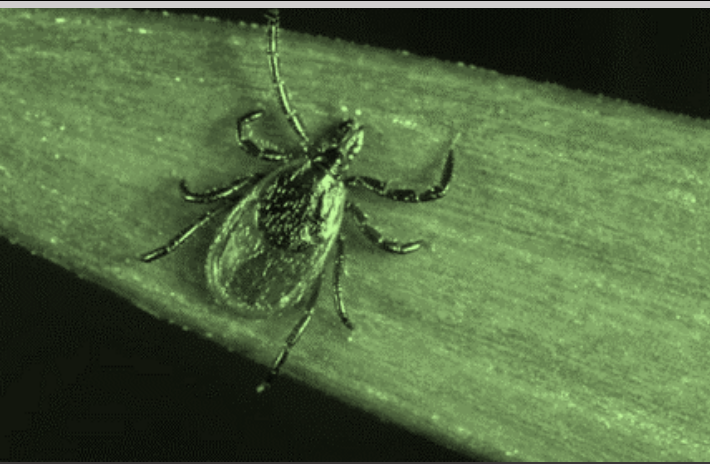


NIH AID

# Current Efforts in Lyme Disease Research, 2015



National Institute of  
Allergy and  
Infectious Diseases

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## Current Efforts in Lyme Disease Research

### Introduction

Ticks are capable of transmitting a variety of disease-causing pathogens including those responsible for Rocky Mountain spotted fever, ehrlichiosis, anaplasmosis, babesiosis, and Powassan disease. Lyme disease, the most prevalent of the tick-transmitted infections, is named after a town in Connecticut where a group of arthritis cases among children appeared in the early 1970s. By the mid-1970s, the symptoms of the disease were being described; however, it was not until 1981 when Willy Burgdorfer, Ph.D., and Alan Barbour, M.D., scientists at the Rocky Mountain Laboratories of the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), with their colleague Jorge Benach, Ph.D., at SUNY Stonybrook, identified the bacteria. Now named *Borrelia burgdorferi*, these bacteria are responsible for Lyme disease and the connection between deer ticks and the disease (1).

Typically, the first symptom of Lyme disease is a rash known as erythema migrans, which starts as a small red spot at the site of the tick bite and gets larger over a period of several days, usually forming a circular or oval-shaped rash. The rash can be all red, or may have some clearing and look like a bull's eye. The rash can range in size and may be accompanied by other symptoms such as fever, headache, stiff neck, body aches, and fatigue. Although these symptoms may be like those of common viral infections, Lyme disease symptoms tend to last longer or may come and go over time. Untreated, the infection can spread, with additional rashes appearing at different sites on the body; the development of facial or Bell's palsy (weakness of the muscles on one or both sides of the face), meningitis, and/or heart involvement (light-headedness, fainting, shortness of breath, heart palpitations, or chest pain) also may occur. Additionally, some people who have Lyme disease may develop arthritis, usually in the large joints, such as the knees (2, 3).

Diagnosis of Lyme disease is based on signs and symptoms of the infection, history of exposure to infected deer ticks, and laboratory tests. Serologic tests are most helpful for patients who have later manifestations of the disease, as it can take several weeks after infection for these tests to become positive. Patients with early Lyme disease should be treated without serologic testing (4).

In most individuals, Lyme disease is effectively treated with antibiotics; however, the disease can be serious if untreated. Furthermore, a small percentage of patients report a range of sometimes debilitating symptoms called Post-Treatment Lyme Disease Syndrome (PTLDS), which may continue years after treatment (5). The reasons for these symptoms are unknown. Some evidence points to an autoimmune disease, perhaps triggered by the initial infection or by remnants of the original infection.

### History of NIAID's Lyme Disease Research Program

Since Burgdorfer and Barbour's seminal discovery in 1981, NIAID has supported an extensive and diverse research portfolio that encompasses basic and clinical research studies conducted by extramural and intramural investigators, including intramural scientists at NIAID's Rocky

Mountain Laboratories in Hamilton, Montana, as well as at NIAID laboratories in Bethesda, Maryland.

