bell's Palsy, reflex sympathetic dystrophy, neuritis, psychiatric illnesses such as schizophrenia, chronic fatigue, heart failure, angina, irregular heart rhythms, fibromyalgia, dermatitis, autoimmune diseases such as scleroderma and lupus, eye inflammatory reactions, sudden deafness, SIDS, ADD and hyperactivity, chronic pain and many other conditions.

Dr. Paul Fink, past president of the American Psychiatric Association, has acknowledged that Lyme Disease can mimic every psychiatric disorder in the Diagnostic Symptoms Manual IV. This includes attention deficit disorder (ADD), antisocial personality, panic attacks, anorexia nervosa, autism and Ausperger's syndrome etc. It might be prudent in any person suddenly found to have psychiatric symptoms to obtain a Q-RIBb blood test to exclude Lyme Disease.

Biology professor, Lida Mattman, author of *Cell Wall Deficient Forms: Stealth Pathogens*, has been able to recover live spirochetes of Bb from mosquitos, fleas, mites, semen, urine, blood, and spinal fluid. A factor contributing to making Bb so dangerous is that it can survive and spread without having a cell wall (cell wall deficient CWD). Many valuable antibiotics kill bacteria by breaking down the cell wall. These antibiotics often prove ineffective against Bb.

Lyme Disease is now thought to be the fastest growing infectious disease in the world. There are believed to be at least 200,000 new cases each year in the U.S. and some experts think that as many as one in every 15 Americans is currently infected (20 million persons). Dr. Robert Rowen knows a family where the mother's infection spread to 5 of her 6 children[1] all of

whom *recovered with appropriate therapy*. It is difficult to believe that these children were all bitten by ticks and seems more plausible that person to person spread within the family caused this problem. Bacteriologist, Dr. Lida Mattman, states “I’m convinced Lyme disease is transmissable from person to person”. In 1995 Dr. Mattman obtained positive cultures for Bb from 43 of 47 persons with chronic illness. Only 1 of 23 control patients had a positive Bb culture. Dr. Mattman has subsequently recovered Bb spirochetes from 8 out of 8 cases of Parkinson’s Disease, 41 cases of multiple sclerosis, 21 cases of amyotrophic lateral sclerosis and *all tested cases of Alzheimer's Disease.* The complete recovery of several patients with terminal amyotrophic lateral sclerosis after appropriate therapy shows the *great importance of establishing the diagnosis of Lyme Disease.*

Some very important information has recently become available about the spread and magnitude of the problem with Lyme Disease. The severity of the Lyme illness is related to the spirochete load in the patient. Few spirochetes produce mild or asymptomatic infection. A study from Switzerland in 1998 pointed out that only 12.5 % of patients testing positive for Bb had developed symptoms. A German boy developed Lyme arthritis *5 years after his tick bite.* Often mycoplasmal infections remain without symptoms until the victim suffers a traumatic event (stress, injury, accident etc.) These stressing events enable the mycoplasma to begin consumption of cholesterol and symptoms may begin to present. The mechanism of this deterioration is thought to be suppression of the immune system secondary to stress.

Many patients with LD have concomitant infections with other parasites (*Ehrlichia* in white blood cells and *Babesia* in red blood cells) Some patients have all 3 parasites. Each requires a different therapy with*Babesia being particularly difficult to eradicate.* Recently, Artemisinin appears effective in Babesia infections. All co-infections must be eliminated .to obtain a successful result.

Dr. Joanne Whitaker relates that nearly every patient with Parkinson’s Disease (PD). has tested positive for Bb. Dr. Louis Romero reports that 3 patients with PD are 99 % better after TAO-free cat’s claw (Uncaria tomentosa) therapy. When Dr. Mattman cultured 25 patients with fibromyalgia *all subjects had positive cultures for the CWD Bb. which causes LD*. She relates that Bb can be found in tears and could thus easily appear on the hands where touching could spread LD. Several families are now documented where nearly every family member is infected. How sick the individual patient becomes probably relates to their initial spirochete dose,

immune system, detoxification capability and stress levels.

Transmission of the disease has been clearly documented after bites by fleas, mites mosquitoes and ticks. There is compelling evidence that Lyme disease (LD) can be spread by sexual and congenital transfer. One physician has cared for 5000 children with LD. *240 of these children were born with the disease*. Dr. Charles Ray Jones, the leading pediatric specialist on Lyme Disease, has found 12 breast fed children who have developed LD. Miscarriage, premature births, stillborn, birth defects, and transplacental infection of the fetus have all been reported. Studies at the Univ. of Vienna have found Bb in urine and breast milk of LD mothers.

Researchers at the Univ. of Wisconsin have reported that dairy cattle can be infected with Bb hence milk could be contaminated. Bb can also be transmitted to lab animals by oral intake such as food.

