Center for Lyme Action

The State of Lyme Disease Research in the United States

An Educational Paper from Center for Lyme Action

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EXECUTIVE SUMMARY

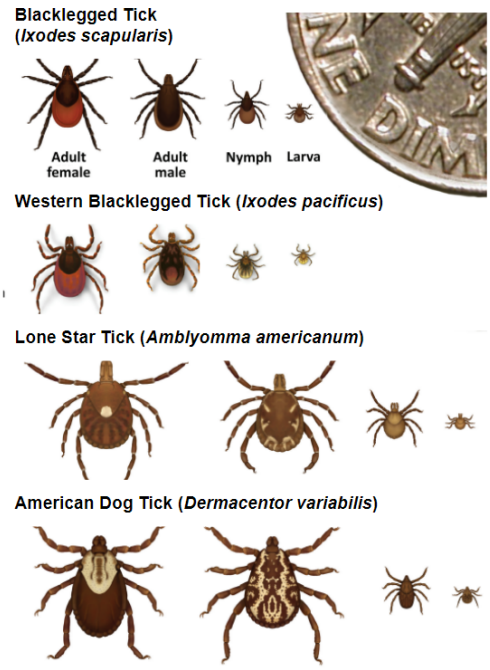
A growing epidemic	Tick-borne illness is a growing health threat to the American public, and ticks capable of transmitting diseases are found in all fifty states. Lyme disease accounts for the vast majority of tick-borne illnesses in the Northern Hemisphere, with an estimated 476,000 Americans diagnosed and treated annually.
Caused by an elusive pathogen	Decades of research on the causative agent of Lyme disease, a bacterium called Borrelia burgdorferi, demonstrates that it is an elusive pathogen capable of evading the immune system. The infection starts locally around the spot of the tick bite, but later disseminates to tissues throughout the body, including the skin, joints, heart, peripheral nervous system, and brain.
With no reliable diagnostic	Patients acquiring Lyme disease have a high probability of misdiagnosis. Lyme disease often comes with an erythema migrans rash, which is the most common method of diagnosis. However, the complexities of rash presentation make this approach unreliable, with a misdiagnosis potential of over 50%. In vitro diagnostic tests are also unreliable with the standard of care incorrectly reporting 39-59% of early Lyme cases.
And ineffective treatment	Treatment is most effective in the early stages of the infection; however, treatment failure still occurs in 14% of patients receiving timely therapy. Patients with delayed treatment have over 2 times the odds of failing treatment and developing chronic, debilitating symptoms. This condition impacts over 2 million Americans.
Resulting in societal burden	The annual direct medical costs associated with Lyme disease are estimated at $914M - $1.7B, with the full economic impact potentially reaching $50 - $100B. Borrelia is also linked to other costly conditions such as neuropsychiatric disorders, dementia, and heart conditions that increase the economic impact.
HHS and NIH request more research	In 2016, the Department of Health and Human Services (HHS) assembled a Tick-Borne Disease Working Group. This group called for the National Institutes of Health (NIH) to develop a plan to address this critical public health problem. In 2019, NIH released a comprehensive and well-researched Strategic Plan to improve tick-borne disease diagnosis, prevention, and treatment.
Yet funding is insufficient	Despite this government support, Lyme research received only $50M in NIH funding in 2022. This amount represents less than 2% of the public funds invested in HIV/AIDS, though the annual case count for Lyme is over an order of magnitude higher. The per-case funding for Lyme is also dwarfed by other vector-borne diseases, receiving 0.1% and 0.5% of that for Malaria and West Nile virus, respectively.
A call to action	In 2020, the Center for Lyme Action published a policy paper calling for a cure for Lyme and other tick-borne diseases by 2030. Achieving this goal will require research funding of $500M - $1B per year. This document summarizes the research to date in Lyme disease to show that this call to action is justified and essential.

BACKGROUND

The Strategic Importance of Tick-borne Disease

Tick-borne illness is increasingly recognized as an escalating public health threat for all Americans. Disease-carrying ticks, such as the black-legged tick, the lone star tick, and the American dog tick, are found in all fifty states. These ticks have significantly increased their geographical footprint, and surveillance maps from the CDC show the spread across the United States in recent years.

Currently, the CDC recognizes sixteen tick-borne pathogens causing human diseases, as summarized in Figure 1. These pathogens are a mixture of bacteria, parasites, and viruses, all of which have been proven to infect humans and pets through a tick bite. Some of the most common tick-borne diseases in the United States include Lyme disease, babesiosis, ehrlichiosis, Rocky Mountain spotted fever, anaplasmosis, Southern tick-associated rash illness, Tick-borne relapsing fever, and tularemia.



Tick-borne conditions recognized by the CDC to be present in the United States

Viral Infections	Bacterial Infections	Parasitic Infections
Bourbon virus Colorado tick fever Heartland virus Powassan virus	Anaplasmosis Borrelia mayonii Borrelia miyamotoi Ehrlichiosis Lyme disease Rickettsia parkeri rickettsiosis Rocky Mountain spotted fever Southern tick-associated rash illness (STARI) Tick-borne relapsing fever (TBRF) Tularemia 364D rickettsiosis	Babesiosis
Syndromes		
Alpha-gal		

FIGURE 1: (Left) Ticks that commonly bite humans. (Right) Tick-borne diseases and syndromes recognized by the CDC to be present in the U.S. Sources: CDC Reference Manual for Healthcare Providers on Tick Borne Disease in the U.S., CDC Information on Diseases Transmitted by Ticks in the U.S. and Bay Area Lyme Foundation.

This list of diseases expands when including pathogens endemic outside the U.S., threatening the health of U.S. military personnel and U.S. civilians living and working abroad. In addition, emerging research implicates other pathogens carried by ticks for which vector competence (i.e., the ability to transmit the pathogen) needs to be established (Chang et al., 2001; Holden et al., 2006; Wechtaisong et al., 2020).

Tick-related threats also include Alpha-gal syndrome, a serious, potentially life-threatening allergic reaction to red meat and other mammalian-derived products. Growing evidence suggests that Alpha-gal is triggered by the bite of the Lone Star Tick, found throughout the eastern, southeastern, and south-central states.

While severe red meat allergy is commonly recognized as the main symptom of Alpha-gal Syndrome (AGS), the common denominator among those infected with AGS is the human body's allergic response to a sugar molecule called alpha gal (galactose-α-1,3-galactose). According to the Alpha gal Syndrome Subcommittee Report to the Tick-borne Disease Working Group, "Alpha-gal allergy is currently best understood as a 'syndrome' because of the ubiquitous presence of mammalian-derived products and sources in seemingly innocuous exposures such as gummy bears and capsules (gelatin), medications (for example, heparin and thyroid hormone), bioprosthetics (for example, porcine heart valves), surgical mesh, select vaccines, and unlabeled 'natural flavorings' in countless foods."

Lyme disease accounts for the vast majority of tick and vector-borne illnesses in the Northern Hemisphere (Schotthoefer, 2015; Rosenberg, 2018). In the United States, Lyme disease is caused primarily by the bacterium *Borrelia burgdorferi*, transmitted to a host through the bite of an infected Ixodes tick (Radolf et al., 2012). The CDC estimates that nearly 500,000 Americans are diagnosed and treated for Lyme disease annually. This number makes Lyme disease the most common vector-borne disease in the United States.

Figure 2 summarizes the various annual case numbers often cited for Lyme disease. Each year over 30,000 cases are reported to the CDC (shown in dark green). However, the CDC notes that these case numbers come through a passive reporting system driven by busy healthcare providers, causing many cases to go unreported. In 2013, the CDC estimated that the incidence is 3-12x higher, with approximately 300,000 cases diagnosed in the U.S. annually (10x reported numbers shown in light green). In 2021, this number was increased to 476,000 Americans diagnosed and treated annually (shown in gray) based on insurance records gathered between 2010 and 2018 (Kugeler, 2021).

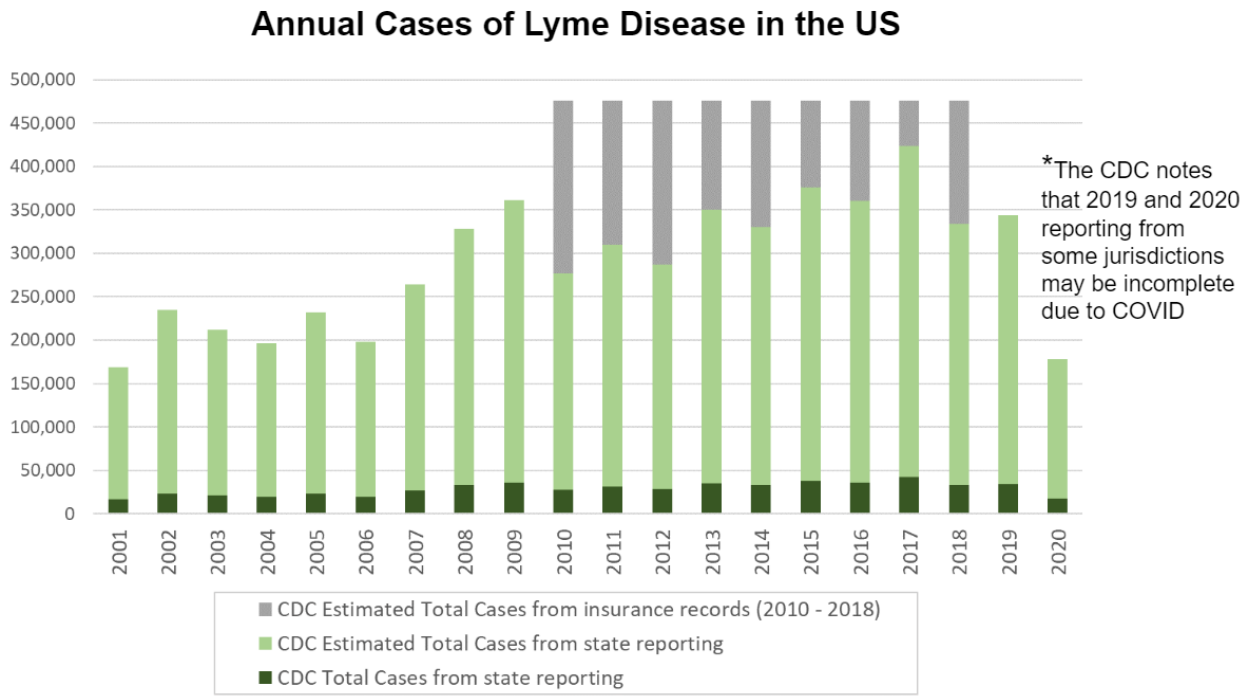


FIGURE 2: Annual cases of Lyme disease in the U.S. as reported and estimated by the CDC. The number of reported cases is a small fraction of the estimated incidence. Source: Generated from [CDC Surveillance Data] and Kugeler, 2021.

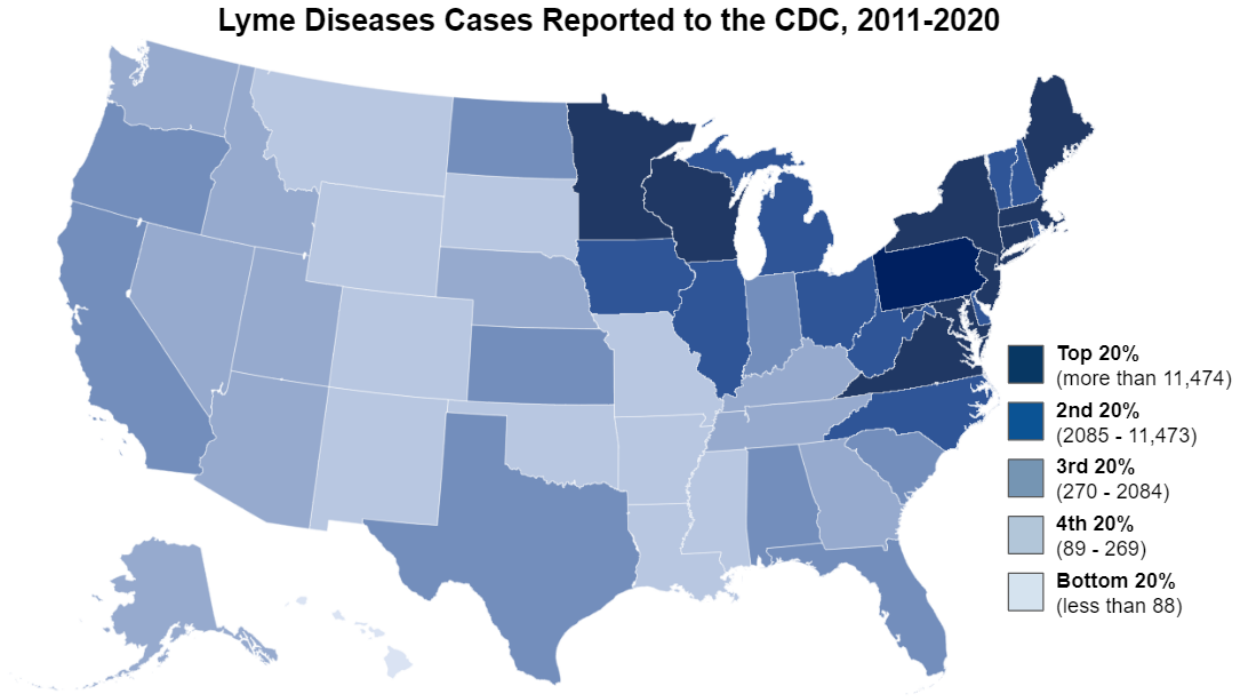
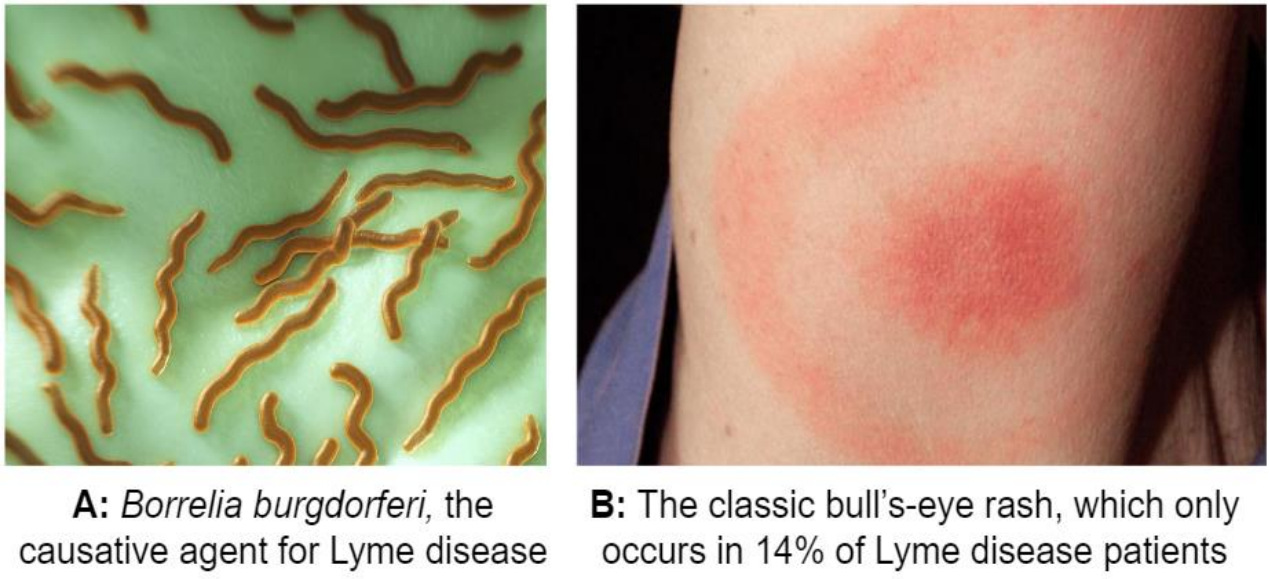


FIGURE 3: Lyme disease cases reported to the CDC by state from 2011-2020. The disease affects the entire population of the continental U.S. with growing incidence nationwide. Source: Generated from [CDC Surveillance data].

Figure 3 shows the Lyme disease cases reported to the CDC by state from 2011-2020. The disease affects the entire population of the United States, with the highest risk regions in the Northeast and Upper Midwest. The western region of the United States is also an area of growing incidence due to the presence of the western black-legged tick (Ixodes pacificus), which is known to carry the pathogen causing Lyme disease (Burgdorfer et al., 1985).

Lyme Disease: Symptoms, Diagnosis, Treatment, and Challenges

Although the causative agent for Lyme disease, *Borrelia burgdorferi* (Figure 4A), was first identified 40 years ago, challenges in diagnosing and treating the disease still exist. The distinctive feature of early Lyme disease is the erythema migrans (EM) rash, which occurs at the site of the tick bite an average of 7–10 days after the bite of an infected tick (Steere, 2001). It has been documented that the EM rash can have various manifestations; however, the classic "bull's-eye" shaped EM is the best known and is characterized by a central clearing or ring-within-a-ring pattern (Figure 4B). While EM is a common manifestation of early Lyme disease, at least 30% of patients do not identify an EM rash (Schwartz et al., 2017), and only 20% of patients with an EM rash (14% of total patients) exhibit the classic bull's eye (Tibbles et al., 2007).