The institute has remained at the forefront of Lyme disease research and continues to address fundamental questions essential for progress in the field. NIAID's many contributions include:

- Helping to sequence the entire genome of *B. burgdorferi* and improving overall molecular tools available for studying the organism and response to infection. Without these early tools, research on the basic biology and disease-causing properties of *B. burgdorferi* would have lagged far behind that of other bacteria.
- Funding and conducting much of the basic research on pathogenesis and transmission, including research on ticks and their role in the disease cycle, and Lyme-associated arthritis.
- Funding and conducting fundamental studies on the human immune response to *B. burgdorferi* and identifying bacterial factors involved in that response. The identification of potential biomarkers of *B. burgdorferi* has informed current diagnostic tests and continues to be explored for development of improved tests.
- Addressing questions concerning the efficacy of long-term antibiotic therapy for the treatment of late or "chronic Lyme disease" through its support of three placebo-controlled clinical trials. The peer-reviewed, published results of these studies demonstrated that prolonged antibiotic therapy does not have a sustained benefit and that risks to the patient outweigh the benefits (<http://www.niaid.nih.gov/topics/lymedisease/Pages/antibiotic.aspx>).

### **Ongoing Research**

The overarching goals of the NIAID Lyme disease research program are to develop better means of diagnosing, treating, and preventing this disease. To accomplish these objectives, the NIAID Lyme disease research portfolio includes a broad range of activities conducted by extramural and intramural investigators, spanning basic science through human clinical research studies designed to increase our understanding of this disease. Many of these projects are investigator-initiated efforts but several are the result of targeted grant and small-business initiatives that NIAID has used to actively grow the research portfolio to address high priority areas such as persistence of infection after antibiotic treatment and the development of both early- and late-stage diagnostics.

NIAID utilizes all of these approaches to ensure it maintains a vibrant and multifaceted Lyme disease research portfolio that is addressing key basic, translational, and clinical research questions. All funded extramural NIH research projects are reviewed by a panel of scientific experts with a second level of review by the NIAID Advisory Council before support is initiated. This process ensures that the highest quality science is supported.

The NIAID Lyme disease research portfolio includes systematic studies on: microbial physiology; molecular, genetic, and cellular mechanisms of pathogenesis; mechanisms of protective immunity; vectors, as well as vector competency, and their influence on the

transmission of disease; effect of co-infections with other tick-borne diseases; efficacy of different modes of antibiotic therapy; and development of more sensitive and reliable diagnostic tests for both early and late Lyme disease, among other areas of research. Detailed information about NIH funding for Lyme disease research is available on the NIH Categorical Spending page, accessible from the NIH Research Portfolio Online Reporting Tools (RePORT) [website](#). Here, project lists are available that show funding amounts for each project and the NIH Institute or Center that has provided the support. These lists can be downloaded for further analysis. Information is also available on the [NIAID website](#).

The following summary highlights major areas of research currently under investigation in NIAID's extramural and intramural Lyme disease research portfolio:

### **Studies on the Vector**

NIAID supports basic research on the tick that transmits Lyme disease and other infections. Ongoing efforts include research on tick feeding and factors in tick saliva that affect transmission. These may include proteins that block blood clotting, the pain response to a tick bite, or other factors that may enhance the tick's chance of passing the bacteria to humans. Other researchers are investigating the tick olfactory system, and the development of ticks with reduced ability to transmit Lyme disease that could be used to interrupt the natural cycle of disease.

### **Studies on Pathogenesis**

Elucidating bacterial pathogenesis can help scientists understand how the bacteria infect the host, multiply, and ultimately cause human disease. NIAID plays a key role in support of basic pathogenesis studies to help understand how Lyme disease develops. Better understanding of this process can help to identify new targets for future diagnostics, therapies, and vaccines.

- A comprehensive analysis of *B. burgdorferi* surface proteins and their cellular counterparts could help scientists understand the unique dissemination characteristics and persistence of the bacterium.
- Structure/function studies of the Lyme bacterium flagellar motor may help scientists understand how *B. burgdorferi* and related bacteria travel through the body and affect multiple body sites.
- Understanding the surface composition of *B. burgdorferi* at the biochemical and molecular level may lead to ways to disrupt the interaction of the bacteria with the host or generate novel vaccine/diagnostic targets for future testing.

