

The existing public health perspective on Lyme disease underestimates human Lyme disease risk and disease burden. This will only get worse, as the number of ticks and tick-borne disease is expected to increase with climate change.

Lyme disease is an Underestimated, Disabling, and Expensive Disease

Pfeiffer, MB. Lyme: *The First Epidemic of Climate Change*. 2018. Island Press, Washington DC

Summary: Lyme disease is spreading rapidly around the globe as ticks move into places they could not survive before. The first epidemic to emerge in the era of climate change, the disease infects half a million people in the US and Europe each year, and untold multitudes in Canada, China, Russia, and Australia. Mary Beth Pfeiffer shows how we have contributed to this growing menace, and how modern medicine has underestimated its danger. She tells the heart-rending stories of families destroyed by a single tick bite, of children disabled, and of one woman's tragic choice after an exhaustive search for a cure. Pfeiffer also warns of the emergence of other tick-borne illnesses that make Lyme more difficult to treat and pose their own grave risks. Lyme is an impeccably researched account of an enigmatic disease, making a powerful case for action to fight ticks, heal patients, and recognize humanity's role in a modern scourge.

Rebman AW, Aucott JN, Weinstein ER, Bechtold KT, Smith KC, Leonard L. Living in Limbo: Contested Narratives of Patients with Chronic Symptoms Following Lyme Disease. *Qualitative Health Research* 2017; 27(4):534-546.

Abstract: Persistent, subjective symptoms of unknown etiology following treatment for Lyme disease have been termed post-treatment Lyme disease syndrome or chronic Lyme disease (PTLDS/CLD). The objective of this study was to give primacy to the patient experience of this medically contested condition by eliciting patient illness narratives and identifying emergent issues through semi-structured interviews conducted among 29 participants. We used thematic narrative analysis to identify three predominant themes: (a) Physical and social limitations lead to a "new normal" characterized by fundamental shifts of ways of being in the world, (b) disease-specific factors contribute to symptom and illness invisibility that affects social support in nuanced ways, and (c) pervasive medical uncertainty regarding PTLDS/CLD promotes an increased sense of personal responsibility for care. Similar to other contested or medically unexplained syndromes, our findings suggest that the social sequelae of PTLDS/CLD can be equally protracted as the physical effects of this illness.

Adrion ER, Aucott J, Lemke KW, Weiner JP. Health Care Costs, Utilization and Patterns of Care following Lyme Disease. Brissette CA, ed. *PLoS ONE*. 2015;10(2):e0116767.

Abstract: Lyme disease is the most frequently reported vector borne infection in the United States. The Centers for Disease Control have estimated that approximately 10% to 20% of individuals may

experience Post-Treatment Lyme Disease Syndrome – a set of symptoms including fatigue, musculoskeletal pain, and neurocognitive complaints that persist after initial antibiotic treatment of Lyme disease. Little is known about the impact of Lyme disease or post-treatment Lyme disease symptoms (PTLDS) on health care costs and utilization in the United States. **Objectives:** 1) to examine the impact of Lyme disease on health care costs and utilization, 2) to understand the relationship between Lyme disease and the probability of developing PTLDS, 3) to understand how PTLDS may impact health care costs and utilization. **Methods:** This study utilizes retrospective data on medical claims and member enrollment for persons aged 0-64 years who were enrolled in commercial health insurance plans in the United States between 2006-2010. 52,795 individuals treated for Lyme disease were compared to 263,975 matched controls with no evidence of Lyme disease exposure. **Results:** Lyme disease is associated with \$2,968 higher total health care costs (95% CI: 2,807-3,128, $p<.001$) and 87% more outpatient visits (95% CI: 86%-89%, $p<.001$) over a 12-month period, and is associated with 4.77 times greater odds of having any PTLDS-related diagnosis, as compared to controls (95% CI: 4.67-4.87, $p<.001$). Among those with Lyme disease, having one or more PTLDS-related diagnosis is associated with \$3,798 higher total health care costs (95% CI: 3,542-4,055, $p<.001$) and 66% more outpatient visits (95% CI: 64%-69%, $p<.001$) over a 12-month period, relative to those with no PTLDS-related diagnoses. **Conclusions:** Lyme disease is associated with increased costs above what would be expected for an easy to treat infection. The presence of PTLDS-related diagnoses after treatment is associated with significant health care costs and utilization.