A: *Borrelia burgdorferi,* the causative agent for Lyme disease

B: The classic bull's-eye rash, which only occurs in 14% of Lyme disease patients

FIGURE 4: (A) Photograph of Borrelia burgdorferi, the causative agent of Lyme disease. (B) Photograph of a classic "bull's-eye" rash often associated with Lyme disease but only occurring in a subset of patients. Sources: (A) Fineartamerica.com. (B) CDC Signs and Symptoms of Lyme Disease.

Early Lyme disease can be accompanied by virus-like infection signs and symptoms, including headache, fever, chills, fatigue, and muscle and joint pain (Aguero-Rosenfeld et al., 2005). These symptoms are nonspecific, and without a well-defined EM rash, accurate diagnosis can be challenging for frontline clinicians. Laboratory testing has traditionally been performed using a two-tiered testing algorithm screening for antibodies to *Borrelia*. However, a systematic review demonstrated that this methodology reports a negative result in 39-59% of early Lyme disease cases (Branda et al., 2018). This misdiagnosis occurs since antibody levels peak weeks after the initial infection and rely upon a robust host immune response. As a result, Lyme can go unnoticed or be misdiagnosed for months to years. In these cases, the infection can disseminate through the bloodstream and cause damage to the skin, joints, heart, peripheral nerves, and brain.

Proper treatment also represents a significant challenge in Lyme disease. The recommended treatment is typically 7-28 days of antibiotics, depending on the length of the infection and clinical symptoms. Treatment is most effective in the early stages of the disease (Torbahn et al., 2018; Yang et al., 2021; Zhang et al., 2023); however, treatment failure in this patient group is still common. A study published in 2022 demonstrated that failure of treatment occurs in 14% of patients receiving timely antibiotic therapy for acute Lyme disease (Aucott et al., 2022). These patients experience persistent or recurrent symptoms, including fatigue, musculoskeletal pain, cognitive issues, and decreased physical and social functioning.

When these symptoms last for 6 months or more, researchers classify it as Post-Treatment Lyme Disease Syndrome (PTLDS), and it is estimated that in 2020 nearly 2 million patients suffered from PTLDS (DeLong et al., 2019). Treatment in the later stages of *Borrelia* infection is often less effective, with an increased risk of treatment failure (Asch et al., 1994; Moody et al., 1994; Shadick et al., 1994). A study published in 2020 found that patients whose treatment was delayed, defined as time to treatment >30 days, had 2.26 times the odds of PTLDS (Hirsch et al., 2020).

With the challenges in diagnosis and treatment, Lyme disease leads to a host of persistent, debilitating symptoms that cause a significant cost to the American public. A 2015 study out of Johns Hopkins University estimated that the direct medical costs due to Lyme disease are between $712 million and 1.3 billion annually (Adrion et al., 2015), adjusting to $914 million to $1.7 billion in 2023 dollars. A comprehensive understanding of the full economic and societal cost of Lyme disease requires further research; however, the federal Tick-borne Disease Working Group in 2018 noted that the full annual impact is potentially a $50-100 billion problem.

TABLE 1: Key facts about the Lyme disease epidemic in the United States. Sources listed in the text.

Key Facts about Lyme Disease in the U.S.
• An estimated 476,000 people are diagnosed and treated annually • 2 million people live with debilitating symptoms due to failure of treatment • Only 14% of patients exhibit the classic "bull's-eye" rash often associated with the disease, others exhibit other rash types or no rash at all • The recommended diagnostic incorrectly reports 39-59% of early Lyme cases • Late-stage disease can damage the skin, joints, heart, peripheral nervous system, and brain • Direct medical costs are estimated at $914M – 1.7B annually (2023 dollars) • The full economic impact has the potential to be $50-100B annually

NIH Strategic Plan for Tick-Borne Disease

In response to the growing epidemic and public health threat, the United States Department of Health and Human Services (HHS) assembled a Tick-Borne Disease Working Group (TBDWG) in 2016 as part of the 21st Century Cures Act. The TBDWG was a six-year process, issuing reports in 2018, 2020, and 2022. Per the recommendations of the 2018 report, the National Institutes of Health (NIH) convened a trans-NIH strategic planning team. This team included twenty-seven subject and policy experts from five NIH institutes and the NIH Office of the Director. In 2019, the team released an NIH Strategic Plan to improve tick-borne disease diagnosis, prevention, and treatment.

The comprehensive and well-researched plan acknowledged that managing the epidemic caused by Lyme and other tick-borne diseases must be multidisciplinary due to the complexity of the pathogens, the zoonotic cycle that the pathogens maintain in nature, and the diversity of human disease that results. It outlined four areas of opportunity in tick-borne disease research: improving fundamental knowledge, detection, prevention, and treatment. The plan also called for investment in research tools and resources as a fifth initiative (Figure 5). Such tools include biobank repositories, genomic resources, animal models, and preclinical services to aid in developing diagnostic, vaccine, and therapeutic candidates.

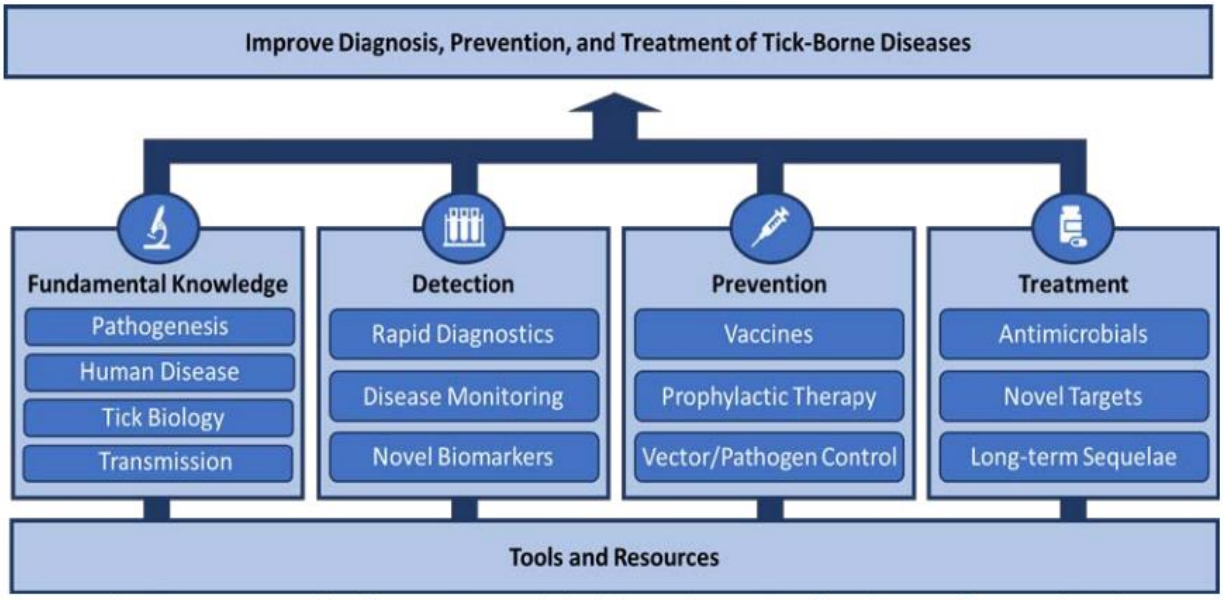


FIGURE 5: Schematic outlining the NIH Strategic Plan for Tick-borne Disease Research, a comprehensive and well-researched plan to address a growing public health threat. Source: www.niaid.nih.gov.

Federal and Private Funding for Tick-borne Disease

Although the NIH strategic plan indicated support for tick-borne disease research, NIH spending has remained limited. In 2022, NIH funding for all tick-borne diseases was only $119M. Lyme disease is one of the country's fastest-growing infectious diseases and leads to persistent and debilitating symptoms in a significant percentage of patients, yet NIH funding for Lyme research was only $50M in 2022. This amount represents less than 2% of the public funds invested in HIV/AIDS, even though the annual case count for Lyme is over an order of magnitude higher. The per-case funding for Lyme is also dwarfed by other vector-borne diseases, receiving 0.1% and 0.5% of that for Malaria and West Nile, respectively (Figure 6).

To date, the NIH funding allocated for Lyme has been predominantly directed at basic research. Figure 7 shows the 2022 NIH spending according to the five strategic priorities outlined in the NIH Strategic Plan. Although the plan called for a comprehensive, multi-disciplinary approach, funding was heavily skewed toward fundamental knowledge, receiving 59% of available funds. The combined investment in diagnostics, treatments, prevention, and research tools and resources was approximately $20M in 2022.

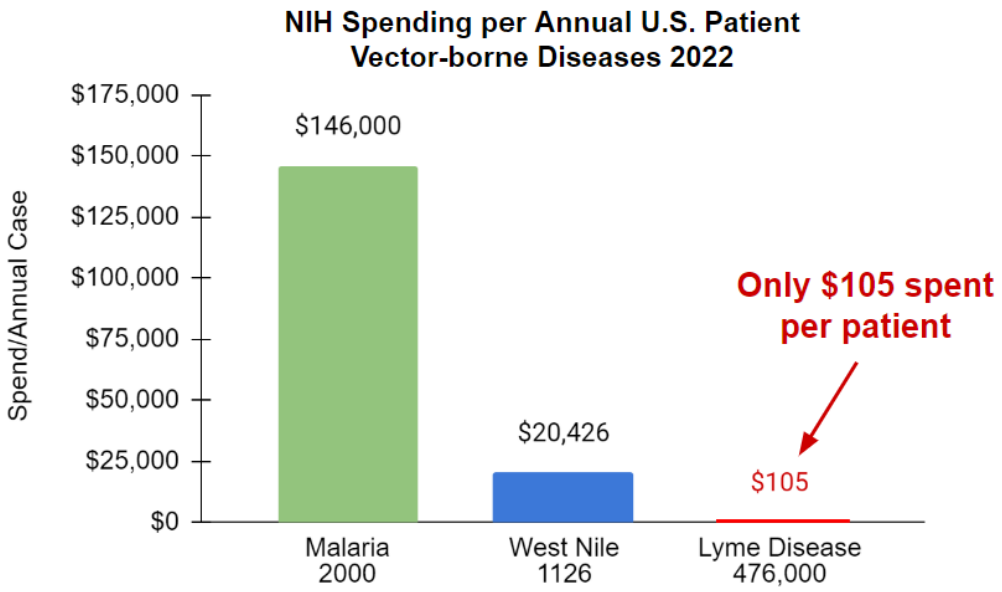


FIGURE 6: NIH spending per annual U.S. patient for vector-borne diseases in 2022. Lyme disease funding per-case is dwarfed by other vector-borne diseases. Sources: NIH Funding estimates and CDC Case counts. Malaria is a global health concern, but virtually all U.S. cases are contracted outside the U.S.

NIH Spending on Lyme Disease in 2022
Grouped by NIH Tick-borne Disease Strategic Priority ($M)

Fundamental Knowledge	Detection	Prevention	Treatment	Tools and Resources
$29.0	$4.8	$11.7	$3.6	$0.3

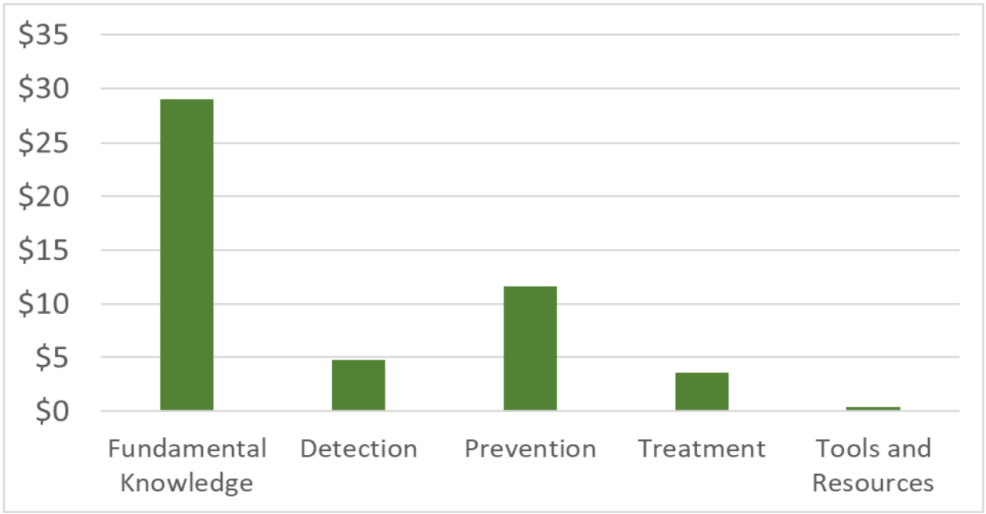


FIGURE 7: NIH Lyme spending for each of the five strategic priorities in the NIH Strategic Plan for Tick-borne Disease Research in 2022. Funding is heavily skewed to fundamental knowledge, and there is little funding for other key initiatives. Sources: NIH Funding reports and Center for Lyme Action Analysis.

In 2016, the Department of Defense established the Tick-Borne Disease Research Program (TBDRP) through the Congressionally Directed Medical Research Programs (CDMRP). This program has provided an additional $41M in aggregate funding in fiscal years 2016 through 2022 and has allocated $7M for 2023. Although funding has been modest, TBDRP has endeavored to improve tick-borne disease prevention, diagnosis, and treatment for civilian and military populations, with these initiatives receiving 66% of allocated funds through 2022.

Due to the overwhelming, unmet clinical need, private foundations have funded additional research on tick-borne illness. These foundations have also funded critical infrastructure needed for research, such as biobanks of patient samples and controls. Non-profit organizations such as the Steven and Alexandra Cohen Foundation, the Bay Area Lyme Foundation, and the Global Lyme Alliance have provided or committed over $100M to tick-borne disease research over the past 10-15 years. This increase in spending has led to an increase in the number of groups working on tick-borne diseases, advancing the fundamental knowledge within the space. Publications on Lyme disease were relatively flat from 1995-2014, but with the influx of private funding, PubMed citations have increased by 46% since 2015 (Figure 8). PubMed is a free resource developed and maintained by the National Center for Biotechnology Information (NCBI) supporting the search and retrieval of biomedical and life sciences literature.

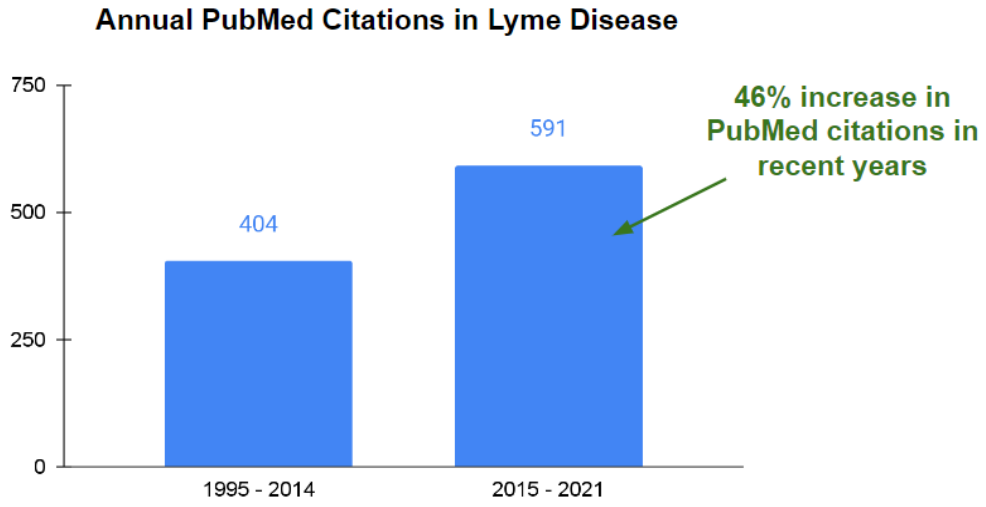


FIGURE 8: PubMed citations in Lyme disease from 1994-2021. Citations have increased due to the increase in available funding from private foundations. Sources: PubMed and Center for Lyme Action analysis.

These recent increases in research funding have led to significant leaps in our understanding of *Borrelia*, the genus of bacteria causing Lyme disease, and its impact on public health. The next section of this document, the Current Status of Lyme Research, will highlight key findings and outline opportunities for further advancement. Summaries will focus on Lyme disease

since it is the most common vector-borne disease; however, additional funding is required across all endemic tick-borne diseases.