### **Studies on Persistence of Infection**

NIAID supports basic research projects to address key questions regarding persistence of infection in animal model systems (mice and non-human primates) and human clinical studies.

- Studies of interactions between the tick and its vertebrate hosts such as mice and deer at different phases of the life cycle may reveal more about how *B. burgdorferi* evades

the host immune system and provide clues on how to limit or prevent immune evasion.

- Analysis of an abundant bacterial surface protein (VlsE), known to vary its surface antigens, may contribute to an understanding of mechanisms *B. burgdorferi* employs to evade the host immune system.
- Real-time imaging analysis of *B. burgdorferi* infection in mice is being used to track the infection at the cellular level and monitor treatment effectiveness.
- A recent NIAID Division of Intramural Research (DIR)/Tufts Medical School collaboration utilizing xenodiagnosis showed the feasibility and safety of using laboratory-reared, disease-free larval ticks to search for persistence of *B. burgdorferi* infection after antibiotic therapy in Lyme patients. Putative positive patients were found by xenodiagnosis in the study, and a larger and more comprehensive study is underway to determine the significance of the finding.

### **Studies on Lyme Disease Diagnostic Testing**

Currently, there is no point-of-care diagnostic test that can substitute for laboratory-based testing. Development of such a diagnostic would enable physicians to make more informed treatment decisions when a patient in a Lyme-endemic area presents with symptoms consistent with Lyme disease. In recent years, NIAID has published targeted research initiatives and utilized the Small Business Innovation Research funding mechanism to inform the research community of its interest in advancing research on new diagnostic tools for Lyme disease. For example, several projects supported under the request for applications [RFA-AI-11-024](#) were transitioned to later-stage development in 2014.

- The development and testing of a new cytokine-based immunoassay for Lyme diagnosis, if successful, could allow for earlier and more rapid diagnosis of Lyme disease.
- Metabolic biomarkers and biosignatures for improved diagnostics are being discovered and characterized. These may contribute to new methods to measure presence of Lyme disease, including earlier-stage diagnosis, accurate staging of disease, or indications of successful treatment.
- Several investigators are working on the development of a new, rapid point-of-care Lyme diagnostic using lateral flow technologies.

### **Studies on Lyme Vaccines**

The first Lyme disease vaccine, LYMERix, was approved for use in humans in 1998, but was voluntarily withdrawn by the manufacturer in 2001 due to market reasons. NIAID was not

directly involved in the design and implementation of the LYMERix vaccine trials. However, patents for cloning the genes used in making the vaccine as well as knowledge of how certain antibodies contribute to protective immunity were derived from basic research grants funded by NIAID. NIAID-supported efforts have continued to build upon these advancements and have helped to spur new approaches currently under investigation.

- Ongoing efforts include multiple research projects in early-stage discovery and characterization of novel vaccine formulations and targets, including approaches that target proteins in tick saliva that are critical for the transmission of the Lyme bacteria to humans.
- In addition, several research groups are investigating the potential of reservoir vaccines—oral “bait” vaccines to prevent mice from becoming infected and continuing the transmission cycle—for reducing Lyme disease in humans and, in some cases, are field testing their products with support from the Centers for Disease Control and Prevention (CDC).
- Researchers have identified tick proteins that facilitate tick transmission of the Lyme disease bacteria or that enhance survival of those bacteria once in their vertebrate hosts. Studies are ongoing to see if vaccines specifically targeting some of these proteins may be used as a strategy for an “anti-tick vaccine” to be used to prevent disease.

### **Autoimmunity and Lyme Disease**

Understanding mechanisms resulting in inflammation disorders that accompany PTLDS and/or antibiotic-refractory Lyme arthritis may inform the development of therapies to help those impacted by these symptoms.

- Auto-antigens, antigens or epitopes thought to initiate or perpetuate an autoimmune response, may be at least partially responsible for post-infectious Lyme disease signs and symptoms. Multiple candidate auto-antigens are being screened with one having been characterized.
- Characterizing the reactivity of nervous system antibodies, which seem to differ between PTLDS patients and controls, may lead to an understanding of why some patients continue to have ongoing symptoms as well as lead to new avenues for treatment.