The Sacramento, California blood bank beleives that LD can be spread by blood transfusions. The CDC (Center for Disease Control) in Atlanta, Georgia states that their data indicates that Bb *can survive without detection by the blood processing techniques used for transfusions in the U.S.*

Lyme Disease is the fastest growing epidemic in the world. LD is grossly underreported so there may be far more than the 200,000 cases reported annually in the U.S. Dr.Harvey and Salvato estimate that *1 billion persons in the world may be infected with LD*. LD is thought to be a *contributing factor in 50 % of patients who have chronic illness.*

Dr. Joanne Whitaker, a Lyme disease victim from childhood, has developed a reliable test for the presence of Lyme disease. This test looks for the Bb organism, not antibodies, and is able to identify the cell wall deficient (CWD) form of the spirochete as well as the actual Bb organism. The test is called Q-RIBb which stands for quantitative rapid identification of Bb. Dr. Lida Mattman has confirmed that Dr. Whitaker's test is sensitive because there has been a *100 % correlation between a postive culture of Bb by Dr. Mattman's lab and a postive Q-RIBb test from Dr. Whitaker's Laboratory.*

Case Reports Illustrating The Critical Importance Of Establishing The Diagnosis Of Lyme Disease

Case 1 Larry Powers, a former Mr. America in 1962, became ill with the symptoms of Parkinson's Disease in 1990. *Sinemet* therapy was taken for eight years but he gradually became worse. He became confined to a wheel

chair and required help with eating. After learning that Lyme Disease might be causing his symptoms of PD he started taking TAO free cat's claw (*Uncaria tormentosa*). Within three weeks he was out of his wheelchair and fishing for 100 pound tarpon.

Case 2 Tom Coffey at age 34 developed diplopia, severe hypertension uncontrolled by drugs, and impaired balance. A diagnosis of amyotrophic lateral sclerosis was made. Surgery was performed to correct the diplopia. By June 2001 he was unable to swallow saliva and feeding tube nutrition was begun. His weight had fallen by 100 pounds. Nutritional support from the tube feedings produced slow resolution of the swallowing problem. Consultation with a Lyme expert uncovered the history of a bulls-eye rash after a tick bite. Therapy with *Rocephin* led to complete recovery.

Case 3 A young male college student developed such severe cognitive difficulties he was forced to drop out of school. A RIBb test was positive for LD and he resumed a normal life after receiving 4 months of antibiotic therapy...

What Causes Neurone Death In Amyotrophic Lateral Sclerosis ALS?

One of the most insidious mimics for Lyme disease is ALS. The neurotoxins released by the Bb organism are capable of causing neurologic dysfunction in the central nervous system that produces symptoms typical of amyotrophic lateral sclerosis. The pathological hallmark of ALS is motor neurone degeneration and death.

Research performed by Dr. Harold Clark and Dr.Garth Nicholson and coordinated by Donald W. Scott[2] has resulted in a breakthrough in our understanding of amyotrophic lateral sclerosis.

Mycoplasma were discovered in 1898. These are living particles of bacterial nucleic acid which do not have a cell wall. In 1971 Rottem et al[3] learned that most species of mycoplasma were absolutely dependent for their growth on the consumption of pre-formed sterols including cholesterol obtained from animal and human host cells. These mycoplasma live harmlessly in host cells until they are stimulated to activity by a stressing traumatic event (bullet wound, bad fall, injury from accident etc.). The growth of the mycoplasma consumes the cell's cholesterol resulting in death of the affected cell. Mycoplasma have been identified in ALS using high resolution blood morphology. In the November 9, 2001 issue of Science Dr. Daniel

Mauch[4] et al revealed that the glial cells surrounding the motor neurone supply the extra cholesterol needed to repair and replace aging synapses. If the repair does not properly occur the motor neurone cells proceed to die from overwork Glial cells are also heavily involved in gathering, processing and storing glutamate. Elevations in glutamate have been found in brain tissue in ALS.

A mycoplasma species, probably fermentans, which was harmlessly sequestered in a glial cell becomes aroused by some traumatic stressful event. This mycoplasma then consumes the glial cholesterol which makes up 40 % of the glial cell membrane causing rupture and death of the glial cell. The death of these glial cells releases large amounts of glutamate which becomes elevated in brain tissue. Within the neurone some of the excess glutamate accesses a urea molecule. The urea molecule gives up an ammonia ion which converts a glutamate molecule into less dangerous glutamine. This leaves the former urea molecule as a cyanate ion which damages the motor neurone's mitochondria. One of the consequences of the damaged mitochondria is a decrease in the energy output available to the neurone. This produces the severe weakness and fatigue seen in patients with ALS. If the mitochondrial injury is severe the neurone dies. The death of motor neurones stops message delivery to muscle cells leading to atrophy of muscle tissue a universal finding in ALS.

This avid consumption of cholesterol may also contribute to the endocrine dysfunction seen in ALS because it decreases the amount of cholesterol available to produce estrogen, testosterone, progesterone, hydrocortisone, and aldosterone. Patients with ALS, fibromyalgia, and chronic fatigue syndrome often have hypothalamic dysfunction which may result in adrenal insufficiency, hypothyroidism, and gonadal failure.