Despite recent progress, Lyme patients still face the lack of reliable diagnostics, ineffective therapies for persistent disease, and no available vaccine. In 2020, the Center for Lyme Action published a Lyme Moonshot policy paper, calling for a cure for Lyme and other tick-borne diseases by 2030. Achieving this goal will require 500 million to 1 billion dollars of annual federal funding. This investment, while significant, still represents a small fraction of the annual estimated impact of the disease.

In 2020, the Center for Lyme Action published a policy paper calling for a cure for Lyme and other tick-borne diseases by 2030.

Achieving this goal will require funding on the order of $500M - $1B per year, a fraction of the estimated annual economic impact.

Terminology and Categorization of Lyme Disease

Before discussing the progress and needs within Lyme research, it is important to understand the terminology used for disease progression, summarized in Table 2. The disease is often characterized as "early" or "late," depending on when the disease is diagnosed in relation to the tick bite and the onset of the infection. Another key distinction is whether the disease is "localized," i.e., contained near the inoculation site, or "disseminated," i.e., circulated through the bloodstream and into tissues.

The stages defined in Table 2 are well understood and agreed upon in the medical literature; however, additional terminology commonly used to describe Lyme outcomes has led to debate, with usage and favor changing over time as shown in Table 3. In the early literature, Chronic Lyme Disease or CLD was utilized to describe Lyme patients with long-standing symptoms, usually in the context of previous treatment. This term became out of favor around the turn of the century (2000), when the Infectious Disease Society of America (IDSA) noted that there was no objective evidence that patients had residual or "chronic" infections, and studies failed to demonstrate the benefit of prolonged antibiotics (Wormser et al., 2000).

TABLE 2: Disease stage definitions for Lyme disease, including clinical findings and microbiological origins. Early-stage disease can present like the flu and late-stage disease is associated with complex, adverse outcomes. Source: Modified from Bamm et al. 2019.

Stage	Microbiology	Clinical Findings
Early Localized Lyme Disease 7-14 days after inoculation	Localized *Borrelia* infection	Can present with a single EM lesion Can present with mild flu-like symptoms or a combination of rash (or rashes) and flu-like symptoms
Early Disseminated Lyme Disease Days to months	*Borrelia* enters the bloodstream and disseminates systemically	Can present as multiple EM lesions, acute neurologic disease, Lyme carditis, borrelial lymphocytoma, and/or systemic symptoms
Late Disseminated Lyme Disease Months to years (untreated)	Ongoing *Borrelia* infection of tertiary organ sites	Intermittent or ongoing objective signs of specific organ damage to joints (arthritis), heart (Lyme carditis), nervous system (encephalitis, polyneuropathy), and/or skin (acrodermatitis chronica atrophicans and lymphocytoma)

TABLE 3: Terminology used to describe Lyme patients with ongoing illness. Historically, several terms have been utilized and the 2020 TBDWG recommended Persistent Lyme Disease as an enduring term. Source: Modified from Bamm et al. 2019.

Stage	Clinical Findings
Chronic Lyme Disease (CLD)	Utilized in early literature to describe ongoing symptoms consistent with Lyme disease Used to describe treated and untreated patients Cause of symptoms and relationship to documented Borrelia burgdorferi infection called into question
Post-Treatment Lyme Disease Syndrome (PTLD or PTLDS or PLDS)	Specific subset of patients who: • Had previous objective evidence of infection • Were treated with antibiotics followed by resolution of objective signs • Subsequently experienced onset of subjective symptoms (i.e., fatigue, pain, cognitive difficulties) that persist for at least 6 months
Persistent Lyme Disease (PLD)	Recommended by the Clinical Aspects of Lyme Disease subcommittee of the TBDWG in 2020 to describe ongoing symptoms consistent with Lyme disease Used to describe treated and untreated patients Cause of symptoms could be bacterial or non-bacterial causes

But objective evidence continued to mount that a significant percentage of patients treated for Lyme disease exhibited ongoing symptoms requiring further study. In 2006, the IDSA proposed the research case definition of Post-Lyme Disease Syndrome (PLDS), which later morphed into Post-Treatment Lyme Disease Syndrome (PTLDS) (Wormser et al., 2006). The term refers to a specific subset of patients treated with antibiotics who resolved objective signs of the disease and later developed the onset of subjective symptoms persisting for longer than six months.

Since 2006, further evidence has associated Lyme with persistent symptoms beyond the PTLDS case definition. In the 2020 Federal Tick-borne Disease Working Group (TBDWG), the Clinical Aspects of Lyme Disease Subcommittee acknowledged the unfavorable reputation of Chronic Lyme combined with the narrowness of PTLDS led to the lack of a centralized term to describe patients with persistent symptoms. The subcommittee recommended using the term persistent Lyme disease and noted that "persistent" does not imply causation. Ongoing symptoms could be due to the persistence of infection or other immune responses. Further study is required to determine causal factors in different patient groups.

Since this report references the literature with its diversity of terms, all the terms in Table 3 will be utilized; however, persistent Lyme disease will be used, when possible, to follow the TBDWG guidance.

It is also worth noting that the Department of Health and Human Services has begun using the term "infection-associated chronic illness" to describe illnesses that include a patient history of infection by viral or bacterial infections followed by long-lasting debilitating symptoms. Examples of infection-associated chronic illnesses include long COVID or post-acute sequelae of COVID-19, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), Post-Treatment Lyme Disease (PTLD), and Multiple Sclerosis (MS).

CURRENT STATUS OF LYME RESEARCH

Leveraging the NIH Strategic Plan

As discussed, the 2019 NIH Strategic Plan for Tick-borne Disease Research is a comprehensive but underfunded approach that will significantly impact the millions of Americans suffering from tick-borne illnesses. The five strategic priorities of the plan are listed in Figure 5 and reiterated in Table 4.

TABLE 4: The strategic priorities listed in the NIH Strategic Plan for Tick-borne Diseases Source: NIH Strategic Plan for Tick-borne Diseases, 2019

NIH Strategic Plan Priorities
Strategic Priority 1: Improve Fundamental Knowledge of Tick-Borne Diseases
Strategic Priority 2: Advanced Research to Improve the Detection & Diagnosis of Tick-Borne Diseases
Strategic Priority 3: Accelerate Research to Improve Prevention of Tick-Borne Diseases
Strategic Priority 4: Support Research to Advance the Treatment of Tick-Borne Diseases
Strategic Priority 5: Develop Tools and Resources to Advance Tick-Borne Disease Research

The Center for Lyme Action believes that investing in Strategic Priorities 2 and 4 (bolded) has the potential for the highest immediate impact to the American public.

As noted in Figure 1, ticks can carry a long list of pathogens, which can lead to infection with more than one microbe (called co-infections). Understanding the interactions between these infections and the impact on human disease is a critical and complex topic that is beyond the scope of this review. Instead, this review will focus on advancements and opportunities within Lyme disease research, since Lyme is the most common tick-borne disease in the U.S.

The structure of the review mirrors the sections of the NIH Strategic Plan. We will start with Strategic Priority 1 since fundamental knowledge is required to understand and advance the other priorities. Next, we will discuss Strategic Priorities 2 and 4 because funding diagnostics and therapies has the highest potential for immediate impact on the American public. Finally, we will discuss Strategic Priorities 3 & 5 to outline progress and needs within those critical areas.

Fundamentals of Lyme Disease and Link to Human Disease

Looking at the stages and presentations of Lyme disease, it is critical to understand the wide variability seen across patients. Some patients exhibit the classical presentation of disease

progression with the appearance of the stereotypical bull's-eye EM rash and respond well to antibiotic therapy. Meanwhile, others do not exhibit the classic EM rash (or any rash at all) and remain symptomatic after treatment. Understanding this variability requires a fundamental understanding of *Borrelia* and its interaction with the host immune system.

How Borrelia Suppresses and Evades the Immune System

Borrelia burgdorferi exists in diverse hosts, from cold-blooded insects and reptiles to warm-blooded birds and mammals. And although Lyme disease was first described in 1977 following a cluster of juvenile arthritis cases near Lyme, Connecticut, evidence shows that the origins of *Borrelia* date back as far as 15 million years (Poinar 2015). As a result, the pathogen has evolved to adapt and survive in various environments.

This adaptability is exhibited as soon as the bacteria are introduced from the tick midgut into the human body. The shift in environment causes the bacteria to experience a dramatic temperature increase and pH decrease. In response, the pathogen alters its genetic composition almost instantaneously, clipping out segments and weaving in other segments (Norris, 2006; Zhang and Norris, 1998). It continues this recombination throughout the infection, changing the outer protein coat to help evade the immune system defenses (Norris et al., 2014). This tactic is one of many used by *Borrelia* to avoid detection.

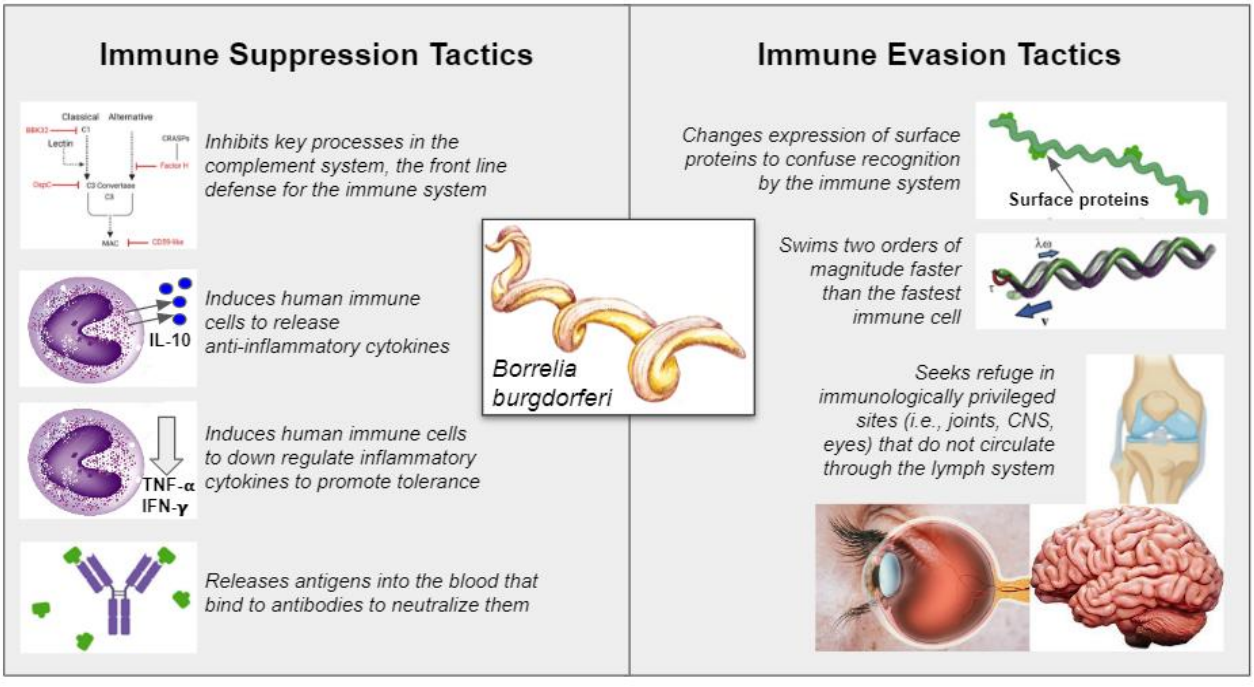


FIGURE 9: A summary of *Borrelia's* immune suppression and evasion tactics. The organism has an array of techniques to avoid detection and destruction by the immune system. Source: Created with information from Embers et al., 2004, Bamm et al., 2019, and Malawista and de Boisfleury Chevance, 2008.

Scientists have discovered several strategies *Borrelia* uses to mount an invasion and avoid detection and destruction by the host, as summarized in Figure 9. Immune suppression strategies include active suppression of the chemical signals used to mount an immune attack and the release of antigens into the blood to bind and neutralize antibodies (Embers et al., 2004; Bamm et al., 2019). Immune evasion tactics include modifying surface proteins to confuse recognition by the immune system and sequestration in immune-privileged sites such as the joints, eyes, and brain (Embers et al., 2004; Bamm et al., 2019). The sheer speed of the bacteria is also useful for immune evasion since the bacteria swim two orders of magnitude faster than the fastest immune cell (Malawista and de Boisfleury Chevance, 2008). As a result, *Borrelia* can evade both the innate and adaptive immune systems and disseminate throughout the body.

Borrelia Dissemination Throughout the Body

The mechanisms that *Borrelia* use to travel throughout the body have also been a subject of study. Once *Borrelia* moves into the dermis from the tick midgut, it can colonize within the local extracellular matrix (ECM), replicate, and disseminate locally (Vig et al., 2014). But the pathogen can also enter the bloodstream using mechanics resembling those used by white blood cells (Ebady et al., 2016). *Borrelia* is a class of bacteria called spirochetes, which have long, slender, spiral-like bodies. Their unique structure makes them particularly mobile, and, unlike many other bacteria, they can swim in highly viscous gel-like media, like that found in connective tissue (Kimsey & Spielman, 1990). As such, *Borrelia* can colonize secondary tissue sites nearly anywhere in the body, including locations that are considered "immune privileged," such as the brain.

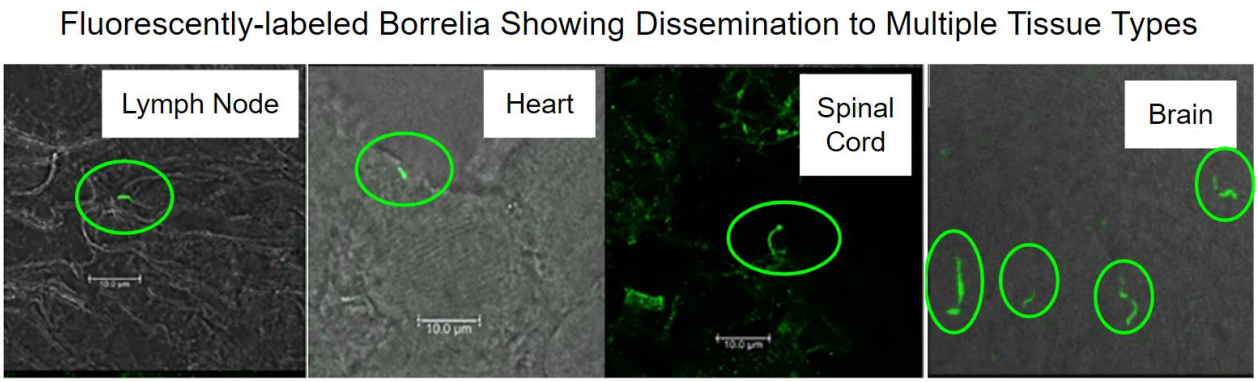


FIGURE 10: Frozen sections of affected tissues when stained with fluorescently labels specific for *Borrelia*. Images are from the necropsy of rhesus macaques infected with *Borrelia* showing dissemination into an array of tissues, including immune privileged sites like the brain. Sources: Embers et al., 2017; Cabello et al., 2022.

Figure 10 shows *Borrelia* labeled with fluorescent antibodies in multiple tissue types in rhesus monkeys, as identified after necropsy (Embers et al., 2017; Cabello et al., 2022). Affected tissue sites exhibited inflammatory processes consistent with active infection, with pathology details in the referenced articles.

Pathogenesis - The Link to Human Disease

Borrelia's ability to evade the immune system and disseminate throughout the body leads to various clinical phenotypes. Research has linked the bacteria to localized and systemic illnesses impacting neurologic, cardiac, dermatologic, and rheumatologic systems, as shown in Figure 11.