### **Clinical Studies**

NIAID intramural physician-scientists conduct a research program that aims to advance scientific knowledge of Lyme disease and to translate these advances into clinical practice. Patients with Lyme disease are studied at the NIH Clinical Center with the goals of ameliorating disease and improving prognosis as well as increasing understanding of the laboratory diagnosis, clinical manifestations, and immunological responses associated with *B. burgdorferi* infection. More than 500 volunteers are currently enrolled in ongoing studies focused on:

- Evaluation, treatment, and follow-up of Lyme disease patients to assess clinical course and outcomes and define immune responses to infection.
- Identification of biomarkers of infection to aid development of new diagnostic tools.
- Investigation of potential causes of PTLDS.
- Exploration of potential persistence of infection after antibiotic therapy for Lyme disease.

Information about these and other studies can be found on [ClinicalTrials.gov](https://ClinicalTrials.gov), a registry and results database of publicly and privately supported clinical studies of human participants. For information on NIAID-conducted studies, refer to the study title or ClinicalTrials.gov identifier provided in the table below:

Study Title	ClinicalTrials.gov Identifier
Xenodiagnosis After Antibiotic Treatment for Lyme Disease	<a href="https://ClinicalTrials.gov/ct2/show/study/NCT02446626">NCT02446626</a>
Evaluation, Treatment, and Follow-up of Patients With Lyme Disease	<a href="https://ClinicalTrials.gov/ct2/show/study/NCT00028080">NCT00028080</a>
Evaluation of Lyme Disease: Clinical, Microbiological, and Immunological Characteristics	<a href="https://ClinicalTrials.gov/ct2/show/study/NCT00001539">NCT00001539</a>
Analysis of Lyme Disease Lesions	<a href="https://ClinicalTrials.gov/ct2/show/study/NCT00132327">NCT00132327</a>

### Recent NIAID Lyme Disease Scientific Advances

NIAID intramural scientists at the Rocky Mountain Laboratories utilize molecular approaches in conjunction with experimental models to analyze *B. burgdorferi*. Of particular interest is how *B. burgdorferi* adapts to the distinct environments it encounters in the tick vector and mammalian host. These basic research studies provide knowledge about key components of the Lyme disease bacterium that are important for tick-borne transmission and infection of mammals, including humans. Some recent published findings of NIAID intramural researchers are summarized below.

- **Do bottlenecks exist during the infectious cycle of *B. burgdorferi*?**  
Changes in the population of Lyme disease bacteria at different stages of the infectious cycle are not well-defined. Humans get infected in the same way as the typical rodent host---through the bite of an infected tick. Researchers developed an experimental system to analyze the constraints that *B. burgdorferi* experiences as it completes the mouse-tick-mouse infectious cycle and found that both acquisition and transmission of the bacteria



by feeding ticks are limiting steps, or bottlenecks, presumably because only a few organisms are transferred. These findings identify potentially vulnerable points during the natural infectious cycle when interventions to prevent transmission and infection could be most effective (6).

- **Why do Lyme disease bacteria change what they look like at different stages of the infectious cycle?**

*B. burgdorferi* cycles between mammalian and tick environments, each of which varies over time. *B. burgdorferi* survival in these diverse conditions requires adaptation, a hallmark of which is a change in the composition of its outer surface. Researchers assessed whether the predominant surface proteins made by *B. burgdorferi* at different points of the infectious cycle were interchangeable and found that they could partially but incompletely substitute for each other. This finding suggests that these unrelated outer surface components share a related basic function but are optimized for the time and place in which they are made (7).

- **How does *B. burgdorferi* change as it moves between mammal and tick?**

Transmission of *B. burgdorferi* between the tick vector and a mammalian host requires the bacterium to sense and adapt to these distinct environments. Researchers investigated how and where BBD18, a regulatory protein they had previously identified, contributed to this adaptive process. They found that this key regulator played a critical role during bacteria acquisition by a feeding tick by shutting off the program needed by the Lyme disease bacteria in the mammal and switching on a different program needed to colonize the tick vector. These studies provide insight into how *B. burgdorferi* regulates its adaptive response during transition from mammalian host to tick vector (8, 9).