Lyme Disease frequently exhibits neurologic abnormalities because the Bb neurotoxins are drawn to the fatty tissue found in the brain and peripheral nerves. As a consequence sudden deafness, Bells palsy, Parkinson's Disease, Multiple Sclerosis, reflex sympathetic dystrophy, peripheral neuritis, chronic pain, and a multitude of other neurologic disorders may appear.

The Influence of Toxins from Bb On The Symptoms and Course of Lyme Disease

Autopsy examinations of young persons (30s) dying from what appeared to be Parkinson's disease PD have frequently failed to confirm the basal ganglion damage that would be expected in the classic PD seen in the elderly.

Some patients with illnesses of many years duration misdiagnosed as Amyotrphic Lateral Sclerosis, Multiple Sclerosis, and Parkinson's Disease have made incredible recoveries within periods as short as 24 to 72 hours when placed on TOA-free uncaria tormentosa (cat's claw) for LD.. This rapid response could not rationally be attributed to improved immune function or bacteriocidal effects on spirochetes. Bb is known to produce a *group of neurotoxins*. The most sensible explanation for this recovery lies in turning off or blocking the neurotoxic effects of Bb on the lipid containing structures that the Bb neurotoxins are attracted to (central nervous system, peripheral nerves, muscles, joints etc.). This sudden improvement appears to be the result of blockage and inhibition of the neurotoxins[5]. The most important example of a "Biotoxin Illness" appears to be Lyme Disease[6]. Patients with symptoms of Parkinson's Disease at a young age caused by neurotoxins would not be expected to show permanent structural destruction in the basal ganglia. These neurotoxins probably act at specific sites such as neurotransmitters-pre- and- post synaptic membranes, altering dopamine, serotonin, GABA, and acetylcholine molecules, thereby blocking surface membrane receptors of various kinds which would interfere with the proper action of enzymes, coenzymes and hormones. This is only one of the damaging mechanisms of action of the neurotoxins.

The TOA free form of cat's claw (*Samento*) may have three direct beneficial effects in humans with LD:

- Immune modulation (correcting immune dysfunction)
-
- Direct broad spectrum anti-microbial effect on spirochetes. Quinovic acid glycosides found in TAO-free cat's claw are similar to the quinilones widely used as antibiotics.
-
- Blocking the adverse neurotoxic effects on cells, enzymes, and hormones

Whether the serious lack of energy and fatigue seen in LD are similar to the cyanate[7] induced damage to the mitochondria's ability to produce energy in the motor neurone found in amyotrophic lateral sclerosis or is due to failure of proper calcium channel function is not clear.

Favorable Therapeutic Results With TAO-Free Cat's Claw In Lyme Disease

A pilot study treated 28 patients with *Advanced*Chronic Lyme Disease with TOA-free Uncaria tomentosa (cat's claw). Conventional cat's claw contains

TOA alkaloids that interfere with the desired immune modulation. The 14 person control group was given antibiotic therapy. At the study's termination 85 % of those receiving the cat's claw preparation **no longer had positive blood tests for Bb**. All 28 persons had experienced a *dramatic improvement in their clinical condition*. No significant changes were seen in the control group.

Currently it is believed that nearly all adults are infected with stealth organisms (Borrelia burgdorfi, yeast, fungi, mycoplasma, anerobic bacteria,) and have picked up toxic metals (mercury, lead, cadmium, aluminum, fluoride, aluminum etc.) both of which lead to detrimental effects on health. *Samento*may be of great value in eliminating some of these infectious (certainly Bb) and has also proven very effective in cancer therapy.

The Prima Una de Gato can be obtained from Allergy Research Group 800-545-9960, Nutramedix (product name Samento Plus) 561-745-2917, Farmacopia at 800-896-1484. and from Natural Health Team 800-416-2806. Dr. Whitaker's lab can be reached by Internet at www.bowen.org or by calling 727-937-9077 to arrange blood Bb testing. Improving nutrition, detoxifying and improving mental health all contribute to good results in treating Lyme Disease. Removal of mercury amalgams and treatment of heavy metals may be needed.

There is convincing evidence that the Lyme Disease epidemic may have originated from the bio-warfare laboratory in Plum Island off the coast of Lyme, Connecticut.

Lyme Disease Numbness:

Possible related medical conditions:

1. Lyme disease
2. Bartonella
3. autoimmune disorders
4. carpal or cubital tunnel or any nerve entrapment syndrome
5. diabetes
6. hypothyroidism
7. pregnancy
8. heavy metal toxicity
9. other environmental toxins
10. vitamin deficiencies
11. immune deficiency
12. mitochondrial dysfunction
13. MS
14. strokes or TIAs
15. anxiety with hyperventilation

Shifting numbness is a classic symptom of Lyme disease.