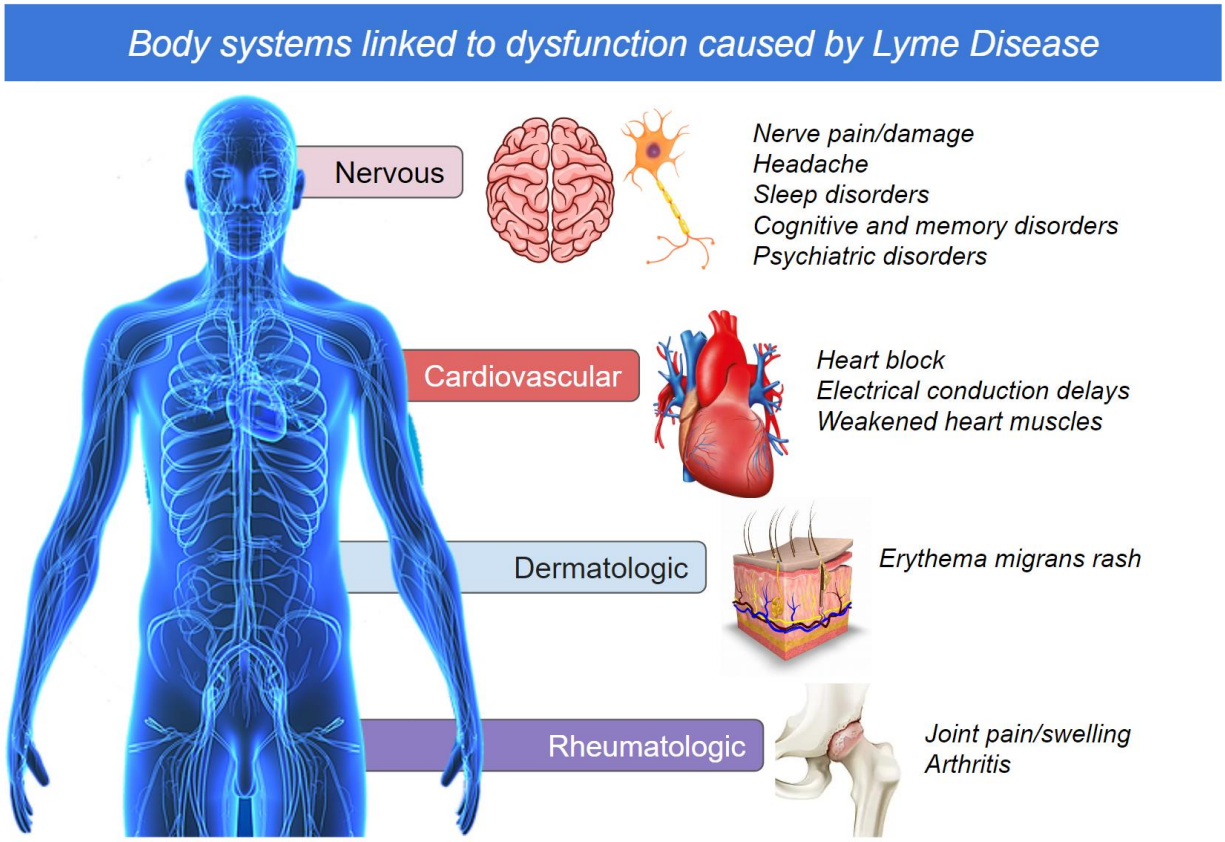


FIGURE 11: The primary body systems where research has linked the presence of *Borrelia* to dysfunction and disease with examples of clinical outcomes.

Dermatologic and rheumatologic conditions associated with Lyme disease are the most prevalent manifestations, with 72% of patients exhibiting the erythema migrans rash and 28% exhibiting arthritis according to a subset of CDC reported cases (Schwartz et al., 2017). While there are some complexities to the EM rash that will be discussed in the diagnostic section, the progression and pathology of the infection are well established in the medical literature

(Berger, 1989; Müllegger, 2004; Müllegger & Glatz, 2008) and thus will not be discussed. Likewise, the link between arthritis and *Borrelia* has been one of the more studied aspects of the disease. Readers are referred to scientific review papers on Lyme arthritis for additional information (Arvikar et al., 2017; Miller & Aucott, 2021; Steere et al., 1987). Instead, this section will focus on two critical organs that *Borrelia* is known to infect but where further research is required to understand disease pathology: the brain and the heart.

Lyme and the Brain

The blood-brain barrier is composed of specialized endothelial cells that help regulate substances in and out of the brain. This barrier is one of the tightest in the body and protects the brain from insults, such as infection. But the system is not infallible, and some microbial pathogens have developed mechanisms to cross the blood-brain barrier and cause infection (Kim 2008). Spirochetes are a class of bacteria with this capability, and syphilis, a disease caused by another spirochete, *Treponema pallidum*, was first discovered in the brain in 1913 by a Japanese scientist, Hideyo Noguchi (Tan, 2014).

Spirochetes enter the brain through a complex expression of surface proteins that allow the bacteria to adhere and crawl between the endothelial cells that establish the blood-brain barrier (Tkáčová et al., 2020). The entry of *Borrelia* into the cerebrospinal fluid and brain has been documented by culture methods, polymerase chain reaction (PCR), and tissue sectioning (Pfister et al., 1984; Wilske et al., 2007; Gadila et al., 2021), and approximately 13% of Lyme patients exhibit neurological symptoms (Schwartz et al., 2017).

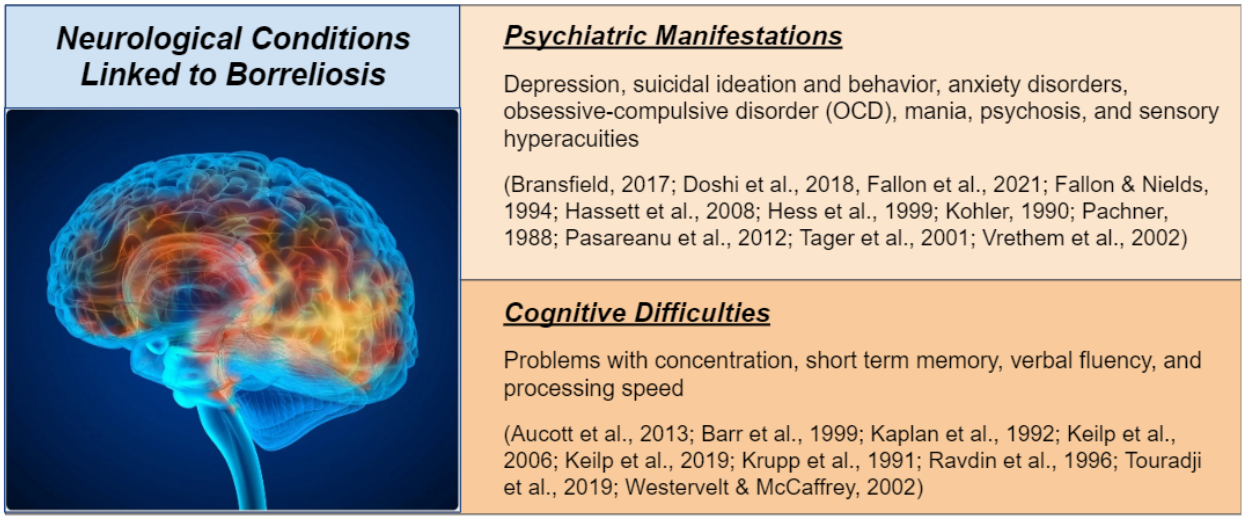


FIGURE 12: Psychiatric and cognitive disorders linked to Lyme disease in the scientific literature. Further study is required to understand the mechanisms of action and causality.

Clinically, borreliosis has been associated with multiple cognitive and neuropsychiatric disorders. The 2022 Tick-borne Disease Working Group summarized their findings, which are included in Figure 12. Despite more than three decades of findings in the medical literature, research on *Borrelia's* link to causality has been limited. The causal mechanisms could be active or post-infectious processes triggering localized or systemic inflammation, autoimmunity, or alterations in the central nervous system metabolism and/or blood flow (Halperin, 2022; Coughlin et al., 2018; Kim et al., 2022). Additional funding and research are required to understand the connection between *Borrelia* and these critical and costly conditions.

Due to the lack of funding in the space, many of the studies highlighted in Figure 12 are case reports or have limited sample sizes. However, one notable foundation-funded, nationwide study was recently completed using a Danish registry (Fallon et al., 2021). The study analyzed the population of Denmark from 1994 to 2016 to determine if individuals with a hospital-based diagnosis of Lyme disease had an increased risk of subsequent psychiatric disorders. The study found a 28% higher rate of any mental disorder, a 42% higher rate of affective disorder, a 2-fold higher rate of suicide attempts, and a 75% higher rate of death by suicide in people diagnosed with Lyme disease (Figure 13). Given the growing challenge of mental health disorders, further studies are required to examine the linkage between Lyme disease and these conditions in the U.S. population.

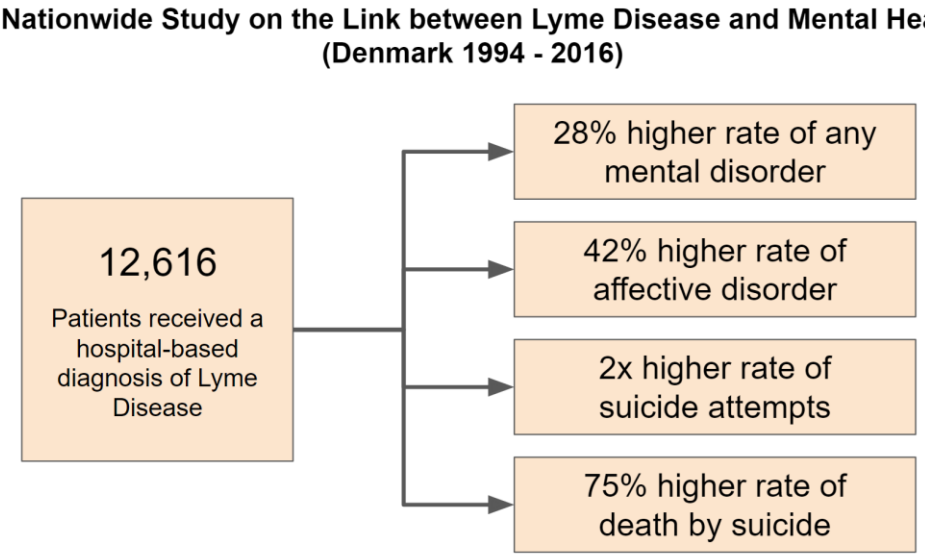


FIGURE 13: Summary of a nationwide study of people living in Denmark from 1994-2016 investigating the link between Lyme disease and mental health. Results demonstrate a higher incidence of subsequent psychiatric disorders in patients with a hospital-based diagnosis of Lyme disease. Source: Generated from Fallon et al. 2021.

Another condition of nationwide importance is dementia. The Population Reference Bureau estimates that more than 7 million people ages 65 and older suffered from dementia in 2020, which is expected to grow to nearly 12 million in 2040. New research reported at the Alzheimer's Association International Conference in 2021 found associations between COVID-19 and persistent cognitive deficits, including the acceleration of Alzheimer's disease pathology and symptoms. These findings have rekindled scientific interest in the link between neurological infections and the progression of dementia, with the National Institute of Aging offering a workshop on the Infectious Etiology of Alzheimer's Disease in October of 2021.

Studies have linked cognitive impairment to both early and late-stage Lyme disease. Concentration problems are reported by about 24% of patients with early disseminated disease (Aucott et al., 2013). Among patients with persistent symptoms (PTLDS), up to 90% complain of cognitive difficulties (Touradji et al., 2019), and 7-30% have objective measurable problems with short-term memory, verbal fluency, and processing speed (Kaplan et al., 1992; Keilp et al., 2006; Krupp et al., 1991; Touradji et al., 2019).

The spirochete causing syphilis has been linked to dementia for decades, but linking Borrelia to dementia has been more challenging due to the lack of a reliable diagnostic (Wadell et al., 2016) and the difficulty in detecting the bacteria in autopsy specimens (Embers et al., 2017). Despite these challenges, linkages have emerged in clinical case reports (Kristoferitsch et al., 2018) and in autopsy analysis of brain specimens (Gadila et al., 2021; MacDonald, 1986). This evidence, combined with the recent learnings from COVID-19, has inspired a consortium of scientists to call for the establishment of a consensus protocol to explore the role of infections, such as Borrelia, in patients with mild cognitive impairment and Alzheimer's Disease (Lathe et al., 2023). Such initiatives are critical to understanding the role of pathogens in this important public health issue.

Lyme and the Heart

Borrelia is also known to infect cardiac tissue and cause a condition known as Lyme carditis. This condition affects approximately 1.5% of Lyme patients according to a subset of CDC reported cases (Schwartz et al., 2017). Although the reported incidence is lower than other clinical manifestations, the severity is high since untreated cases can be fatal (Forrester et al., 2014).

In Lyme carditis, the spirochete infiltrates the connective tissue associated with collagen fibers at the base of the heart, basal interventricular septum, perivascular regions, outer or inner membranes, and more rarely infiltrates blood vessels, valves, or the heart muscle (Steere et al., 1980; Cadavid et al., 2004; Haddad and Nadelman, 2003; Motamed et al., 2022). Atrioventricular (AV) block is the most common presentation of Lyme carditis (90%), with

high-degree AV block accounting for approximately two-thirds of the cases (van der Linde, 1991; McAlister et al., 1989).

Early intervention for high-degree AV block typically resolves within the first 10 days of antibiotic treatment, and other less severe conduction disturbances within 6 weeks (Yeung and Baranchuk, 2019). However, since the EM rash is less common in Lyme carditis cases (40% versus 70%) (Krause and Bockenstedt, 2013) and due to challenges with serological diagnosis of Lyme (to be discussed below), many patients go prolonged periods without an accurate diagnosis. Untreated carditis can lead to symptomatic bradycardia requiring temporary or inappropriate permanent pacemaker use in otherwise healthy individuals (Figure 14). Untreated carditis can also lead to rare cases of death due to loss of consciousness causing an accident or trauma, or a complete heart block (Marx et al., 2020).

It is also worth noting that half of non-Lyme-related, high-degree AV block is caused by "idiopathic fibrosis," where the cause is unknown (Kashou et al., 2022). A study published in 2022 suggests that undiagnosed Lyme disease could contribute to a significant percentage of this cohort. In this study, 130 patients with AV block without obvious cardiac causes were tested for Lyme disease. Results demonstrated that 30 patients (23%) tested positive for *Borrelia* antibodies, with 16 patients (12%) showing antibodies associated with recent infection (Kaczmarek et al., 2022). There have not been large population-based studies on the prevalence of AV block; however, it is one of the most common conditions leading to the implantation of nearly 250,000 pacemakers in the U.S. annually (Mond et al., 2011).

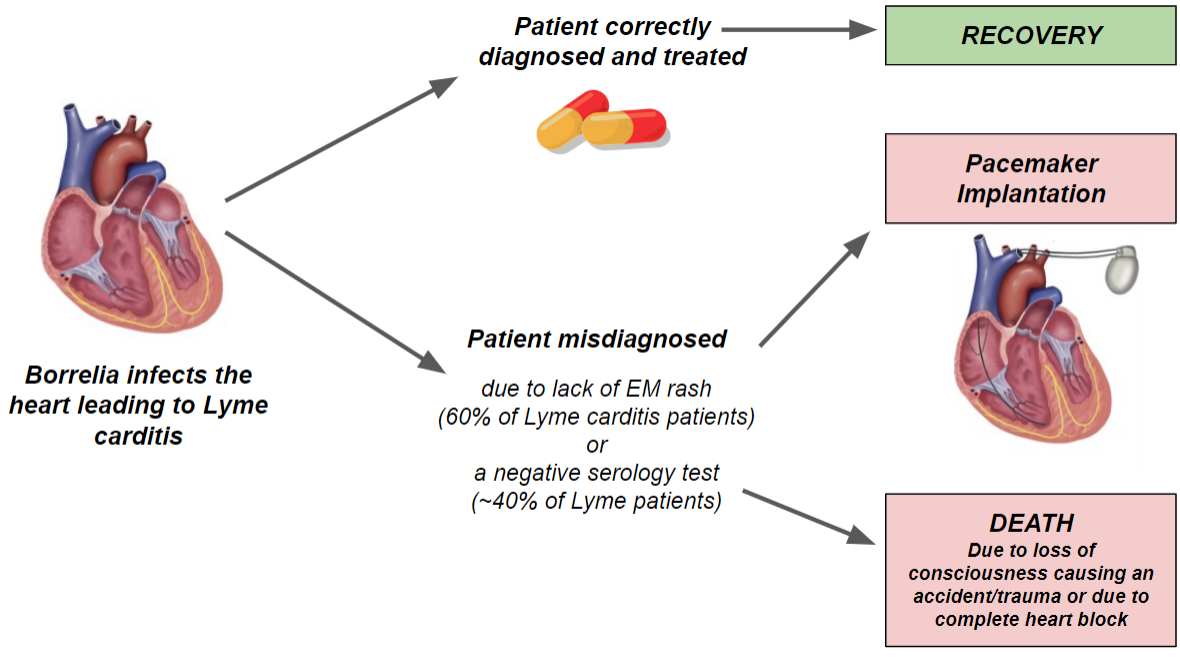


FIGURE 14: Summary of possible outcomes for Lyme carditis patients. Cases diagnosed and treated early usually resolve with antibiotic therapy. Cases misdiagnosed can lead to the need for a pacemaker implant or death. Source: Adapted from Yeung and Baranchuk, 2019

Late-stage Lyme has also been proposed as a causal agent for many persistent cardiac presentations, such as electrical conduction delays and dilated cardiomyopathy (DCM). A systematic review of research into late disseminated Lyme and DCM suggested that DCM could result from undiagnosed or poorly treated Lyme carditis; however, further research is required to establish causality (Motamed, 2022).

Other Pathogenesis - Maternal to Fetal Transmission of Borrelia

Pregnant women are a vulnerable population where infections can impact the mother and the unborn child. The first confirmed case of Lyme disease in a pregnant woman was described in 1985, with the mother acquiring Lyme in the first trimester. The child was delivered at 35 weeks and died of congenital heart disease (Schlesinger et al., 1985). Since then, reported outcomes following *Borrelia* infections acquired during pregnancy have ranged from minor self-limiting illnesses to birth defects, spontaneous miscarriage, and newborn death (Lambert 2020).