- **Can we facilitate laboratory investigations of *B. burgdorferi*?**

Many components of the Lyme disease bacterium are unique and of unknown significance to pathogenesis. Molecular genetics provide a powerful means with which to investigate novel and potentially important elements of bacterial pathogens, but the tools currently available for such studies in *B. burgdorferi* are somewhat limited. Researchers have developed a convenient and widely applicable new tool for molecular genetic investigations in *B. burgdorferi* (10).

NIAID also supports extramural scientists around the country who are investigating multiple aspects of Lyme disease spanning basic, translational, and clinical studies. Some of the most recent extramurally-supported research is described here, highlighting the diversity of studies funded.

- **Why do different *Borrelia* strains behave differently?**

The Lyme disease bacteria spread to targeted tissues within both tick and mammalian hosts, but strain-specific differences in their distribution within the host and ability to cause disease have been observed. Researchers identified protein molecules on the surface of the Lyme bacteria that are involved in attaching to different tissue structures

within mammalian and tick hosts and discovered that variations in those proteins can help direct where the bacteria go following infection. These studies may help explain different, strain-specific clinical manifestations of Lyme disease (11).

- **How prevalent is *Borrelia miyamotoi*?**

Investigators have conducted epidemiologic studies in the Northeastern United States of *Borrelia miyamotoi*, a bacterium that causes relapsing fever and is transmitted by the same ticks as Lyme disease and occurs in all Lyme-endemic areas of the United States. They determined that *B. miyamotoi* infection may be common in southern New England and that some infected individuals falsely test positive for Lyme disease (12).

- **Can biomarkers lead to a better diagnostic test for early Lyme disease?**

Lyme disease can be difficult to diagnose early in infection because current laboratory tests look for antibodies that take time to rise to detectable levels. In response to a specific NIAID solicitation for better early-stage Lyme diagnostics, researchers looked for biomarkers—non-antibody molecules that arise in response to infection—to see if a subset could be identified that was specific for Lyme disease. The first publication from that study reports a group of biomarkers that can differentiate between Lyme and a group of other diseases with high sensitivity, and that could accurately indicate Lyme disease in many blood samples that had tested negative through the currently accepted two-stage antibody test (13).

- **Which *B. burgdorferi* genes are switched on, and when?**

Understanding the genetic control of the Lyme bacteria life cycle is important for developing new diagnostic, treatment, and prevention measures. Scientists recently conducted a comprehensive study to determine which *B. burgdorferi* proteins are produced in tick nymphs and larvae as well as in bacteria that had been adapted to mammalian hosts. The results showed clear differences in which *B. burgdorferi* genes are switched on in response to different developmental stages and environmental clues, and provides the first detailed molecular blueprint of the carefully regulated Lyme bacteria life cycle. These studies will now provide the framework for further research on the genes critical for *B. burgdorferi* survival and pathogenesis (14).

- **Why do symptoms persist in some people after antibiotic treatment?**

- Lyme disease is generally responsive to antibiotic therapy. In some patients, however, musculoskeletal symptoms may continue for months after treatment. Although ongoing infection is considered an unlikely explanation for persistent symptoms or disease, it cannot be definitively excluded because *B. burgdorferi* is difficult to detect by culture except in early infection when the tell-tale erythema migrans rash is present. NIAID-supported researchers used a mouse model to suggest that inflammatory, immunogenic antigens, but not infectious bacteria, can persist near cartilage after treatment and might contribute to persistent symptoms (15).