- Mayo Clinic: **Neurological problems.** Weeks, months or even years after infection, you might develop inflammation of the membranes surrounding your brain (meningitis), temporary paralysis of one side of your face (Bell's palsy), numbness or weakness in your limbs, and impaired muscle movement.
- The Foundation for Peripheral Neuropathy:

Early diagnosis and treatment are important to stop the progression of the disease. If untreated, the disease can result in neurological disorders such as peripheral neuropathy, including Bell's palsy, as well as pain, numbness or weakness in the limbs. The onset of peripheral neuropathy typically develops weeks, months or years later, if the disease is left untreated.

While potentially serious, Lyme disease can be treated, especially in the early stages. It is important to take preventive measures when outdoors in areas known to have infected deer ticks. Some helpful steps include: wearing enclosed shoes and light colored clothing; checking clothing and exposed skin frequently for ticks; and using insect repellant containing DEET (Diethyl-meta-toluamide) on skin or clothes.

SYMPTOMS

(Not all symptoms and signs may be present.)

Lyme disease progresses in three stages of severity:

- First Stage:
- Fatigue
- Fever and chills
- Muscle and joint pain
- Red circular rash
- Stiff neck
- Swollen lymph nodes
- Second Stage:
- Facial paralysis (Bell's palsy)
- Irregular heartbeat
- Meningitis (fever, stiff neck, severe headaches)
- Numbness and pain in arms and legs
- Stiff neck
- Poor coordination
- Third Stage:
- Chronic arthritis and swelling in large joints, especially the knees
- Chronic pain in muscles
- Problems with sleeping
- Numbness and pain in arms and legs
- Nervous system problems
- Difficulty concentrating
- Memory loss
- Numbness and tingling
- Peripheral neuropathy
- Pain, numbness and tingling in limbs
- Paralysis of facial muscles (Bell's palsy)
-

EVALUATION AND TESTS

(Not all evaluation and tests may be necessary.)

- Neurological exam
- Electromyography

- Nerve conduction velocity test
- Blood tests, including tests for antibody against the agent that causes Lyme disease and tests to detect the agent itself.

TREATMENT AND THERAPY

(Not all treatments and therapies may be indicated.)

- Antibiotics
- Intravenous therapy

For Bell's palsy (facial paralysis)

- Eye drops for affected eye
- Medications such as steroids to reduce inflammation of nerve and decrease pain
- Surgery (in rare cases to improve appearance)
- Treatment of underlying inflammatory condition, if present

If experiencing weakness, pain, or inflammation (in limbs or knees)

- Ask your doctor about special therapeutic shoes or a knee brace (which may be covered by Medicare and other insurance)
- Take safety measures to compensate for loss of sensation

Lyme disease is curable, if treated early

SUMMARY

Lyme disease is the most common vector-borne disease in the Australia. The Centers for Disease Control (CDC) in USAestimates that 329,000 cases occur in the United States each year. CDC studies have found that reported cases underestimate its true incidence by a factor ten, indicating that tens of thousands of residents in Australia may be infected with Lyme disease each year.
Tick borne diseases (TBD) also create a significant economic burden in Australia. Over $7 million in annual medical expenses have been attributed to Lyme disease as well as up to $10,000 per

patient annually in lost productivity. Lyme patients require 87 percent more visits to the doctor, and 71 percent more visits to the emergency room in comparison to those without Lyme disease. The rapid expansion of TBDs in Australia is further complicated by a lack of consensus among researchers and healthcare professions in many critical areas. Two organizations have published guidelines for the diagnosis and treatment of Lyme and other TBD's: the Infectious Diseases Society of America (IDSA), and the International Lyme and Associated Diseases Society (ILADS) These organizations vary greatly in their approach to diagnosis and treatment of this disease. In September 2014, 15 states in USA, with current or past legislation establishing task forces, commissions, and/or working groups focused on aligning policy, resources, and programs to support education and awareness, prevention, surveillance, and treatment of tick-borne diseases. The Task Force members included representation from the opposing medical views (IDSA and ILADS), considered both medical perspectives, and the diversity of these views is reflected in the report and its core recommendations. The task force was subdivided in three main subgroups: Prevention, Education & Awareness, and Surveillance. Their recommendations are as follows:

Prevention:

1) Develop Protocol and Funding Strategies for High Risk Areas: Develop and implement a protocol and funding strategy for schools located in high-risk areas to implement personal protection and property actions.

2) Develop Park Staff Protocols: Develop and implement a protocol for federal, state, and local properties to include communicating risk awareness, and taking property actions, and other methods to reduce the risk of TBDs to the staff and public.

3) Develop Standard Brochures for Healthcare Provider Distribution: Develop and provide a standard brochure that healthcare providers ideally should provide to patients when they are evaluated for tickborne infection.

4) Develop a Strategy for Reducing Transfusion Transmitted Tick-Borne Babesiosis: Develop and implement a strategy to reduce risk of transfusion-transmitted Babesiosis.

Education & Awareness:

1) **Develop a Public Awareness Campaign:**
   Develop a comprehensive multimedia public awareness campaign targeting in the general public and at-risk population to improve awareness and understanding of TBD in Pennsylvania. Page 52 of 64 2) Provide Information for Healthcare Professionals: Provide information that will give health care professionals options for developing and making recommendations for tick-borne disease, prevention of tick-borne disease, and prevention of disease progression.