Implications of Immune System Manipulation by *Borrelia burgdorferi*

Embers ME, Hasenkampf NR, Jacobs MB, et al. Variable manifestations, diverse seroreactivity and post-treatment persistence in non-human primates exposed to *Borrelia burgdorferi* by tick feeding. Stevenson B, ed. *PLoS ONE*. 2017;12(12):e0189071.

Abstract: The efficacy and accepted regimen of antibiotic treatment for Lyme disease has been a point of significant contention among physicians and patients. While experimental studies in animals have offered evidence of post-treatment persistence of *Borrelia burgdorferi*, variations in methodology, detection methods and limitations of the models have led to some uncertainty with respect to translation of these results to human infection. With all stages of clinical Lyme disease having previously been described in nonhuman primates, this animal model was selected in order to most closely mimic human infection and response to treatment. Rhesus macaques were inoculated with *B. burgdorferi* by tick bite and a portion were treated with recommended doses of doxycycline for 28 days at four months post-inoculation. Signs of infection, clinical pathology, and antibody responses to a set of five antigens were monitored throughout the ~1.2 year study. Persistence of *B. burgdorferi* was evaluated using xenodiagnosis, bioassays in mice, multiple methods of molecular detection, immunostaining with polyclonal and monoclonal antibodies and an *in vivo* culture system. Our results demonstrate host-dependent signs of infection and variation in antibody responses. In addition, we observed evidence of persistent, intact, metabolically-active *B. burgdorferi* after antibiotic treatment of disseminated infection and showed that persistence may not be reflected by maintenance of specific antibody production by the host.

Elsner RA, Hastey CJ, Olsen KJ, Baumgarth N. Suppression of Long-Lived Humoral Immunity Following *Borrelia burgdorferi* Infection. Coburn J, ed. *PLoS Pathogens*. 2015;11(7):e1004976.

Abstract: Lyme Disease caused by infection with *Borrelia burgdorferi* is an emerging infectious disease and already by far the most common vector-borne disease in the U.S. Similar to many other infections,

infection with *B. burgdorferi* results in strong antibody response induction, which can be used clinically as a diagnostic measure of prior exposure. However, clinical studies have shown a sometimes-precipitous decline of such antibodies shortly following antibiotic treatment, revealing a potential deficit in the host's ability to induce and/or maintain long-term protective antibodies. This is further supported by reports of frequent repeat infections with *B. burgdorferi* in endemic areas. The mechanisms underlying such a lack of long-term humoral immunity, however, remain unknown. We show here that *B. burgdorferi* infected mice show a similar rapid disappearance of *Borrelia*-specific antibodies after infection and subsequent antibiotic treatment. This failure was associated with development of only short-lived germinal centers, micro-anatomical locations from which long-lived immunity originates. These showed structural abnormalities and failed to induce memory B cells and long-lived plasma cells for months after the infection, rendering the mice susceptible to reinfection with the same strain of *B. burgdorferi*. The inability to induce long-lived immune responses was not due to the particular nature of the immunogenic antigens of *B. burgdorferi*, as antibodies to both T-dependent and T-independent *Borrelia* antigens lacked longevity and B cell memory induction. Furthermore, influenza immunization administered at the time of *Borrelia* infection also failed to induce robust antibody responses, dramatically reducing the protective antiviral capacity of the humoral response. Collectively, these studies show that *B. burgdorferi*-infection results in targeted and temporary immunosuppression of the host and bring new insight into the mechanisms underlying the failure to develop long-term immunity to this emerging disease threat.