- Researchers are continuing to search for persistence of infection after antibiotic therapy for Lyme disease, including an ongoing collaboration of NIAID intramural scientists with Tufts University and New York Medical College extramural researchers. This study is investigating xenodiagnosis using live, disease-free, laboratory-bred ticks to see if Lyme disease bacteria can be detected in people after completing antibiotic therapy and whether persistence is more common in people who continue to experience symptoms, such as fatigue and joint pain. The initial study showed that xenodiagnosis was safe and well-tolerated. The results of the study were published in 2014. The phase 2 study is now open (see [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02446626), identifier [NCT02446626](https://clinicaltrials.gov/ct2/show/study/NCT02446626)) (16).
- **How important is antigenic variation in Lyme disease?**  
Researchers conducted studies on a *B. burgdorferi* surface protein, VlsE, and provided the most direct evidence to date highlighting the importance of that protein in maintaining the Lyme bacteria life cycle in nature and describing the significant role played by changes in that protein—“antigenic variation”—in maintaining infections in mammalian hosts (17).
- **Does high cholesterol place you at increased risk of Lyme disease?**  
Because Lyme bacteria utilize cholesterol from their host to help construct their own membranes, researchers looked at whether individuals with lipid disorders such as high cholesterol might be at increased risk of Lyme disease. Using two different mouse models, the investigators determined that genetic conditions leading to elevated serum cholesterol may be a risk factor for increased severity of disease. Dietary cholesterol did not appear to lead to the increased risk, and because high cholesterol can stem from multiple genetic factors, additional work will be needed to identify the mechanism behind the results (18).
- **How do the Lyme bacteria cause arthritis?**  
A research group studying the factors leading to and sustaining Lyme arthritis have identified a genetic locus associated with both interferon, which is known to be associated with an increase in *B. burgdorferi*-induced arthritis, and Lyme disease pathogenesis (19). The same group also recently began characterizing the role of micro RNAs—small molecules that help to regulate which genes are switched on and off—in regulating Lyme arthritis (20).

### **NIH collaborations with other federal agencies and external organizations**

NIH participates in the Health and Human Services (HHS) Lyme and Other Tick-Borne Diseases Working Group along with representatives from the Office of the Secretary of HHS, CDC, and the U.S. Food and Drug Administration (FDA) to facilitate coordination and planning among participating agencies. The Working Group convenes twice a year to review the state of the science in Lyme disease research and has conducted four public webinars over the past few years to brief the scientific and patient communities on topics of interest including the state of

diagnostics, the persistence of infection in animal model systems, emerging tick-borne diseases, and vaccine research and development efforts.

NIH is partnering with the CDC, FDA, university researchers, and industry to develop improved diagnostics using the CDC Lyme disease serum repository for new test validation. The repository contains serum from Lyme disease patients and other disease etiologies that can be used as positive and negative controls. NIH provided support for the development of the repository, which is being used as a reference standard for the evaluation and calibration of new and existing assays (21).

### **Funding opportunities and research resources**

NIAID maintains a funding opportunities website to inform the research community about current grant opportunities and contract solicitations:

<http://www.niaid.nih.gov/researchfunding/ann/pages/opps.aspx>. In addition, NIAID has a comprehensive set of product development services and research tools and technologies to facilitate development and evaluation of vaccines, diagnostics, and therapeutics:

<http://www.niaid.nih.gov/labsandresources/resources/dmid/Pages/default.aspx>. These services make critical data, expertise, standardized research materials, and state-of-the art technologies available to eligible investigators worldwide at no charge. The purpose of these resources is not to assist researchers in developing a particular product from start to finish, but rather to lower the financial risk to product developers by providing limited, but critical, information to fill specific gaps in the product development pipeline. Currently, NIAID is utilizing its preclinical resources to develop an improved methodology for culturing *B. burgdorferi*. This organism grows slowly and is very difficult to culture, particularly from patient samples. By developing better ways to grow the bacteria, NIAID hopes to enable faster and more efficient laboratory research on the pathogen.

### **Future Plans/Directions**

NIAID's Lyme disease research portfolio will continue to encompass basic, translational, and clinical research studies. Specifically, efforts will continue to be centered on the study of basic biology and pathogenesis of *Borrelia burgdorferi*, which will help gain insight into Lyme disease. Research areas will include persistence of infection after antibiotic treatment using a variety of animal models, clinical studies, and improvement of Lyme disease diagnostics.

### **References**

1. Burgdorfer W et al. Lyme Disease – A Tick-Borne Spirochetosis? Science. 1982 Jun 18;216(4552):1317-9.
2. Bockenstedt LK and Wormser GP. Unraveling Lyme Disease. Arthritis Rheumatol. 2014 Sep;66(9):2313-23. doi: 10.1002/art.38756.
3. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet. 2012 Feb 4;379(9814):461-73.
4. Marques AR. Laboratory Diagnosis of Lyme disease - Advances and Challenges. Infect Dis Clin North Am. 2015 Jun;29(2):295-307.