2. Surveillance:

1) Notifiable Disease List Updating and Reporting: Adjust and periodically review the Australian notifiable disease list and specifically include Babesiosis and Powassan virus. Healthcare providers should encourage providers to report new and emerging TBDs not included on the list.

2) Perform a Statewide Environmental Survey: Increase the public, medical and scientific community's awareness of tick populations, and the disease s they carry through a broad and comprehensive statewide environmental survey.

3) Fund Research and Information Sharing: Earmark state budgeted appropriations to conduct research and share information for tick distribution, control, infectivity rates, and pathogen load.

4) Fund Observational Epidemiological Studies: Obtain funding to support observational epidemiologic studies to provide more detailed data on the burden and costs of TBDs among Pennsylvania residents.
5) Annual Updates on Diagnostic Testing: Provide annual updates for, and enhance availability of, a broad array of diagnostic test for tick-borne disease, as well as encourage the development of innovative diagnostic tests.
6) Expand Surveillance Network: Improve healthcare provider and veterinarian participation in tick-borne disease surveillance by disseminating annual advisories on the recognition, diagnosis, and reporting of TBD's in PA and by utilizing technology to streamline and enable electronic tick-borne disease case reporting.
7) Expand and Standardize Data Collection During Case Investigations: Enhance and ensure tick-borne disease surveillance case investigations used by local health department and health district staff in Pennsylvania include questions that can identify potential co-infections with other tick borne pathogens, and help identify potential risk factors for infection.
8) Develop a Surveillance Data Website: Use a centralized, publically accessible website to disseminate summaries of human, other animal, and ecologic tick-borne disease surveillance data at a statewide and county level.
Other: 1) Convene an Advisory Body: Convene a task force that report to the Secretary of Health and operates an independent advisory group on Lyme disease and other TBDs.

2) Obtain Independent Implementation Cost Analysis: Utilize the Legislative Budget and Finance Committee to provide an estimate for implementation of recommendations. It should be noted that despite the diversity of background and opinion in the members of the Lyme Task Force. There was uniform agreement on the growing threat of Lyme disease in Australia. The disease is increasing in frequency, is often difficult to diagnose, and requires significant further study. There is strong agreement that there is need for better education, better prevention, better diagnosis, and better treatment of the disease. Despite the many controversies in Lyme disease, there is uniform agreement among members of the committee of the need to implement the above recommendations.

APPENDIX A: ACT 83 OF 2014 FULL TEXT

LYME AND RELATED TICK-BORNE DISEASE SURVEILLANCE, EDUCATION,

PREVENTION AND TREATMENT ACT -
ENACTMENT
Act of Jun. 29, 2014, P.L. 808, No. 83 Cl. 35
An Act
Establishing a task force on Lyme disease and related maladies; and providing for powers and duties of the task
force, the Department of Health, the Department of Conservation and Natural Resources and the Pennsylvania
Game Commission to execute surveillance, prevention and education strategies.
The General Assembly of the Commonwealth of Pennsylvania hereby enacts as follows:
Section 1. Short title.
This act shall be known and may be cited as the Lyme and Related Tick-Borne Disease Surveillance, Education,
Prevention and Treatment Act.
Section 2. Findings.
The General Assembly finds that:
(1) Lyme disease and other tick-borne diseases are carried primarily by ticks and pose a serious threat
to the health and quality of life of many citizens of this commonwealth.
(2) The most common way to acquire Lyme disease is to be bitten by a tick that carries the spirochete.
(3) In 2009 and 2011, this Commonwealth ranked highest in the country in the number of confirmed
cases of Lyme disease. From 2002 through 2011, this commonwealth has reported a total of 42,032 confirmed

cases of Lyme Disease.
(4) The World Health Organization (WHO) states that Lyme disease will increasingly become a public
health threat in the United States.
(5) In August 2013, the Centers for Disease Control and Prevention (CDC) released a report that
preliminary estimates indicate approximately 300,000 Americans are diagnosed with Lyme disease each year.
This is approximately 10 times higher than the number of cases previously reported to the CDC every year.
(6) Lyme disease is most prevalent in Southeastern Pennsylvania, but it is found and is increasing across
this commonwealth.
(7) With proper precautions taken while engaged in outdoor activities, people can greatly reduce their
chances of tick pathogen transmission by making sure that frequent tick checks are made and ticks are
removed and disposed of promptly and properly.
(8) The early clinical diagnosis and appropriate treatment of these tick-borne disorders and diseases can
greatly reduce the risks of continued symptoms which can affect every system and organ of the human body
and often every aspect of life.
(9) Left untreated, Lyme disease can cause a number of signs and symptoms which can become quite
severe.
Section 3. Legislative intent.