Further study has shown that gestational Lyme disease promptly diagnosed and treated with the appropriate antibiotics results in a favorable prognosis for the fetus. However, untreated disease increases the risk of adverse outcomes. A systematic review of studies published from 1969-2017 in the U.S., Europe, and Asia found that untreated, gestational disease had a higher rate (50%) of adverse pregnancy outcomes than treated gestational disease (11%) (Waddell et al., 2018, Table 5 (left)). Similarly, a study of 95 women with Lyme during pregnancy in Hungary showed adverse outcomes in 12.1% of women treated with IV antibiotics, 31.6% of women treated with oral antibiotics, and 60% of women left untreated (Lakos & Solymosi, 2010, Table 5 (right)).

TABLE 5: Results from two different studies on gestational Lyme disease. Untreated disease correlates with a 5x increase in adverse outcomes. Sources: Waddell et al., 2018 and Lakos & Solymosi, 2010.

Adverse Outcomes in Gestational Lyme Disease

Waddell et al., 2018

	Adverse Outcomes
Gestational Lyme - Treated	11%
Gestational Lyme - Untreated	50%

Lakos & Solymosi, 2010

	Adverse Outcomes
Gestational Lyme - Treated with IV antibiotics	12.1%
Gestational Lyme - Treated with oral antibiotics	31.6%
Gestational Lyme - Untreated	60%

Autopsy studies of children exposed to gestational Lyme have revealed the presence of *Borrelia* spirochetes in fetal and neonate tissues (Horowitz & Yunker, 2003; Lavoie et al., 1987; MacDonald, 1986, 1989; MacDonald et al., 1987; Maraspin et al., 1999; Neubert, 1987; Schlesinger et al., 1985; Weber et al., 1988). These results confirm *in-utero* transmission of *Borrelia*, adding the placenta to the list of immune barriers that the spirochete can penetrate. The bacteria migrate through endothelial surfaces surrounding the umbilical cord and the amniotic membrane (Agus, 1995; Garcia-Monco & Benach, 1989; Szczepanski et al., 1990), where they can then infect the developing fetus.

Evidence of infection *in utero* has led to speculation of congenital Lyme infections in otherwise healthy newborns. This phenomenon has been documented for syphilis, a similar spirochetal infection. Many infants born to untreated mothers with syphilis appear healthy and without evidence of infection at birth (Wicher & Wicher 2001). However, left untreated, these children may develop manifestations of the disease months to years later (Cooper & Sanchez, 2018), causing concern that congenital Lyme may lead to illness or developmental delays later in life.

The Clinical Presentation and Pathogenesis Subcommittee of the 2022 Tick-Borne Disease Working Group reviewed summary findings on congenital Lyme and provided a summary in their report. They noted a lack of high-quality research and recommended prospective cohort studies of women infected with Lyme disease during pregnancy and their offspring. These studies should be aimed at understanding the effects of the infection on maternal health, as well as child health and development.

Fundamental Knowledge and the Link to Human Disease - Key Research Needs

Borrelia is a highly adaptable, persistent pathogen capable of infecting multiple tissue types throughout the body, including critical organs like the heart and brain. The high incidence of Lyme disease combined with the high rates of misdiagnosis and treatment failure makes it critical to understand disease progression and intervention. Key areas in fundamental knowledge, pathogenesis, and pathophysiology requiring funding and further study are adapted from the recommendations of the Tick-borne Disease Working Group and summarized in Table 6. Studies should focus on understanding infection mechanisms in the research laboratory as well as answering translational research questions in a clinical setting.

TABLE 6: Critical areas requiring further study in fundamental knowledge and the link to human disease. Actions are adapted from the recommendations of the Tick-borne Disease Working Groups in 2020 and 2022.

Areas in Lyme Disease Fundamental Knowledge and the Link to Human Disease Requiring Funding and Further Study
Research on the variable host response (in humans and animal models) to Borrelia and the impact on detection and persistence.
Research on the causal mechanisms and host interactions in neuroborreliosis.
Research on neuropsychiatric disease related to tick-borne infections.
Research on the role of infections, such as Borrelia, in patients with mild cognitive impairment and dementia.
Research on the prevalence of undetected Lyme disease in illnesses with known or suspected linkages to Borrelia, such as psychiatric illness, dementia, and cardiomyopathy.
Research on the prevalence of undetected tick-borne illness in underserved, high-risk groups such as individuals in psychiatric facilities, prisons, and homeless shelters.
Research on maternal to fetal transmission mechanisms of Borrelia.
Prospective cohort studies of women infected with Lyme disease during pregnancy and their offspring to understand the effects of this infection on maternal health, offspring health, and offspring development.

Advance Research to Improve the Detection and Diagnosis of Lyme Disease

The root of many challenges within the Lyme disease epidemic is the lack of a reliable and accurate diagnostic. Both the Tick-borne Disease Working Group and the NIH Strategic Plan acknowledged that the current standard of care test is insufficient and that new technologies are required to provide definitive evidence of infection. Despite this overwhelming consensus, diagnostics received only $4.8 million in NIH funding in 2022 (Figure 7). Understanding the importance of this underfunded need requires an understanding of the current methods and the complexities of detecting *Borrelia* in human samples.

The History of the Two-Tier Assay, the Current Standard of Care

The currently accepted standard for care for Lyme disease diagnosis is a two-tiered testing protocol, with each step measuring different antibodies associated with *Borrelia*. This two-tiered testing protocol for Lyme disease was adopted at the Second National Conference on Serologic Diagnosis of Lyme disease held in Dearborn, Michigan, in 1994.

The goal of the Dearborn Conference was to address the poor specificity of serologic assays being used at the time, which resulted in many false positives. This improvement was achieved by combining the poorly specific but reasonably sensitive enzyme immunoassays with a second protein separation technique called a Western blot. The combined test is called the standard two-tier test or STTT. The Western blot improved the specificity of the combined result; however, the improvement came at the expense of poor sensitivity (Engstrom et al., 1995; Dressler et al., 1993), meaning that people who had the disease would be reported as negative. Figure 15 describes sensitivity and specificity in the context of a diagnostic assay.

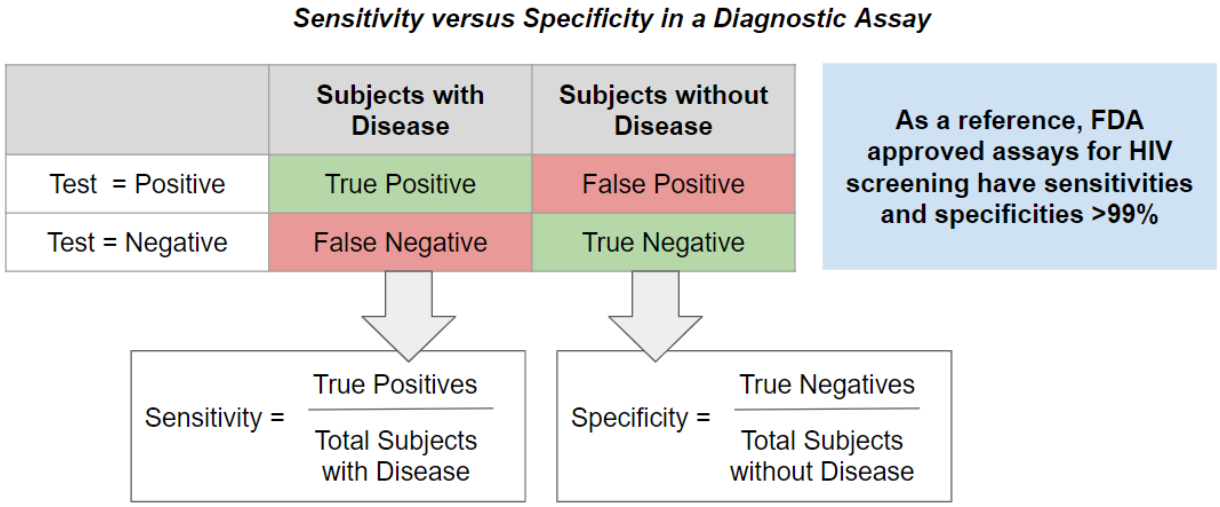


FIGURE 15: Sensitivity and specificity for a diagnostic assay. Sensitivity detects the presence of disease, and specificity reduces the chance of false positives.

The Challenges with Antibody Testing (Serology)

There are several fundamental challenges with using antibodies, also known as serology, to diagnose infectious diseases. Antibody responses typically peak 4-6 weeks after infection, making early diagnosis (when Lyme disease treatment is most effective) difficult. A review of five different studies on the performance of the standard two-tier test (STTT) revealed an average sensitivity of 39.4% for early-stage Lyme disease (Table 7, Branda et al., 2018). This

means that over 60% of early-stage patients with Lyme disease will test negative with the standard two-tier test.

In 2019, the CDC endorsed a modified two-tier test (MTTT), replacing the Western blot with a second enzyme immunoassay targeting a different antigen from the first tier. This modification improves early-stage sensitivity by about 20%; however, MTTT sensitivity is still poor, averaging less than 60% across five studies (Table 7, Branda et al., 2018).

TABLE 7: Sensitivity for the serological tests for early-stage Lyme disease (when it is the most treatable). STTT stands for the standard two-tier test implemented in 1994, and MTTT stands for the modified two-tier test endorsed by the CDC in 2019. Both exhibit poor sensitivity for early-stage Lyme disease. Source: Modified from Branda et al., 2018.

				Sensitivity	
	Study	Sample Size & Location	Disease Manifestations	STTT	MTTT
Early Lyme Disease	Branda et al., 2011	N = 140 US	Erythema migrans Neuroborreliosis Lyme carditis	48%	61%
	Wormser et al., 2013	N = 318 US	Erythema migrans Neuroborreliosis	41%	60%
	Branda et al., 2013	N = 35 Europe	Erythema migrans Neuroborreliosis	29%	74%
	Molins et al., 2016	N = 57 US	Erythema migrans Neuroborreliosis Lyme carditis	54%	61%
	Branda et al., 2017	N = 55 US	Erythema migrans	25%	38%
Average				39.4%	58.8%

Another limitation of antibody testing is that, as discussed in the pathology section, the immune response to *Borrelia* varies significantly among patients. This host variability was effectively demonstrated in an animal model when 10 rhesus monkeys were injected with the same strain of *Borrelia* (Embers et al., 2017). Each animal exhibited widely different antibody responses that changed over the course of the ~1.2-year study. Notably, 2 of the 10 monkeys exhibited negligible antibody titers at late-stage time points where they tested positive for *Borrelia* infection through other methodologies. The sensitivity of STTT and MTTT has been reported to be high for late-stage disease (95.7-99.9%, according to a systematic review by Waddell et al., 2016). However, the wide range of antibody responses seen in animal models

combined with the challenges with the clinical diagnosis of late-stage Lyme suggests that the patient population may be broader than is typically studied.

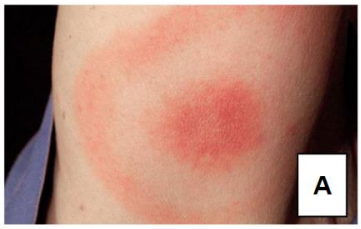
Finally, antibodies indicate that a host has been exposed to a pathogen but do not distinguish between current and past infections. This distinction is important for patients who have had previous infections or have been vaccinated. For this reason, for other infectious diseases like COVID-19, the CDC recommends against antibody testing for the diagnosis of current infection. Although a vaccine is not currently available for Lyme disease, Pfizer has a candidate in Phase 3 Clinical Trials and could submit for Food and Drug Administration (FDA) approval as early as 2025. Additionally, in April of 2023, Moderna announced that they were advancing two vaccine candidates for Lyme disease. Launching these vaccines will further complicate the serological diagnosis of Lyme disease.

The Challenges of Diagnosis with EM Rashes

In the absence of a reliable in vitro diagnostic, clinicians must rely upon other signs and symptoms to diagnose patients. The best-known indicator of Lyme disease is the erythema migrans (EM) rash, which occurs an average of 7–10 days after the bite of an infected tick (Steere, 2001). According to CDC reporting, approximately 72% of patients exhibit an EM rash (Schwartz et al., 2017); however, only 20% of patients with an EM rash (14% of total patients) exhibit the classic bull's-eye with a central clearing (Tibbles et al., 2007). The other 80% of EM rashes (58% of total patients) appear uniformly red or bluish in color, have central blistering, or have multiple EMs (Figure 16). These other EM rashes are misdiagnosed by general practitioners 36% of the time (Lipsker et al., 2004) and by the general public over 70% of the time (Aucott et al., 2012). Misdiagnoses include spider bites (Aucott et al., 2009), herpes zoster (Mazori et al., 2015), cellulitis (Li et al., 2007), and annular skin disorders (Mullegger & Glatz, 2008).

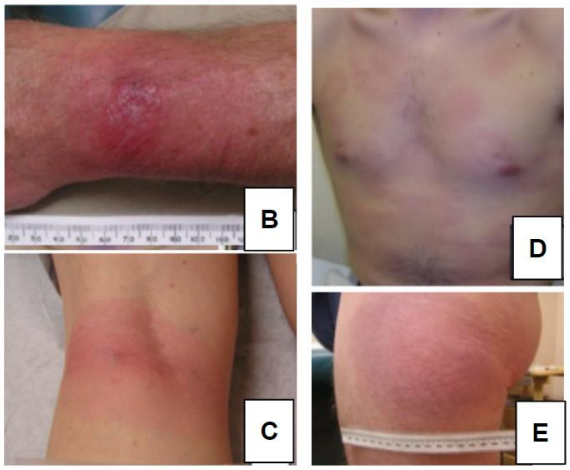
The reliance upon skin presentations may also lead to disparities in diagnosis based on sex, body location, and age. A study published in 2021 found that EM size was 2.18 cm larger in males than in females, the odds of a red versus blue EM were three times higher on the pelvis, torso, or arm compared to the leg, and every 10-year increase in age decreased the odds of central clearing (in the characteristic bull's-eye) by 25% (Rebman et al., 2021).

Race is also an important factor in EM rash diagnosis. A recent study in the Journal of General Internal Medicine reported that patients with black skin, where rashes may be harder to identify (Figure 17), were more likely to present with advanced disease. A comparison of black and white patients revealed higher incidences of disseminated disease (41.3% versus 16.2%) and neurologic manifestations (34% versus 9%) in black patients at initial diagnosis (Ly, 2022).



Classic Bull's Eye Rash caused by Lyme Disease

- Occurs in 14% of Lyme patients (1&2)
- Misdiagnosed by 20% of general practitioners (3) and 27% of the general public (4)



Other EM Rashes caused by Lyme Disease

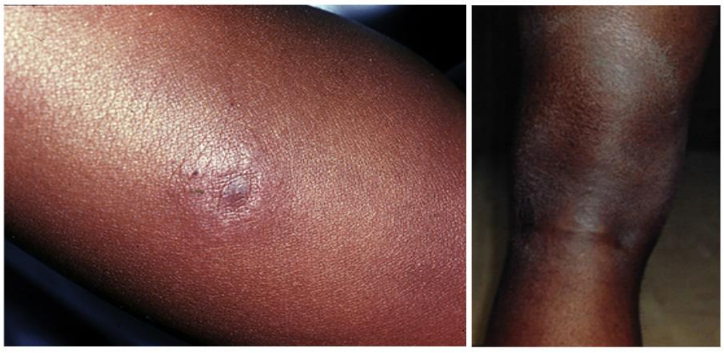
- Occurs in 58% of Lyme patients (1&2)
- Misdiagnosed by 36% of general practitioners (3) and >70% of the general public (4)

Rash	Potential Misdiagnosis
B	Herpes zoster (5)
C&E	Cellulitis (6)
D	Erythema multiforme, urticaria, annular skin disorders (7)

References

1 - Tibbles et al., 2007; 2 - Schwartz et al., 2017; 3 - Lipsker et al., 2004; 4 - Aucott et al., 2012; 5 - Mazori et al., 2015; 6 - Li et al., 2007; 7 - Mullegger & Glatz, 2008

FIGURE 16: Images of various EM rashes, including the likelihood of correct identification by general practitioners and the general public and possible misdiagnoses. Source: Images from the CDC Lyme Disease Website (A) and Aucott et al., 2012 (B-E).



Comparison of Black and White Patients at Initial Lyme Disease Diagnosis

	White Patients	Black Patients
Disseminated Disease	16.2%	41.3%
Neurologic Manifestations	9%	34%

FIGURE 17: Examples of EM rashes on dark skin (left) and data comparing Lyme disease progression in white and black patients at initial diagnosis (right). In black patients, EM rashes are harder to diagnose, and late-stage disease is more common upon initial diagnosis. Sources: Images from CDC Lyme Disease Website and data summarized from Ly, 2022.