5. Marques AR. Lyme disease: A Review. *Curr Allergy Asthma Rep.* 2010 Jan;10(1):13-20.
6. Rego R et al. Population Bottlenecks during the Infectious Cycle of the Lyme Disease Spirochete *Borrelia burgdorferi*. *PLoS One* 2014;9(6):e101009.
7. Tilly K, Bestor A, and Rosa PA. Lipoprotein Succession in *Borrelia burgdorferi*: Similar but Distinct Roles for OspC and VlsE at Different Stages of Mammalian Infection. *Mol. Microbiol.* 2013 Jul;89(2):216-27. doi: 10.1111/mmi.12271.
8. Hayes BM et al. Regulatory Protein BBD18 of the Lyme Disease Spirochete: Essential Role during Tick Acquisition? *mBio.* 2014 Apr 1;5(2):e01017-14. doi: 10.1128/mBio.01017-14.
9. Dulebohn DP, Hayes BM, and Rosa PA. Global Repression of Host-Associated Genes of the Lyme Disease Spirochete through Post-Transcriptional Modulation of the Alternative Sigma Factor RpoS. *PLoS One.* 2014 Mar 26;9(3):e93141. doi: 10.1371/journal.pone.0093141.
10. Kasumba IN et al. Use of an Endogenous Plasmid Locus for Stable in trans Complementation in *Borrelia burgdorferi*. 2015 *Appl. Environ. Microbiol.* 81(3):1038-1046.
11. Lin YP et al. Strain-Specific Variation of the Decorin-Binding Adhesin DpbA Influences the Tissue Tropism of the Lyme Disease Spirochete. *PLoS Pathog.* 2014;10(7):e1004238.
12. Krause PJ et al. *Borrelia miyamotoi* sensu lato Seroreactivity and Seroprevalence in the Northeastern United States. *Emerg Infect Dis.* 2014;20(7):1183-1190.
13. Molins CR et al. Development of a Metabolic Biosignature for Detection of Early Lyme Disease. *Clin Infect Dis.* 2015 Jun 15;60(12):1767-75. doi: 10.1093/cid/civ185.
14. Iyer R et al. Stage-specific Global Alterations to the Transcriptomes of Lyme disease Spirochetes during Tick Feeding and Following Mammalian Host Adaptation. *Mol Microbiol.* 2015;95(3):509-538.
15. Bockenstedt LK et al. Spirochete antigens persist near cartilage after murine Lyme borreliosis therapy. *J Clin Invest.* 2012;122(7):2652-2660.
16. Marques AR et al. Xenodiagnosis to Detect *Borrelia burgdorferi* Infection: A First-in-human Study. *Clin Infect Dis.* 2014 Apr;58(7):937-45
17. Rogovosky AS et al. Evaluation of the Importance of VlsE Antigenic Variation for the Enzootic Cycle of *Borrelia burgdorferi*. *PLoS One.* 2015;10(4):e0124268.
18. Toledo A et al. Hypercholesterolemia and ApoE deficiency result in severe infection with Lyme disease and relapsing-fever *Borrelia*. *Proc Nat Acad Sci.* 2015;112:5491-5496.
19. Ma Y et al. *Borrelia burgdorferi* arthritis-associated locus Bbaa1 regulates Lyme arthritis and K/B $\times$ N serum transfer arthritis through intrinsic control of type I IFN production. *J Immunol.* 2014 Dec 15;193(12):6050-60. doi: 10.4049/jimmunol.1401746.
20. Lochhead RB et al. MicroRNA-146a Provides Feedback Regulation of Lyme Arthritis but not Carditis During Infection with *Borrelia burgdorferi*. *PLoS Pathog.* 2014;10(6):e1004212.
21. Molins CR et al. Collection and characterization of samples for establishment of a serum repository for lyme disease diagnostic test development and evaluation. *J Clin Microbiol.* 2014 Oct;52(10):3755-62.