It is the intent of the General Assembly:
(1) To provide the public with information and education to create greater public awareness of the
dangers of and measures available to prevent, diagnose and treat Lyme disease and related maladies.
(2) To ensure that:
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(i) Health care professionals, insurers, patients and governmental agencies are educated about
the broad spectrum of scientific and treatment options regarding all stages of Lyme disease and related
tick-borne illnesses.
(ii) Health care professionals provide patients with information about the broad spectrum of
scientific and treatment options regarding all stages of Lyme disease and related tick-borne illnesses to
enable patients to make an informed choice as part of informed consent and to respect the autonomy
of that choice.
(iii) Government agencies in this commonwealth provide information regarding the broad
spectrum of scientific and treatment options regarding all stages of Lyme disease and related tick-borne
illnesses.
(iv) A system is established for tick surveillance.
Section 4. Definitions.
The following words and phrases when used in this act shall have the

meanings given to them in this section
unless the context clearly indicates otherwise:
"Department." The Department of Health of the commonwealth.
"Health care professional." A licensed physician, a physician's assistant, a certified registered nurse
practitioner or other licensed health care professional.
"Lyme disease." The clinical diagnosis of a patient by a licensed physician, physician's assistant or certified
registered nurse practitioner of the presence of signs or symptoms compatible with acute, late-stage, persistent
infection with Borrelia burgdorferi or complications related to such infection or with such other strains of Borrelia
that are recognized by the Centers for Disease Control and Prevention as a cause of Lyme disease. The term
includes infection that meets the surveillance criteria established by the Centers for Disease Control and
Prevention and other acute and persistent manifestations of such an infection as determined by a physician.
"Related tick-borne illness." A case of Bartonella, babesiosis/piroplasmosis, anaplasmosis, ehrlichiosis or
other tick-transmissible illness. The term does not include Lyme disease.
"Secretary." The Secretary of Health of the commonwealth.
"State officials." The term includes the Secretary of Environmental Protection of the commonwealth.
"Task force." The task force established by this act.
Section 5. Task force.
(a) Establishment.--The department shall establish a task force on Lyme

disease and related tick-borne
diseases.
(b) Purpose.--The task force shall investigate and make recommendations to the department regarding:
(1) The surveillance and prevention of Lyme disease and related tick-borne illnesses in this
commonwealth.
(2) Raising awareness about the long-term effects of the misdiagnosis of Lyme disease.
(3) Development of a program of general public and health care professional information and education
regarding Lyme disease which shall include the broad spectrum of scientific and treatment options regarding
all stages of Lyme disease and related tick-borne illnesses.
(4) Cooperation with the Pennsylvania Game Commission to disseminate the information required
under paragraph (3) to licensees of the commission and the general public.
(5) Cooperation with the Department of Conservation and Natural Resources to disseminate the
information required under paragraph (3) to the general public and visitors of State parks and lands.
(6) Cooperation with the Department of Education to:
(i) Disseminate the information required under paragraph (3) to school administrators, faculty
and staff, parents, guardians and students.
(ii) Determine what role schools may play in the prevention of Lyme

disease, including, but not
limited to, integrated pest management strategies, prompt removal and reporting of tick removals to
parents, guardians and State officials.
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(iii) Update policies to recognize signs or symptoms of Lyme disease and related tick-borne
illnesses as health conditions potentially requiring accommodations.
(7) An active tick collection, testing, surveillance and communication program as provided under
subsection (f)(2).
(c) Composition.--The task force shall be composed of the following individuals:
(1) The secretary or a designee.
(2) The Secretary of the commonwealth or a designee.
(3) The Secretary of Education or a designee.
(4) The Deputy Secretary for Parks and Forestry in the Department of Conservation and Natural
Resources or a designee.
(5) The Director of the Bureau of Information and Education of the Pennsylvania Game Commission or a
designee.
(6) Two physicians licensed in this commonwealth who are knowledgeable concerning treatment of
Lyme disease and related tick-borne illness and who are members of the International Lyme and Associated
Diseases Society.

(7) Two physicians licensed in this commonwealth who are knowledgeable concerning treatment of
Lyme disease and related tick-borne illness and who are members of the Infectious Diseases Society of
America.
(8) An epidemiologist licensed in this commonwealth who has expertise in spirochetes and related
infectious diseases.
(9) Two individuals who represent Lyme disease patient groups and who may be a Lyme disease patient
or a family member of a Lyme disease patient.
(10) One individual who is a Lyme disease patient or family member of a Lyme disease patient.
(11) Two registered nurses licensed in this commonwealth, one of whom is a certified registered nurse
practitioner and both of whom are knowledgeable concerning Lyme disease and related tick-borne illness.
(12) The Director of Vector Management of the Department of Environmental Protection.
(13) An entomologist with the Department of Entomology of The Pennsylvania State University who has
experience in tick identification and tick-borne diseases.
(14) A registered school nurse licensed in this commonwealth who is knowledgeable concerning Lyme
disease and related tick-borne illness.
(15) Two veterinarians licensed in this commonwealth, at least one of whom is a veterinary

epidemiologist and both of whom are knowledgeable concerning Lyme disease and related tick-borne illness.

(16) A representative from the Northeast DNA Laboratory of East Stroudsburg University who is knowledgeable about vector-borne diseases.