Rebman AW, Crowder LA, Kirkpatrick A, Aucott JN. Characteristics of seroconversion and implications for diagnosis of post-treatment Lyme disease syndrome: acute and convalescent serology among a prospective cohort of early Lyme disease patients. Clin. Rheumatology. 2015 Mar;34(3):585-589.

Abstract: Two-tier serology is often used to confirm a diagnosis of Lyme disease. One hundred and four patients with physician diagnosed erythema migrans rashes had blood samples taken before and after 3 weeks of doxycycline treatment for early Lyme disease. Acute and convalescent serologies for *Borrelia burgdorferi* were interpreted according to the 2-tier antibody testing criteria proposed by the Centers for Disease Control and Prevention. Serostatus was compared across several clinical and demographic variables both pre- and post-treatment. Forty-one patients (39.4%) were seronegative both before and after treatment. The majority of seropositive individuals on both acute and convalescent serology had a positive IgM western blot and a negative IgG western blot. IgG seroconversion on western blot was infrequent. Among the baseline variables included in the analysis, disseminated lesions ($p < 0.0001$), a longer duration of illness ($p < 0.0001$), and a higher number of reported symptoms ($p = 0.004$) were highly significantly associated with positive final serostatus, while male sex ($p = 0.05$) was borderline significant. This variability, and the lack of seroconversion in a subset of patients, highlights the limitations of using serology alone in identifying early Lyme disease. Furthermore, these findings underline the difficulty for rheumatologists in identifying a prior exposure to Lyme disease in caring for patients with medically unexplained symptoms or fibromyalgia-like syndromes.

*The existing diagnostic assays for Lyme disease are specific but insensitive. Serological tests demonstrate only that a person has had a recent or past exposure to a single type of tick-borne pathogen, *Borrelia burgdorferi**

The Insidious Life of *Borrelia* and Implications for Diagnostics and Treatment

Meriläinen L, Herranen A, Schwarzbach A, Gilbert L. Morphological and biochemical features of *Borrelia burgdorferi* pleomorphic forms. Lan R, ed. *Microbiology*. 2015;161(Pt 3):516-527.

Abstract: The spirochaete bacterium *Borrelia burgdorferi sensu lato* is the causative agent of Lyme disease, the most common tick-borne infection in the northern hemisphere. There is a long-standing debate regarding the role of pleomorphic forms in Lyme disease pathogenesis, while very little is known about the characteristics of these morphological variants. Here, we present a comprehensive analysis of *B. burgdorferi* pleomorphic formation in different culturing conditions at physiological temperature. Interestingly, human serum induced the bacterium to change its morphology to round bodies (RBs). In addition, biofilm-like colonies in suspension were found to be part of *B. burgdorferi*'s normal *in vitro* growth. Further studies provided evidence that spherical RBs had an intact and flexible cell envelope, demonstrating that they are not cell wall deficient, or degenerative as previously implied. However, the RBs displayed lower metabolic activity compared with spirochaetes. Furthermore, our results indicated that the different pleomorphic variants were distinguishable by having unique biochemical signatures. Consequently, pleomorphic *B. burgdorferi* should be taken into consideration as being clinically relevant and influence the development of novel diagnostics and treatment protocols.

Crossland NA, Alvarex X, Embers ME. Late Disseminated Lyme Disease: Associated Pathology and Spirochete Persistence Posttreatment in Rhesus Macaques. *Am. J. Pathol.* 2018 Mar;188(3):672-682.