Emerging Diagnostic Assays for Lyme Disease

The inconsistency of rash presentation and the limitations of serology leave clinicians and patients with an unreliable standard of care to navigate the growing Lyme disease epidemic. Fortunately, several new approaches have emerged to detect and diagnose Lyme disease. These approaches can be divided into four major categories, as shown in Table 8: direct detection assays; host-focused assays; imaging; and next-generation serological assays.

TABLE 8: Categories of assays emerging for the detection of Lyme disease. Newer technologies provide promise for improvements over the current standard of care. Source: Bobe et al. 2021.

Assay Category	Technical Approach
Direct Detection	Detection of DNA, RNA, or proteins directly associated with the pathogen (e.g., PCR, RT-PCR, and antigen testing)
Host-Focused	"Omics" approaches that identify pathogen-specific patterns at each stage of the disease (e.g., genomics, transcriptomics, proteomics, and metabolomics)
Imaging	Labeling of the pathogen to image within infected tissues OR Use of algorithms to better identify EM lesions
Next Generation Serology	Serology using engineered proteins and multiplexing to assess a wide range of antibodies

A complete analysis of these emerging techniques is beyond the scope of this review, and a comprehensive list of tests in development with references is provided elsewhere (Bobe et al., 2021) and summarized by the Diagnostics Subcommittee of the 2022 Tick-borne Disease Working Group. To provide examples of the advancements in development, this section will focus on two of the four categories in Table 8, direct detection and imaging.

Direct Detection of Borrelia

Serology analyzes proteins generated by the host's immune system, thus leading to additional complexity and variability, as discussed above. In direct detection, the assay detects DNA, RNA, or proteins that are directly associated with the pathogen, and thus a positive result indicates that the pathogen is present. Nucleic acid amplification techniques such as Polymerase Chain Reaction (PCR) and Reverse Transcription Polymerase Chain

Reaction (RT-PCR) are clinically used to detect many infectious diseases, including human immunodeficiency virus (HIV), Methicillin-resistant *Staphylococcus aureus* (MRSA), influenza, and COVID-19.

The challenge with using PCR techniques to detect *Borrelia* is that the pathogen is in low abundance in biological samples. In fact, the number of spirochetes in blood is estimated to be as low as 0.1 per ml (Wormser et al., 2001), 3-4 orders of magnitude below the detection limits of conventional PCR.

But newer methods demonstrate the potential to lower these detection limits. Combining advanced, higher sensitivity detection methods with techniques that enrich or concentrate the sample allows assays to detect *Borrelia* at clinically relevant levels. For example, the development of droplet digital PCR (ddPCR) in combination with sample enrichment methods shows promise for the detection of *Borrelia* and other low-abundance pathogens (King et al., 2017; Maggi et al., 2020). Agent-specific probes that enrich nucleic acids in combination with next-generation sequencing can also provide far superior detection capability and identification of multiple tick-borne pathogens (Briese et al., 2015; Jain et al., 2021; Sanchez-Vicente et al., 2022).

The concentration of Borrelia in blood is 3-4 orders of magnitude below the detection limits of conventional PCR

Sample concentration techniques can be used to increase the sensitivity

	Initial Sample Size	Concentrated Sample Size
Sample Concentration	40 mL	0.015 mL

Sample concentration leads to a 2,667x increase in sampled volume

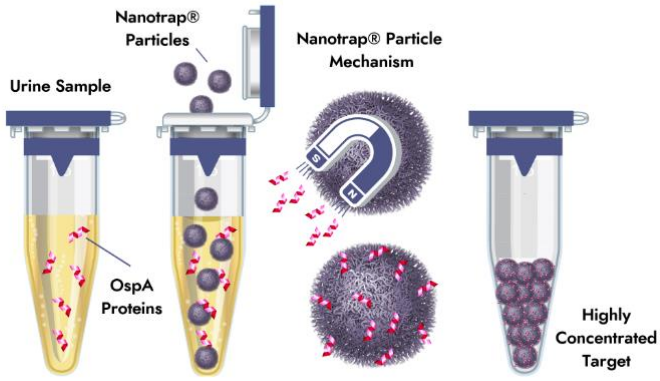


FIGURE 18: The first antigen assay for Lyme disease using sample concentration of urine. Results demonstrate 100% sensitivity in acute Lyme patients with EM rash, a significant improvement over antibody-based approaches (the current standard of care). Sources: Sample concentration data from Magni et al., 2015, image from Galaxy Diagnostics.

Sample concentration of pathogen-specific antigens in urine has also shown improved sensitivity over conventional methods. *Borrelia* tends to colonize in the bladder, thus making urine a viable sample for analysis. For example, a novel antigen assay developed by George

Mason University and commercialized by Galaxy Diagnostics has demonstrated 100% sensitivity and 98% specificity in acute Lyme patients with EM rash (Figure 18, Magni et al., 2015). This assay is currently available for clinical use and represents a significant improvement over the current standard of care. The George Mason team has continued to build upon this sample concentration technique and, in 2020, published a study extending the method to additional vector-borne pathogens, including *Babesia*, *Anaplasma*, *Rickettsia*, *Ehrlichia*, *Bartonella*, *Francisella*, Powassan virus, tick-borne encephalitis virus, and Colorado tick fever virus (Magni et al., 2020).

Imaging of Borrelia

Detection of *Borrelia* in blood or urine indicates a patient is infected, but it does not provide any information on where the infection is present in the body. Since the bacteria can infect the skin, joints, heart, peripheral nervous system, and brain, the ability to visualize the infection *in vivo* would be a powerful tool for clinicians in diagnosis and treatment.

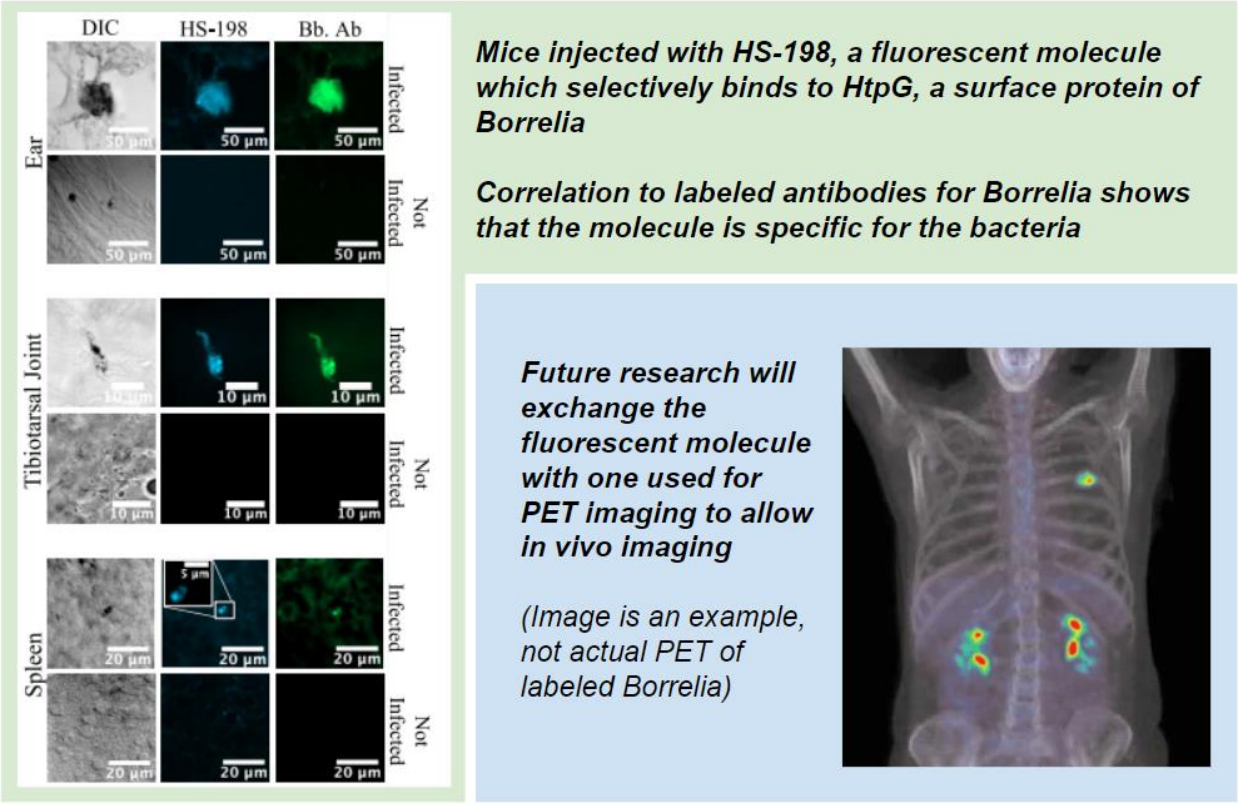


FIGURE 19: Imaging of *Borrelia* in mice by injecting a fluorescent molecule into the animals, which selectively binds to surface proteins of Borrelia (left). Future research will use the approach with an agent detectable via PET to allow non-invasive imaging (right). Sources: Sell et al., 2021 for labeled Borrelia and example PET image from Devoogdt et al., 2012.

Researchers at Duke University have applied the advanced imaging and treatment tools used in cancer research to imaging borrelial infections (Sell et al., 2021). The approach targets a highly conserved protein, high-temperature protein G (HtpG), expressed on the surface of *Borrelia* cells. Imaging agents that bind to HtpG are then utilized to selectively label *Borrelia* for subsequent detection. The approach has been demonstrated in a mouse model using fluorescence (Figure 19, left), with future work labeling with markers for non-invasive positron emission tomography (PET) imaging (Figure 19, right). This approach requires significant research and development for commercialization; however, it could be a powerful tool to pinpoint the location and extent of infection in challenging cases.

Funding Required to Drive Diagnostics to Commercialization

Several promising new approaches exist for diagnosing Lyme disease; however, commercialization of medical diagnostics is fraught with obstacles. Proper adoption requires that the diagnostic demonstrate clinical utility for specific applications, receive insurance codes and reimbursement, be incorporated into clinical guidelines, and be scaled for broad commercial use (Lai-Goldman & Faruki, 2008). This process can take decades, with costs ranging from $20 to $106 million for tests requiring approval from the Food and Drug Administration.

Funding pools for federal and foundation-based grant sources for Lyme diagnostics are highly competitive and small. In 2022, the LymeX Diagnostics Prize, a public-private partnership between HHS and the Steven and Alexandra Cohen Foundation, received 52 applicants for innovations in Lyme Diagnostics and awarded $1 million split among ten winners. Phase 2 of the program launched in February of 2023, inviting winners to participate in a Virtual Accelerator. This accelerator offers access to virtual learning, mentorship, biorepository subject matter expertise, and networking opportunities designed to help the teams progress toward FDA review. Initiatives like the LymeX prize are essential to support proof of concept but insufficient to support a diagnostic through commercialization.

Private venture funding is also difficult to access for Lyme diagnostics. The CDC recommends against novel diagnostic approaches for Lyme, creating an active disincentive for investors, physicians, patients, insurance companies, researchers, and diagnostic companies to advance tick-borne testing. Unfortunately, the most promising diagnostic advances become stuck in the development process (Figure 20), failing to move from the research lab into widespread clinical practice.

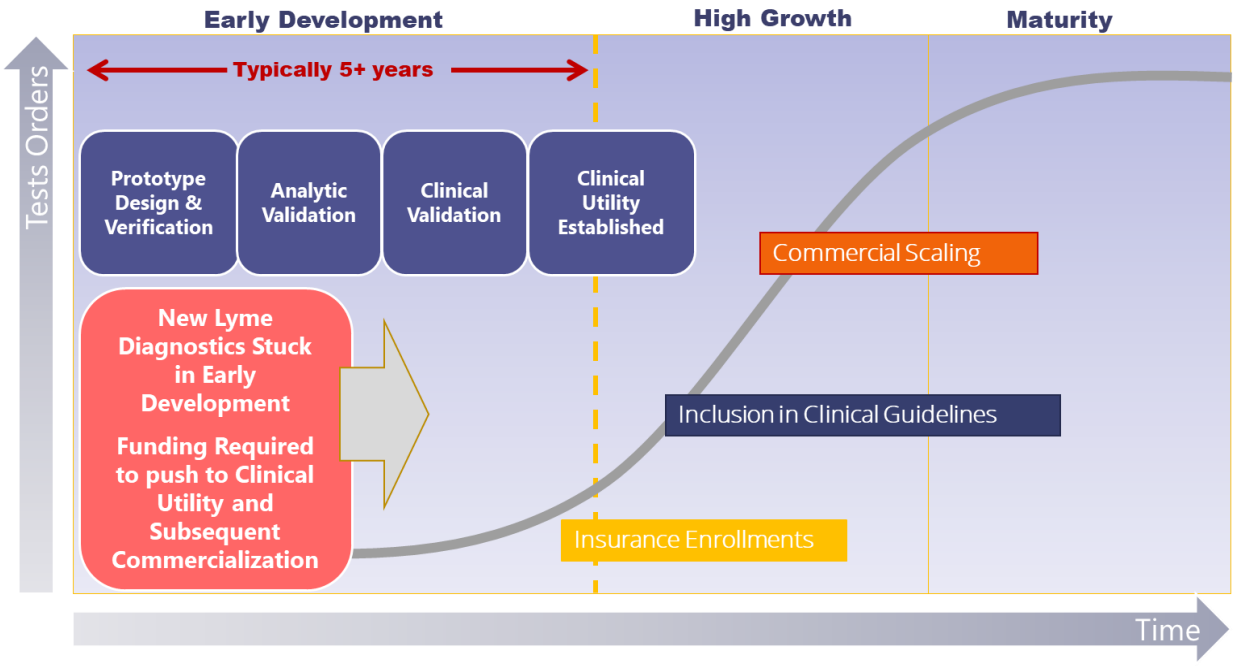


FIGURE 20: The commercialization path for an in vitro diagnostic. Several obstacles must be overcome for widespread adoption, including insurance enrollments, inclusion in clinical guidelines, and commercial scaling. Promising Lyme diagnostics get stuck in early development due to the lack of federal and private funding. Source: Adapted from Lai-Goldman & Faruki, 2008.

Detection and Diagnosis - Key Research Needs

Without accurate diagnostic tools, clinicians are forced to make diagnostic and treatment decisions based on clinical presentation and symptoms. As described in the pathology section, Lyme and other tick-borne diseases can manifest with multiple phenotypes with non-specific symptoms, making this approach problematic and fueling the confusion associated with Lyme. Even if a patient is known to have been affected by a tick bite, there are many tick-borne pathogens or tick bite-associated medical conditions that cause human disease. Many of these other tick-borne illnesses manifest with similar symptoms and also suffer from diagnostic challenges. Clinicians must know which infections are present to provide proper and effective treatment.

NIH and the Tick-borne Disease Working Group have identified that our current diagnostic methods for Lyme are inadequate. Funding is needed to support research and the commercialization of alternate approaches as a top priority. Key areas in diagnostics requiring funding or government action are adapted from the recommendations of the Tick-borne Disease Working Group and summarized in Table 9.

TABLE 9: Critical areas requiring further study or government action in detection and diagnosis for Lyme disease and other tick-borne illnesses. Actions are adapted from the recommendations of the Tick-borne Disease Working Group in 2022.

Areas in Lyme Disease Detection and Diagnosis Requiring Funding or Government Action
Revise CDC guidance on tick-borne disease diagnostics to reflect the limitations of current tests and remove obstacles to developing novel tests.
Increase research funding for the development of diagnostics that are sensitive and specific for the detection of Borrelia and other tick-borne diseases.
Create a clinical trials network to improve federal research in diagnostic solutions for Lyme and tick-borne diseases.
Fund clinical utility studies for new diagnostics in Lyme disease and other tick-borne infections. Clinical utility studies determine whether using the diagnostic leads to an improved health outcome.
Increase funding for Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) for tick-borne disease diagnostics.

Support Research to Advance Treatment of Lyme Disease

The challenges in Lyme diagnosis combined with the complexities of Lyme pathogenesis lead to a wide range of clinical presentations that physicians must decipher and treat. The Infectious Diseases Society of America (IDSA) clinical guidelines for treating Lyme were reviewed and rereleased in 2020 (Lantos et al., 2021). In general, acute, early-stage disease is treated with 7-14 days of oral antibiotics, whereas invasive infections and later clinical manifestations are treated with 14-28 days of oral or intravenous (IV) antibiotics. This guidance seems straightforward; however, the evidence supporting the effectiveness of these treatments for the various manifestations of Lyme disease is much more complex.

IDSA Guidelines - Strength of Recommendation and Quality of Evidence

IDSA clinical guidelines include qualifiers that rate the strength of the recommendation and the quality of evidence supporting it. This systematic process is called the GRADE approach (Grading of Recommendations Assessment, Development, and Evaluation) and leverages set definitions for each rating (Schunemann et al., 2015). The ideal recommendations are listed as "strong recommendation, high-quality evidence," meaning that advice is gathered from unbiased observational studies and further research is unlikely to change the confidence in treatment recommendations.