(d) Meetings.--

(1) Within 45 days of the effective date of this section, the secretary shall appoint the members of the task force. The secretary shall appoint a chairman of the task force.

(2) The task force shall convene within 90 days of the effective date of this section and shall meet at least quarterly. The task force may convene meetings via teleconference.

(3) The task force shall issue a report with recommendations to the secretary within one year of its first meeting. The report shall also be transmitted to the Public Health and Welfare Committee of the Senate, the Health Committee of the House of Representatives and the Human Services Committee of the House of Representatives.

(4) Nothing in this act shall be construed to prohibit the task force from making interim reports or taking interim actions.

(e) Compensation and expenses.--The members of the task force shall receive no compensation for their services but shall be allowed their actual and necessary expenses incurred in performance of their duties. Reimbursement shall be provided by the department.

(f) Duties of department.--The department shall:

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(1) Develop a program of general public and health care professional information and education
regarding Lyme disease which shall include the broad spectrum of scientific and treatment options regarding
all stages of Lyme disease and related tick-borne illnesses.

(2) Develop an active tick collection, testing, surveillance and communication program, subject to the
availability of funds, in cooperation with the Department of Environmental Protection, to provide a better
understanding of, including, but not limited to, the full range of tick-borne diseases, geographic hot spots and
levels of infectivity to be used in targeting prevention, information and education efforts. This effort may
include the exploration of and recommendations regarding the use of veterinary data on tick-borne disease
prevention, specifically dogs and horses and perhaps other animals, as the Centers for Disease Control and
Prevention has recommended. The surveillance data shall be communicated to health care professionals via
public health alerts and shall be published on the department's publicly accessible Internet website. The
department may enter into a contract, memorandum of understanding or other agreement with another
governmental or nongovernmental entity to develop an active tick collection, testing, surveillance and

communication program.
(3) Cooperate with the Pennsylvania Game Commission to disseminate the information required under
paragraph (1) to licensees of the Pennsylvania Game Commission and the general public.
(4) Cooperate with the Department of Conservation and Natural Resources to disseminate the
information required under paragraph (1) to the general public and visitors of State parks and lands.
(5) Cooperate with the Department of Education to:
(i) Disseminate the information required under paragraph (1) to school administrators, school
nurses, faculty and staff, parents, guardians and students.
(ii) Determine what role schools may play in the prevention of Lyme disease, including, but not
limited to, integrated pest management strategies and prompt removal and reporting of tick removals
to parents, guardians and State officials.
(iii) Update policies to recognize signs or symptoms of Lyme disease and related tick-borne
illnesses as health conditions potentially requiring accommodations.
(6) Cooperate with professional associations of health care professionals to provide the education
program for professionals required under paragraph (1).
(7) Cooperate with The Pennsylvania State University, Department of Entomology, cooperative
extension program for integrated pest management, to disseminate

educational resources about ticks, related
diseases and integrated pest management for disease prevention as required under paragraph (1) to health
care professionals and the general public.
(8) Identify and apply for public and private grants and funding in order to carry out the provisions of
this act.
(9) Within 45 days of the effective date of this section, make available current data on tick surveillance
programs in this commonwealth conducted by other entities, including the Northeast DNA Laboratory of East
Stroudsburg University and the Department of Entomology of The Pennsylvania State University, until such
time as the department publishes the results of the active tick collection, testing, surveillance and
communication program as provided for in paragraph (2). The data shall be communicated via public health
alerts to health care professionals and made available on the department's publicly accessible Internet
website.
Section 6. Effective date.
This act shall take effect immediately

APPENDIX C: OTHER REFERENCES & RESOURCES There are well over 20,000 peer-reviewed research studies published on Lyme disease alone, not including coinfections. This Task Force did not evaluate these references. Therefore, this Appendix should not be considered a Task Force-approved recommendation. It is merely a random selection of references and a starting point for independent review and research.

References supporting IDSA and ILADS published guidelines for Lyme and TBD may be referred to for a more comprehensive consideration of the broad spectrum of perspectives.

Research is evolving rapidly, as such Task Force members should be leveraged for comprehensive up-to-date research references as well as cited material.

Lyme Disease References

Other Tick-Borne Diseases References

Other References

ENDNOTES

1

Task Force membership was defined category in Act 83 and named by the Secretary of Health in 2014. Membership of the
task force changed as a result of administration changes and state agency staff changes. One Nurse practitioner became unable
to participate due to personal concerns and was not replaced.

2

Nelson CA, Saha S, Kugeler KJ, Delorey MJ, Shankar MB, Hinckley AF, Mead PS. Incidence of clinician-diagnosed Lyme disease, United States, 2005-2010. Emerg Infect Dis. 2015 Sep;21(9):1625-31. http://wwwnc.cdc.gov/eid/article/21/9/15-0417_article

3

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4

"CDC provides estimate of Americans diagnosed with Lyme disease each year," Centers for Disease Control and Prevention, August 19, 2013, accessed July 26, 2015, http://www.cdc.gov/media/releases/2013/p0819-lyme-disease.html.