Abstract: Nonhuman primates currently serve as the best experimental model for Lyme disease because of their close genetic homology with humans and demonstration of all three phases of disease after infection with *Borrelia burgdorferi*. We investigated the pathology associated with late disseminated Lyme disease (12 to 13 months after tick inoculation) in doxycycline-treated (28 days; 5 mg/kg, oral, twice daily) and untreated rhesus macaques. Minimal to moderate lymphoplasmacytic inflammation, with a predilection for perivascular spaces and collagenous tissues, was observed in multiple tissues, including the cerebral leptomeninges, brainstem, peripheral nerves from both fore and hind limbs, stifle synovium and perisynovial adipose tissue, urinary bladder, skeletal muscle, myocardium, and visceral pericardium. Indirect immunofluorescence assays that combined monoclonal (outer surface protein A) and polyclonal antibodies were performed on all tissue sections that contained inflammation. Rare morphologically intact spirochetes were observed in the brains of two treated rhesus macaques, the heart of one treated rhesus macaque, and adjacent to a peripheral nerve of an untreated animal. *Borrelia* antigen staining of probable spirochete cross sections was also observed in heart, skeletal muscle, and near peripheral nerves of treated and untreated animals. These findings support the notion that chronic Lyme disease symptoms can be attributable to residual inflammation in and around tissues that harbor a low burden of persistent host-adapted spirochetes and/or residual antigen.

Sapi E, Balasubramanian K, Poruri A, et al. Evidence of *In Vivo* Existence of *Borrelia* Biofilm in Borreliac Lymphocytomas. *European Journal of Microbiology & Immunology*. 2016;6(1):9-24.

Abstract: Lyme borreliosis, caused by the spirochete *Borrelia burgdorferi sensu lato*, has grown into a major public health problem. We recently identified a novel morphological form of *B. burgdorferi*, called biofilm, a structure that is well known to be highly resistant to antibiotics. However, there is no evidence of the existence of *Borreliabiofilm in vivo*; therefore, the main goal of this study was to determine the presence of *Borrelia* biofilm in infected human skin tissues. Archived skin biopsy tissues from borreliac

lymphocytomas (BL) were reexamined for the presence of *B. burgdorferi sensu lato* using *Borrelia*-specific immunohistochemical staining (IHC), fluorescent *in situ* hybridization, combined fluorescent *in situ* hybridization (FISH)–IHC, polymerase chain reaction (PCR), and fluorescent and atomic force microscopy methods. Our morphological and histological analyses showed that significant amounts of *Borrelia*-positive spirochetes and aggregates exist in the BL tissues. Analyzing structures positive for *Borrelia* showed that aggregates, but not spirochetes, expressed biofilm markers such as protective layers of different mucopolysaccharides, especially alginate. Atomic force microscopy revealed additional hallmark biofilm features of the *Borrelia*/alginate-positive aggregates such as inside channels and surface protrusions. In summary, this is the first study that demonstrates the presence of *Borrelia* biofilm in human infected skin tissues.

Feng J, Wang T, Shi W, et al. Identification of novel activity against *Borrelia burgdorferi* persisters using an FDA approved drug library. *Emerging Microbes & Infections*. 2014;3(7):e49-.

Although antibiotic treatment for Lyme disease is effective in the majority of cases, especially during the early phase of the disease, a minority of patients suffer from post-treatment Lyme disease syndrome (PTLDS). It is unclear what mechanisms drive this problem, and although slow or ineffective killing of *Borrelia burgdorferi* has been suggested as an explanation, there is a lack of evidence that viable organisms are present in PTLDS. Although not a clinical surrogate, insight may be gained by examining stationary-phase *in vitro* *Borrelia burgdorferi* persisters that survive treatment with the antibiotics doxycycline and amoxicillin. To identify drug candidates that can eliminate *B. burgdorferi* persisters more effectively, we screened an Food and Drug Administration (FDA)-approved drug library consisting of 1524 compounds against stationary-phase *B. burgdorferi* by using a newly developed high throughput SYBR Green I/propidium iodide (PI) assay. We identified 165 agents approved for use in other disease conditions that had more activity than doxycycline and amoxicillin against *B. burgdorferi* persisters. The top 27 drug candidates from the 165 hits were confirmed to have higher anti-persister activity than the current frontline antibiotics. Among the top 27 confirmed drug candidates from the 165 hits, daptomycin, clofazimine, carbomycin, sulfa drugs (e.g., sulfamethoxazole), and certain cephalosporins (e.g. cefoperazone) had the highest anti-persister activity. In addition, some drug candidates, such as daptomycin and clofazimine (which had the highest activity against non-growing persisters), had relatively poor activity or a high minimal inhibitory concentration (MIC) against growing *B. burgdorferi*. Our findings may have implications for the development of a more effective treatment for Lyme disease and for the relief of long-term symptoms that afflict some Lyme disease patients.