TABLE 10: Summary of the 2020 IDSA treatment recommendations for the various manifestations of Lyme disease, including their assessment of the strength of the recommendation and the quality of evidence. Source: Extracted from Lantos et al., 2021.

Clinical Presentation	Treatment	Strength of Recommendation	Quality of Evidence
Dermatological			
Erythema Migrans (EM) rash	Oral antibiotic 7-14 days	Strong	Moderate
Borrelial Lymphocytoma (nodule or plaque typically on the ear of children & breast of adults)	Oral antibiotic 14 days	Weak	Low-quality
Rheumatological			
Initial Lyme Arthritis	Oral antibiotic 28 days	Strong	Moderate
Lyme Arthritis with partial response to initial treatment	No recommendation	Knowledge Gap	
Lyme Arthritis with no or minimal response to initial treatment	IV antibiotic 14-28 days	Weak	Low-quality
Lyme Arthritis with residual symptoms after oral and IV antibiotics	Rheumatological interventions	Weak	Very low-quality
Neurological			
Acute neurologic manifestations without involvement of the brain or spinal cord	IV antibiotic 14-21 days	Strong	Moderate
Neurologic manifestations with involvement of the brain or spinal cord	IV antibiotic 14-21 days	Strong	Moderate*
Cardiac			
Lyme Carditis	Oral or IV antibiotics 14-21 days	Weak	Very low-quality

*** Although this treatment group is listed as having moderate quality evidence, the evidence summary notes that "treatment in this population has never been systematically studied."**

Table 10 summarizes the treatment recommendations and ratings in the 2020 IDSA Clinical Practice Guidelines for Lyme disease. High-quality evidence is lacking for all disease states, and several disease manifestations have weak recommendations with low or very low-quality evidence. The GRADE definition for "weak recommendation, low-quality evidence" notes that "further research is very likely to have an important impact on our confidence in the estimate of effect," thus highlighting the need for further study.

Failed Treatment and Post-Treatment Lyme Disease Syndrome (PTLDS)

Although the full treatment landscape remains unclear, studies show that antibiotics are most effective when used in the early stages of infection (Torbahn et al., 2018; Yang et al., 2021; Zhang et al., 2023). Early diagnosis relies heavily on EM rash presentation since the sensitivity of recommended diagnostics is poor for early-stage disease (Waddell et al., 2016) and since other acute Lyme symptoms (e.g., fatigue, fever, achiness) mirror those of other infections, including COVID-19.

Figure 21 estimates the likelihood that patients will receive a proper, rash-based diagnosis by combining the prevalence and misdiagnosis statistics for EM rashes cited above in Figure 16 (Schwartz et al., 2017; Tibbles et al., 2007; Lipsker et al., 2004). This analysis reveals that less than half of infected patients have the potential for early treatment using the EM rash as a means of diagnosis.

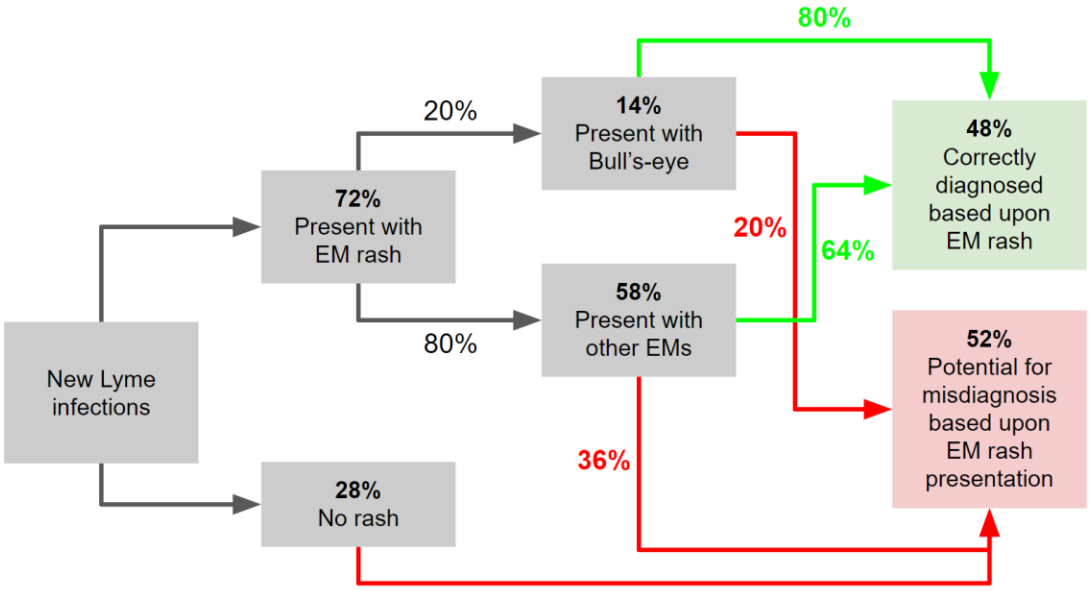


FIGURE 21: The likelihood of an early diagnosis of Lyme disease based upon EM rash presentation. Analysis demonstrates a 52% potential for misdiagnosis. Source: Center for Lyme Action analysis using percentages from prevalence and misdiagnosis studies (Schwartz et al., 2017; Tibbles et al., 2007; Lipsker et al., 2004).

Those receiving early antibiotic therapy have the highest likelihood of returning to health; however, studies report that 10-20% of these patients report persistent symptoms after treatment with antibiotics (Marques, 2008). In 2022, a prospective study assessed the prevalence of Post-Treatment Lyme Disease Syndrome (PTLDS) in patients ideally treated for Lyme disease and compared the prevalence of similar symptoms in those without a history of Lyme disease (Aucott et al., 2022). Results demonstrate that 13.7% of ideally treated Lyme disease patients experience symptoms consistent with PTLDS (Figure 22), an incidence over three times higher than the control group. These symptoms include severe fatigue, cognitive issues, sleep disturbances, and musculoskeletal pain that functionally impair their quality of life. The estimated cumulative prevalence of PTLDS in 2020 was 2 million people (DeLong et al., 2019).

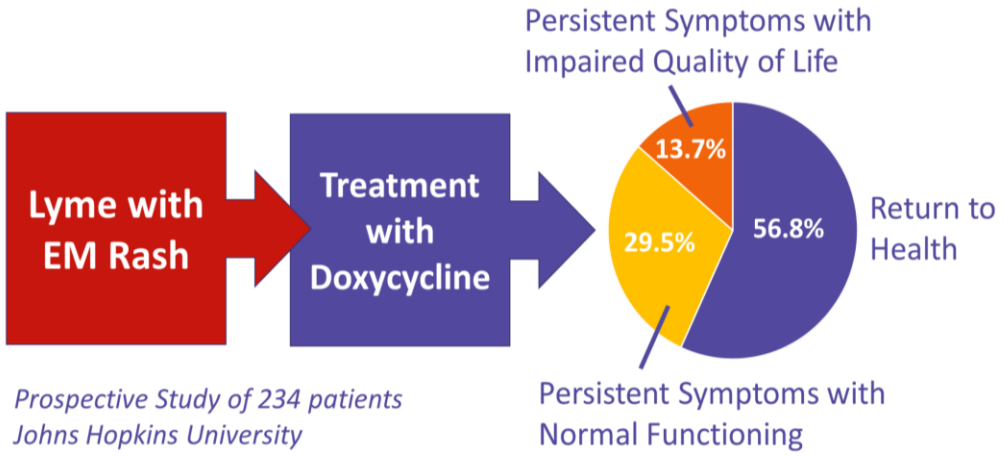


FIGURE 22: A prospective study on ideally treated Lyme disease patients shows that 13.7% of patients experience ongoing, debilitating symptoms after treatment. Source: Aucott et al., 2022.

But most Lyme patients are not ideally treated. As shown in Figure 21, up to 52% of patients are likely to be misdiagnosed based upon EM rash presentation, allowing the bacteria to disseminate to other areas of the skin, joints, heart, peripheral nerves, and brain. Late, untreated disease can present weeks to months after the initial infection, and delayed treatment may be less effective and increase the risk for PTLDS (Asch et al., 1994; Shadick et al., 1994; Moody et al., 1994). Later manifestations of Lyme are also more likely to have weak recommendations for treatment with low-quality evidence in IDSA guidelines (Table 10). More research is required to provide better treatment options for this growing group of patients.

Persistent Lyme - A Growing Public Health Problem with an Unknown Cause

It is critical to understand why so many Lyme patients fail treatment. Unfortunately, the molecular mechanisms underlying PTLDS and other forms of persistent Lyme remain unknown. Scientists have postulated several potential causes, with the top contenders

summarized in Table 11. The list includes inflammation due to persistent *Borrelia* infection, inflammation due to other infections or bacterial debris, and other immune dysfunction. These proposed mechanisms are not mutually exclusive, and different patients may suffer from different mechanisms. In addition, multiple mechanisms can affect the same patient, either in combination or at different time points.

TABLE 11: Postulated mechanisms leading to PTLDS or persistent Lyme. Several root causes may contribute to dysfunction in different patient groups and within the same patient.

Postulated Mechanism for PTLDS or Persistent Lyme	References
Infection-induced immune dysfunction	Strle et al., 2014 Aucott et al., 2016
Autoantigens and/or central nervous system sensitization	Maccallini et al., 2018
Inflammation due to persistent Borrelia bacteria	Crossland et al., 2018 Embers et al., 2012 Embers et al., 2017 Hodzic et al., 2014
Inflammation due to Borrelia bacterial debris	Bockenstedt et al., 2012 Jutras et al., 2019
Inflammation due to other infections	Horowitz et al., 2019 Touradji et al., 2019

The 2020 IDSA guidelines recognize that further studies are required to understand the origins of persistent symptoms and to develop therapeutic strategies (Lantos et al., 2021). The Study of Lyme disease Immunology and Clinical Events (SLICE) is a privately funded research study conducted by the Johns Hopkins University Lyme Disease Research Center. The study aims to examine risk factors, symptom severity, and immunologic biomarkers in patients diagnosed with PTLDS. More studies of this nature are required to determine the underlying causes and resulting treatments for PTLDS.

Evidence of Persistent Infection

A leading but debated theory for the cause of PTLDS is *Borrelia's* persistence after antibiotic treatment. Doxycycline, the most commonly prescribed antibiotic for Lyme disease, is a

bacteriostatic drug that stops the bacteria from reproducing rather than killing the bacteria itself (Loree & Lappin, 2022). As a result, the drug relies upon the host immune system to resolve the infection, slowing bacterial growth so the immune system can gain the upper hand.

But as discussed in the pathology section, *Borrelia* has an array of techniques to suppress and evade the immune system. *In vitro* studies also demonstrate that *Borrelia* transforms into a slow-growing, more tolerant form when exposed to antibiotics (Caskey et al., 2015). This evidence, combined with the high levels of treatment failure, has led researchers to postulate that ongoing *Borrelia* infection is a potential cause of persistent Lyme.

To test this theory, scientists have used animal models to determine if *Borrelia* persists *in vivo* after standard courses of antibiotics. Definitive evidence of persistence has now been established in murine, canine, and non-human primate models (Bockenstedt et al., 2002; Eisner et al., 2015; Embers et al., 2017; Hodzic et al., 2008; Hodzic et al., 2014; Straubinger, 2000). Recent animal studies also demonstrate that the persistent spirochetes are metabolically active and express bacterial genes, implying that they are not only present but also infectious (Embers et al., 2017; Hodzic et al., 2019).

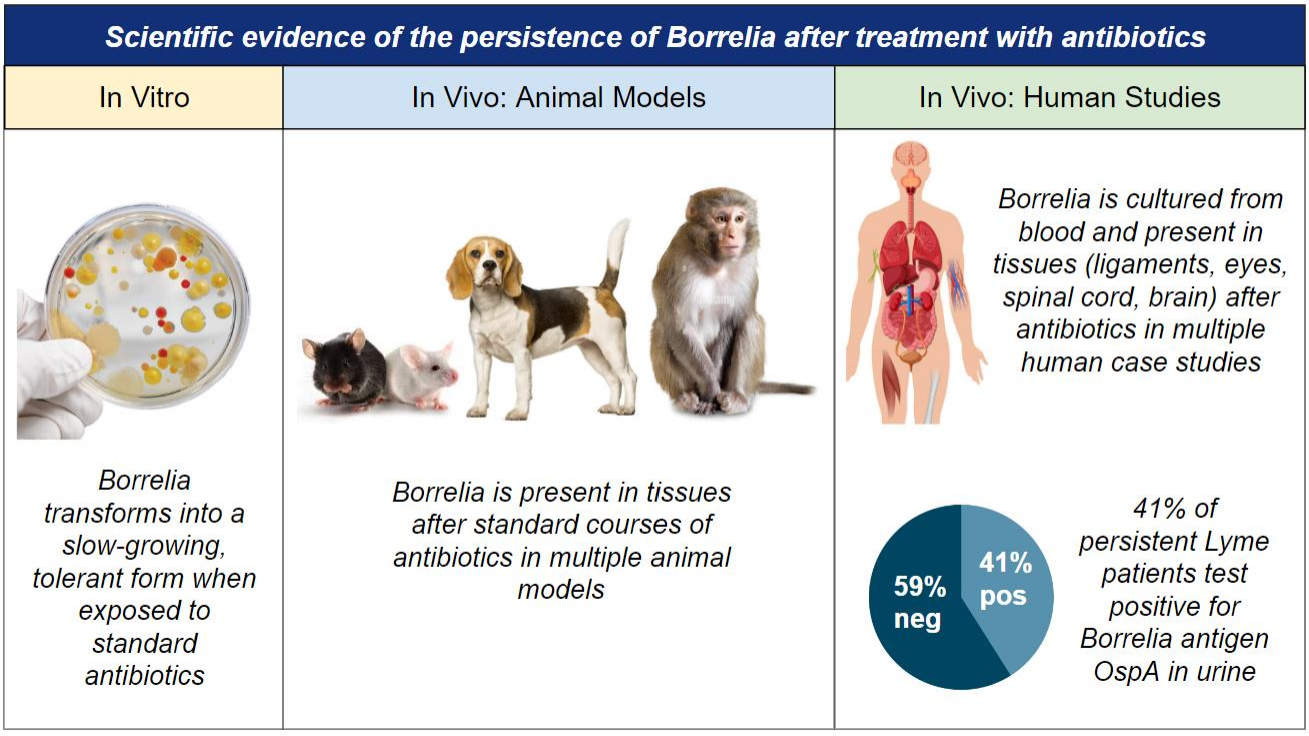


FIGURE 23: A summary of scientific evidence of *Borrelia* persistence after antibiotics. Persistence of the bacteria after treatment has been demonstrated in cell cultures, animal models, and humans. Sources: Listed in Text.

Case studies in humans have also demonstrated the persistence of bacteria after extended courses of antibiotics (Häupl et al., 1993; Hudson et al., 1998; Marques et al., 2014; Oksi et al., 1999; Pfister et al., 1989; Preac-Mursic et al., 1989; Preac-Mursic et al., 1993, Gadila et al., 2021). These studies include evidence of persistent *Borrelia* cultured from blood and identified in tissues extracted from ligaments, eyes, spinal cord, and brain through surgery or autopsy.

Unlike antibody-based methods, new direct detection diagnostics can assess ongoing infection in persistent Lyme patients. The methodology highlighted in Figure 18 tests for *Borrelia*-specific antigens in urine. Since this test measures antigens instead of antibodies, it can be used to determine whether a patient still suffers from active infection. A study of 100 patients under surveillance for persistent Lyme revealed that 41 were positive for the *Borrelia* antigen (a borrelial surface protein called OspA), suggesting that persistent infection is the cause for some, but not all, of persistent Lyme cases (Magni et al., 2015).

The major criticism of the persistent infection hypothesis is that double-blinded studies have failed to show a sustained difference between patients treated with prolonged antibiotics and placebo controls (Berende et al., 2016; Fallon et al., 2008; Klempner et al., 2001; Krupp et al., 2003). An explanation of these results could be the lack of persistent infection, but it could also be that current antibiotic regimens are ineffective for persistent Lyme. The heterogeneous result of urine antigen testing suggests that both hypotheses could be true in different patient groups.

Exploring Alternative Therapies

Given the high treatment failure rate, several researchers have investigated alternative therapies, including novel antibiotics and combination therapies. *In vitro* models have shown that the slow-growing, antibiotic-tolerant form of *Borrelia*, can be eradicated with certain combinations of antibiotics (Alvarez-Manzo et al., 2020; Feng et al., 2015).

Novel small-molecule approaches often utilized in cancer treatment are also being applied to Lyme disease. HtpG is a surface protein of *Borrelia,* and the selective binding of small molecules to this protein can be used for imaging, as shown in Figure 19. A research collaboration between Duke University and the University of North Carolina has also used this binding mechanism as a potential therapeutic (Carlson et al., 2023). HS-291 is a toxin that binds to HtpG and triggers cell death. This approach has demonstrated the ability to effectively kill *Borrelia* in cell cultures, with future research testing the approach in animal models.