5

http://www.portal.state.pa.us/portal/server.pt/community/newsroom/14287?id=20732&typeid=1

6

Kiersten J. Kugeler, et al., "Geographic Distribution and Expansion of Human Lyme Disease, United States," Emerging Infectious Diseases 21 (August 2015): 1456, accessed July 26, 2015, doi: http://dx.doi.org/10.3201/eid2108.141878

7

Co-infection with multiple tick-borne pathogens may make the diagnosis and treatment particularly challenging. Numerous studies demonstrate increased intensity and duration of symptoms. Please see appendix for listing of relevant research.

8

Emily Adrion, et al., "Health care costs, utilization and patterns of care following Lyme disease," PLOS One (February 4, 2015), accessed July 26, 2015, doi: 10.1371/journal.pone.0116767

9

For a full description of terms used in this report – including definitions for the aforementioned TBDs – please see the "Glossary of Terms" in the Appendix.

10 Estimates of the percentage of patients diagnosed with Lyme disease who continue to experience symptoms of Lyme disease post-treatment vary significantly. While the CDC estimates approximately 10-20 percent, some studies have found prevalence of persistent symptoms in as many as 63 percent of patients. For more information, please see the references listed in the Appendix. Multiple studies since the 1980s have demonstrated symptom relapse post-treatment (CITE: [The Long-Term Follow-up of Lyme Disease: A Population-Based Retrospective Cohort Study. Authors: Shadick NA; Phillips CB; Sangha O et al. Ann Intern Med 1999 Dec 21;131(12):919-26]).

11Note: Definitions from CDC and/or Merriam Webster Dictionary. Please see Appendix B for a comprehensive list and definitions of TBDs found in the U.S.

12 Source: Last JM, editor. Dictionary of epidemiology. 4th ed. New York: Oxford University Press; 2001. p. 61.

13 http://www.cdc.gov/vaccines/about/terms/glossary.htm

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15 http://www.aabb.org/programs/publications/bulletins/Documents/ab14-05.pdf

16 Dermatology Research and Practice, Volume 2012 (2012), http://dx.doi.org/10.1155/2012/451727, Bull's-Eye and Nontarget Skin Lesions of Lyme Disease: An Internet Survey of Identification of Erythema Migrans,

John N. Aucott<http://www.hindawi.com/93717358/>, Lauren A. Crowder<http://www.hindawi.com/87970831/>, Victoria Yedlin<http://www.hindawi.com/19596060/>, and Kathleen B. Kortte<http://www.hindawi.com/86930793/>

17 Infection. 1996 Mar-Apr;24(2):182-6. Physician preferences in the diagnosis and treatment of Lyme disease in the United States. Ziska MH1, Donta ST, Demarest FC. - J Am Board Fam Pract. 2002 Jul-Aug;15(4):277-84. Lyme disease knowledge, beliefs, and practices of New Hampshire primary care physicians. Magri JM1, Johnson MT, Herring TA, Greenblatt JF.

18 Johnson, L., Wilcox, S., Mankoff, J. and Stricker, RB (2014) Severity of Chronic Lyme Disease Compared to Other Chronic Conditions: A Quality of Life Survey. PeerJ, DOI 10.7717/peerj.322. (Open access.)

19 Marques, Adriana, Infect Dis Clin North Am. 2008 Jun; 22(2):341–360, Chronic Lyme Disease: An Appraisal. Aucott, John N; et al, Quality of Life Research, Feb 2013, Vol 22, Issue 1, pp 75-84, Post-treatment Lyme disease syndrome symptomatology and the impact on life functioning: is there something here?

20 IOM's report specifically recommended a comprehensive program to bring target audiences up-to-date with current knowledge, to level-set the understanding of the state of the science using an evidence-aware approach, to share the evolving and multiple views guiding prevention – of all types. Please see: http://iom.nationalacademies.org/Reports/2011/CriticalNeeds-and-Gaps-in-Understanding-Prevention-Amelioration-and-Resolution-of-Lyme-and-Other-Tick-Borne-Diseases.aspx

21 Leverage other universities technology like URI – tick spotter, and

potentially NASAs technology developed to quickly review
heat fields to easily locate hot spots??
22
See "Dogs and Ticks" www.dogsandticks.com/diseases_in_your_area.php
23 Companion Animal Parasite Council
www.capcvet.org/diseases_in_your_area.php
24 See "Dogs and Ticks"
www.dogsandticks.com/diseases_in_your_area.php
25 "clinical manifestations of simultaneous Lyme disease and Babesiosis tend to be more severe than would be expected if these infections occurred separately",
http://cid.oxfordjournals.org/content/33/5/676.long , Clinical Infectious Diseases, Vol 33, Iss 5,
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pp 676-685, "Coinfecting Deer-Associated Zoonoses; Lyme Disease, Babesiosis, and Ehrlichiosis", 2001, Authors: Charles Thompson, Andrew Spielman, and Peter J. Krause

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