Feng J, Zhang S, Shi W, Zubcevik N, Miklossy J, Zhang Y. Selective Essential Oils from Spice or Culinary Herbs Have High Activity against Stationary Phase and Biofilm *Borrelia burgdorferi*. *Frontiers in Medicine*. 2017;4:169. doi:10.3389/fmed.2017.00169.

Abstract: Although the majority of patients with acute Lyme disease can be cured with the standard 2–4 week antibiotic treatment, about 10–20% of patients continue suffering from chronic symptoms described as posttreatment Lyme disease syndrome. While the cause for this is debated, one possibility is that persister bacteria are not killed by the current Lyme antibiotics and remain active in the system. It has been reported that essential oils have antimicrobial activities and some have been used by patients with persisting Lyme disease symptoms. However, the activity of essential oils against the causative agent *Borrelia burgdorferi* (*B. burgdorferi*) has not been well studied. Here, we evaluated the activity of 34 essential oils against *B. burgdorferi* stationary phase culture as a model for persister bacteria. We found that not all essential oils had activity against the *B. burgdorferi* stationary phase culture, with top five essential oils (oregano, cinnamon bark, clove bud, citronella, and wintergreen) at a low concentration of 0.25% showing high anti-persister activity that is more active than the known persister drug daptomycin. Interestingly, some highly active essential oils were found to have excellent anti-

biofilm ability as shown by their ability to dissolve the aggregated biofilm-like structures. The top three hits, oregano, cinnamon bark, and clove bud completely eradicated all viable cells without any regrowth in subculture in fresh medium, whereas but not citronella and wintergreen did not have this effect. Carvacrol was found to be the most active ingredient of oregano oil showing excellent activity against *B. burgdorferi* stationary phase cells, while other ingredients of oregano oil p-cymene and α -terpinene had no apparent activity. Future studies are needed to characterize and optimize the active essential oils in drug combination studies in vitro and in vivo and to address their safety and pharmacokinetic properties before they can be considered as a novel treatment of persistent Lyme disease.

Middelveen MJ, Sapi E, Burke J, Filush KR, Agustin F, Fesler MC, Stricker RB. Persistent *Borrelia* Infection in Patients with Ongoing Symptoms of Lyme Disease. *Healthcare* 2018, 6(2), 33; doi:10.3390/healthcare6020033

Abstract: Introduction: Lyme disease is a tickborne illness that generates controversy among medical providers and researchers. One of the key topics of debate is the existence of persistent infection with the Lyme spirochete, *Borrelia burgdorferi*, in patients who have been treated with recommended doses of antibiotics yet remain symptomatic. Persistent spirochetal infection despite antibiotic therapy has recently been demonstrated in non-human primates. We present evidence of persistent *Borrelia* infection despite antibiotic therapy in patients with ongoing Lyme disease symptoms. **Methods:** In this pilot study, culture of body fluids and tissues was performed in a randomly selected group of 12 patients with persistent Lyme disease symptoms who had been treated or who were being treated with antibiotics. Cultures were also performed on a group of ten control subjects without Lyme disease. The cultures were subjected to corroborative microscopic, histopathological and molecular testing for *Borrelia* organisms in four independent laboratories in a blinded manner. **Results:** Motile spirochetes identified histopathologically as *Borrelia* were detected in culture specimens, and these spirochetes were genetically identified as *Borrelia burgdorferi* by three distinct polymerase chain reaction (PCR)-based approaches. Spirochetes identified as *Borrelia burgdorferi* were cultured from the blood of seven subjects, from the genital secretions of ten subjects, and from a skin lesion of one subject. Cultures from control subjects without Lyme disease were negative for *Borrelia* using these methods. **Conclusions:** Using multiple corroborative detection methods, we showed that patients with persistent Lyme disease symptoms may have ongoing spirochetal infection despite antibiotic treatment, similar to findings in non-human primates. The optimal treatment for persistent *Borrelia* infection remains to be determined.

Anchor bias, grounded in medical observations made over 40 years ago, continues to overly influence epidemiological and public health Lyme disease paradigms

Improving the Diagnostic Accuracy of Laboratory Tests for Lyme disease

Citera M, Freeman PR, Horowitz RI. Empirical validation of the Horowitz Multiple Systemic Infectious Disease Syndrome Questionnaire for suspected Lyme disease. *International Journal of General Medicine*. 2017;10:249-273.

Abstract: Lyme disease is spreading worldwide, with multiple *BORRELIA* species causing a broad range of clinical symptoms that mimic other illnesses. A validated Lyme disease screening questionnaire would be clinically useful for both providers and patients. Three studies evaluated such a screening tool, namely the Horowitz Multiple Systemic Infectious Disease Syndrome (MSIDS) Questionnaire. The purpose was to see if the questionnaire could accurately distinguish between Lyme patients and healthy individuals. **Methods:** Study 1 examined the construct validity of the scale examining its factor structure and reliability of the questionnaire among 537 individuals being treated for Lyme disease. Study 2 involved an online sample of 999 participants, who self-identified as either healthy (N=217) or suffering from Lyme now (N=782) who completed the Horowitz MSIDS Questionnaire (HMQ) along with an outdoor activity survey. We examined convergent validity among components of the scale and evaluated discriminant validity with the Big Five personality characteristics. The third study compared a sample of 236 patients with confirmed Lyme disease with an online sample of 568 healthy individuals. **Results:** Factor analysis results identified six underlying latent dimensions; four of these overlapped with critical symptoms identified by Horowitz – neuropathy, cognitive dysfunction, musculoskeletal pain, and fatigue. The HMQ showed acceptable levels of internal reliability using Cronbach's coefficient alpha and exhibited evidence of convergent and divergent validity. Components of the HMQ correlated more highly with each other than with unrelated traits. **Discussion:** The results consistently demonstrated that the HMQ accurately differentiated those with Lyme disease from healthy individuals. Three migratory pain survey items (persistent muscular pain, arthritic pain, and nerve pain/paresthesias) robustly identified individuals with verified Lyme disease. The results support the use of the HMQ as a valid, efficient, and low-cost screening tool for medical practitioners to decide if additional testing is warranted to distinguish between Lyme disease and other illnesses.

Somiari SB, Somiari RI. The Future of Biobanking: A Conceptual Look at How Biobanks Can Respond to the Growing Human Biospecimen Needs of Researchers. Adv. Exp. Med. Biol. 2015;864:11-27.

Biobanking of human biological specimens has evolved from the simple private collection of often poorly annotated residual clinical specimens, to well annotated and organized collections setup by commercial and not-for-profit organizations. The activities of biobanks is now the focus of international and government agencies in recognition of the need to adopt best practices and provide scientific, ethical and legal guidelines for the industry. The demand for more, high quality and clinically annotated biospecimens will increase, primarily due to the unprecedented level of genomic, post genomic and personalized medicine research activities going on. Demand for more biospecimens provides new challenges and opportunities for developing strategies to build biobanking into a business that is better able to supply the biospecimen needs of the future. A paradigm shift is required particularly in organization and funding, as well as in how and where biospecimens are collected, stored and distributed. New collection sites, organized as Research Ready Hospitals (RRHs) and new public-private partnership models are needed for sustainability and increased biospecimen availability. Biobanks will need to adopt industry-wide standard operating procedures, better and "non-destructive" methods for quality assessment, less expensive methods for sample storage/distribution, and objective methods to manage scarce biospecimens. Ultimately, the success of future biobanks will rely greatly on the success of public-private partnerships, number and diversity of available biospecimens, cost management and the realization that an effective biobank is one that provides high quality and affordable biospecimens to drive research that leads to better health and quality of life for all.