Testing of alternative therapies in animal models has been limited but encouraging. In 2019, researchers demonstrated that Lyme bacteria caused severe and persistent symptoms after standard antibiotic treatment in a mouse model. These slow-growing bacteria were

effectively eliminated in mice with a combination of three antibiotics: daptomycin, doxycycline, and ceftriaxone (Feng et al., 2019). Researchers have also applied high-throughput screening to identify new treatment compounds (Pothineni et al., 2016). Further study of top candidates revealed that Azlocillin effectively treats doxycycline-tolerant *Borrelia* in both cell cultures and mice (Pothineni et al., 2020). Hygromycin A has also been identified as an antimicrobial that acts selectively against *Borrelia*, clearing the infection in mice (Leimer et al., 2021). Additional research and double-blind, placebo-controlled trials are required to determine if these alternative therapies are useful as a frontline treatment for acute Lyme or for resolving persistent Lyme disease.

Treatment Beyond Antimicrobials

In 2022, Johns Hopkins University announced a new clinical trial testing the efficacy of Psilocybin as an effective treatment for Persistent or Post-treatment Lyme disease Syndrome (PTLDS). The trial is currently in Phase 1 and is estimated to conclude by the end of 2024. A separate recently published case study highlighting an "immunocompetent male" with a well-documented case of neuropsychiatric Lyme disease shows he experienced significant improvement after microdosing with Psilocybin 3x weekly (Kinderlehrer 2023). The role of Psilocybin as an effective therapy for Lyme disease symptoms warrants further research.

A study led by the Lyme & Tick-borne Disease Research Center at Columbia University's Irving Medical Center examined the benefits of treating post-treatment Lyme disease (PTLD) using Kundalini Yoga (Murray et al., 2022). Participants with higher levels of anxiety experienced the greatest improvement in fatigue, symptom burden, and depression. This study is the first of its kind to explore a behavioral intervention for PTLDS and shows promising results as an alternative to antibiotics.

Treatment - Key Research Needs

The CDC estimates that 476,000 people in the U.S. are diagnosed and treated for Lyme disease every year. Many of those are initially misdiagnosed, and of those promptly diagnosed and treated, 10-20% remain ill with debilitating symptoms. Current treatment guidelines lack high-quality evidence, and further research is required to help the growing population of Americans suffering from persistent Lyme. Key areas in Lyme treatment requiring funding are adapted from the recommendations of the Tick-borne Disease Working Group and summarized in Table 12.

TABLE 12: Critical areas requiring further study in treating Lyme disease. Actions are adapted from the recommendations of the Tick-borne Disease Working Groups in 2018, 2020, and 2022.

Areas in Lyme Disease Treatment Requiring Funding or Government Action
Increase research and clinical studies funding to better understand the mechanisms for Borrelia persistence and its tolerance to antibiotics.
Increase research funding to determine the causes of PTLDS and persistent Lyme and develop appropriate therapeutics.
Increase research funding for alternative therapeutics to treat acute, late-stage, and persistent Lyme disease.
Create a clinical trials network to improve federal research in therapeutic solutions for Lyme and tick-borne diseases.
Fund double-blind, placebo-controlled trials to test the effectiveness of alternative treatments for acute and persistent Lyme disease.
Fund double-blind, placebo-controlled trials to test the effectiveness of alternative treatments for Lyme carditis, Neurological Lyme, Lyme Arthritis, and other manifestations of late-stage Lyme disease.

Accelerate Research to Improve Prevention of Lyme Disease

New treatments are needed to help the millions suffering from persistent Lyme; however, it is also important to prevent millions more from contracting the disease. The number of Lyme disease cases reported annually to the CDC is about three times that of the 1990s, and more research is required to combat this growing public health threat. Two of the most promising prevention techniques, vaccines, and personal protection, take very different approaches. Vaccines provide immunity to the bacteria once infected, whereas personal protection seeks to repel ticks to prevent infection before it occurs.

Vaccines and other Preventative Treatments

Vaccines have been used to prevent infectious diseases for over 200 years. A vaccine for Lyme disease, called Lymerix, was introduced to the market in 1998, and vaccinated individuals showed a 76% reduction in Lyme disease in the year following vaccination (Steere et al., 1998). However, 3 years later, the vaccine was withdrawn from the market due to concerns about vaccine side effects (Nigrovic & Thompson, 2007). Despite this withdrawal, future vaccine approaches are promising and may help to combat Lyme's increasing impact.

The Tick-Borne Disease Working Group provided an overview of the current status of vaccines and other drug-based preventative measures in their 2022 Disease Prevention and Treatment Subcommittee Report. Table 13 summarizes the top vaccine and prevention methods in development, with further information in the Subcommittee Report.

TABLE 13: Promising vaccines and other preventative therapeutics in development as extracted from the 2022 Tick-Borne Disease Working Group Subcommittee Report.

Approach (Developer)	Technology	Status	References
VLA15 (Valneva & Pfizer)	Vaccine containing artificially produced OspA (a surface protein of Borrelia)	Phase III Clinical Trials	Comstedt et al., 2017
Lyme PrEP (MassBiologics)	Human monoclonal antibodies to OspA (a surface protein of Borrelia)	Phase I Clinical Trials	Schiller et al., 2021 Wang et al., 2016
Chimeritopes (Virginia Commonwealth University)	Vaccine combining several immunologically relevant proteins into a new protein	Pre-clinical Testing	Camire et al., 2021 O'Bier et al., 2021
mRNA-1982 and mRNA-1975 (Moderna)	mRNA technology	Research & Development	Moderna Press Release
Tick Salivary Proteins (Yale University)	Vaccine containing tick salivary proteins	Research & Development	Matias et al., 2021 Rego et al., 2019 Sajid et al., 2021

These approaches represent powerful tools against Lyme disease, and additional research and funding are required to bring them to market. Fortunately, vaccines are one of the few areas within tick-borne illness that can access private funding. Pfizer and Valneva entered into a $308 million dollar co-development deal to support the commercialization of the VLA15 vaccine, of which $130 million was provided upfront. VLA15 received Fast Track designation from the FDA in 2017, a practice that should be encouraged for all vaccines targeting tick-borne illnesses.

The majority of vaccines summarized in Table 13 utilize borrelial or tick-based proteins to stimulate the human immune response. The Tick-Borne Disease Working Group recommended expanding research to include other vaccine platforms such as viral vectors, peptide-based vaccines, attenuated/mutant strains of *Borrelia*, and next-generation vaccine platforms like mRNA (announced in April of 2023 as in development by Moderna). Other opportunities include research on the adaptive immune response to *Borrelia*, where key gaps in understanding exist, particularly in the early disease stages. This fundamental knowledge is critical to developing an effective, long-lasting vaccine.

The potential for private vaccine funding is encouraging, and NIH should support vaccine research as outlined in its Strategic Plan. However, it is important to note that vaccines should serve as one prong in a multi-prong strategy against tick-borne illness. Ticks carry a plethora of bacterial, viral, and parasitic pathogens, each of which requires an effective immune response. Furthermore, even with the extensive campaigns for the COVID vaccine, as of May 2023, over 30% of the U.S. population was not fully vaccinated. Finally, vaccines offer no assistance to the millions already suffering from persistent Lyme and other tick-borne infections, emphasizing the need for effective diagnostics and treatment to complement vaccine research and development.

Personal Protection against Tick Bites

A cornerstone of tick-borne illness prevention is using protective clothing or repellants to reduce tick bites. Despite decades of education on prevention measures, recent studies have shown that compliance in Lyme endemic areas is as low as 31% for the regular use of insect repellants (Niesobecki et al., 2019). Part of the reason for this low compliance is that current repellents are perceived as toxic, exhibit strong smells, or are expensive.

The CDC developed a promising new repellent in a series of federally funded laboratory studies (Bharadwaj et al., 2012; Dietrich et al., 2006; Dolan et al., 2009; Flor-Weiler et al., 2011; Jordan et al., 2012; Panella et al., 2005). Nootkatone, from Alaskan yellow cedar and grapefruit essential oil, has been shown to repel and kill four tick species. The substance is non-toxic to humans and is commonly used in foods and fragrances. It can also be mass-produced using a low-cost yeast fermentation process, thus providing the trifecta of repellant attributes: effectiveness, safety, and affordability. Finally, since the product is acaricidal, or capable of killing ticks, it can be utilized to reduce tick populations in endemic areas.

The 2022 Ecology and Personal Protection Subcommittee of the Tick-Borne Disease Working Group noted that the path from proof of concept in the laboratory to commercial product is roughly 15 years. Although studies on Nootkatone were funded between 1997 and 2012, the substance was not registered with the Environmental Protection Agency (EPA) as a

biopesticide until 2020, and consumer products are still unavailable. This timeline illustrates the critical need for a more effective pathway to develop, register, and commercialize tick bite prevention and control products.

Prevention - Key Research Needs

As outlined in the NIH Plan, prevention is a critical piece of a multi-faceted strategy to combat tick-borne diseases and must be advanced along with improvements in diagnosis and treatment. Research needs in prevention are adapted from the recommendations of the Tick-borne Disease Working Group and summarized in Table 14. In addition to funding prevention research, government agencies such as the Food and Drug Administration (FDA), Environmental Protection Agency (EPA), and Biomedical Advanced Research and Development Authority (BARDA) can facilitate deployment by streamlining testing and commercialization pathways for new vaccines and prevention products.

TABLE 14: Critical areas requiring further study in preventing Lyme disease. Actions are adapted from the recommendations of the 2022 Tick-borne Disease Working Group.

Areas in Lyme Disease Prevention Requiring Funding or Government Action
Fund research on the adaptive immune response to Borrelia, particularly in early-stage disease.
Fund research on vaccines using alternative vaccine technologies such as viral vectors, peptide-based vaccines, attenuated/mutant strains of Borrelia, and next-generation vaccine platforms like mRNA.
Increase Small Business Innovation Research funding for the research, development, and evaluation of new tick bite prevention and control products.
Fund studies to reduce tick populations using acaricidal products such as Nootkatone.

Develop Tools and Resources to Advance Lyme Disease Research

The NIH Strategic Plan wisely noted that improvements to basic research tools were needed to support the other four targeted research areas (Figure 5). Advances in fundamental knowledge, diagnosis, treatment, and prevention of tick-borne disease require that investigators have access to the resources required to complete their studies. Two such resources rise to the top: 1) access to well-characterized biological samples and patient groups; and 2) access to animal models that manifest the complexities of human infection.

The Need for Biobanks and Well-Characterized Samples and Patient Groups

As discussed above, improving diagnostics is a top priority in combating the growing problem of Lyme disease. But new diagnostics require access to well-characterized human biological samples to develop and validate each assay. Researchers studying *Borrelia* and developing new treatments also require access to samples to study infection status and disease biomarkers. Unfortunately for researchers, access to well-characterized samples has been a challenge.

Since the current "gold standard" test for Lyme is the two-tier methodology with poor sensitivity, developing a repository of samples where the analytical results are clear and understood is not trivial. Many researchers have utilized the Lyme Serum Repository collected and maintained by the CDC (Molins et al., 2014). Although this repository is a key resource, it comes with limitations. First, all samples have reported positive with at least one test methodology (two-tier, PCR, and/or culture). Since each method has sensitivity limitations, more challenging samples are excluded from the repository. Second, samples are all serum and do not include other matrices like blood, urine, and cerebral spinal fluid, which may be more suitable for diagnosing certain disease manifestations. Finally, sample availability is limited, with additional collections required to support an expansion of research.

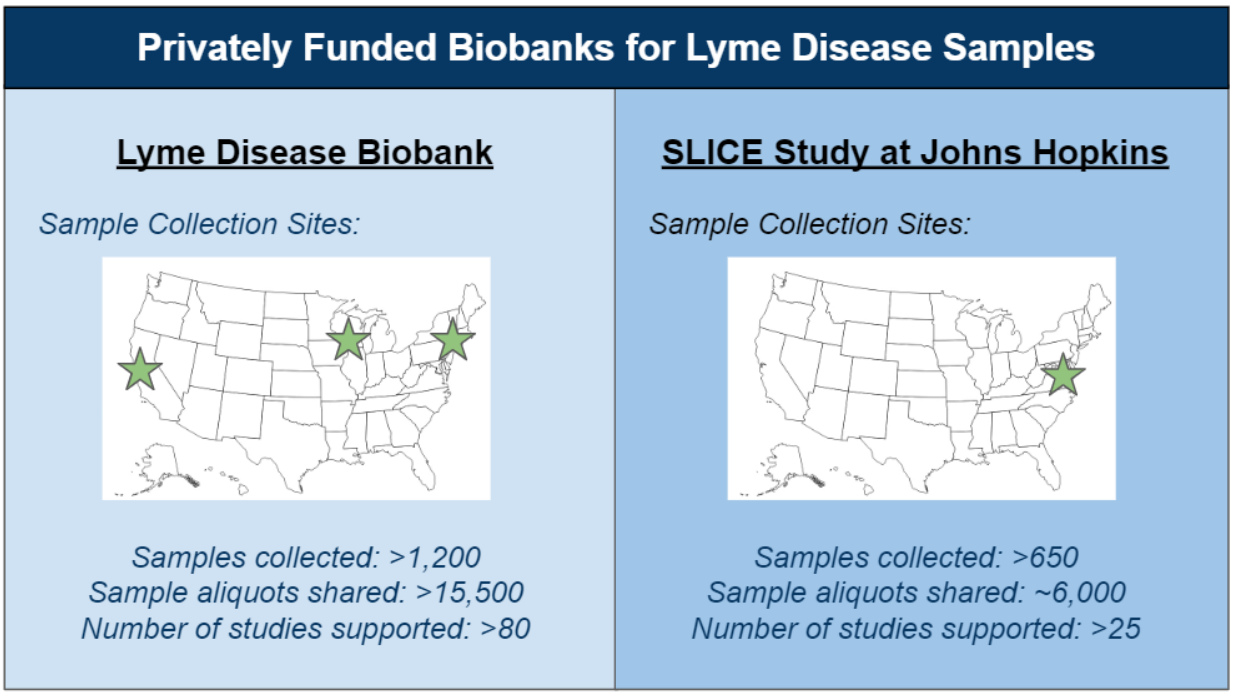


FIGURE 24: A summary of statistics on two privately funded Lyme disease sample repositories. These repositories are essential for diagnostic and therapeutic research. Sources: Bobe et al., 2021 and discussions with repository coordinators.

Two privately funded, centralized repositories also exist, each with distinct collection sites, inclusion and exclusion criteria, and sample characterization methods (Figure 24). The Lyme Disease Biobank has collected more than 1200 human biological samples to facilitate research on Lyme and other tick-borne infections (Horn et al., 2020). Whole blood, serum, and urine samples are collected from individuals in the various stages of Lyme disease along with unaffected controls. Robust clinical information accompanies the samples, including medical histories, photos of EM rashes (if present), test results for Lyme and other tick-borne infections, and demographics.

The second repository of longitudinally collected samples is from the Study of Lyme disease Immunology and Clinical Events (SLICE) at the Johns Hopkins Lyme Disease Research Center (Rebman et al., 2015). The SLICE repository includes a number of different sample types, including skin biopsies for culture and microbiologic validation of infection, whole blood, serum, peripheral blood mononuclear cells, plasma, skin and fecal swabs, and, most recently, urine.

Since sample access is vital for conducting research, the 2022 Tick-Borne Disease Diagnostic Subcommittee recommended that the CDC build a national biorepository of human samples supported by a network of qualified laboratories and physician clinics as a top priority. Funding additional research without such a repository will quickly deplete banked sample supplies, thus stalling the desired progress.

The development of new therapies will also require access to well-characterized patient groups that can be utilized for treatment trials. The SLICE study at Johns Hopkins and the Lyme Disease Biobank have established patient groups to support clinical translational research. This research applies discoveries made in the laboratory setting to human studies (bench to bedside) to determine clinical utility. For conditions like persistent Lyme disease, which may have multiple root causes, characterizing risk factors, symptom severity, and key biomarkers may help pair patients with appropriate therapies as they are developed. The SLICE study and Lyme Disease Biobank have enrolled over 800 patients and similar patient groups will be required as new therapies emerge for clinical testing.

The Need for Animal Models to Study Disease Progression and Treatment

The study of infection and treatment in laboratory animals is an important and established research tool. Small mammals such as mice, rats, and rabbits are the most commonly used models, making up the vast majority of laboratory animals (Hickman et al., 2016). However, smaller hosts such as rodents, squirrels, and hares have evolved as natural reservoirs for *Borrelia* and are the preferred feeding source for young ticks (Gray, 2002). As a result, these

animals are more tolerant of the bacteria and do not develop the same disease manifestations as humans, which are considered incidental hosts.

Inbred laboratory mice that are immune deficient have served as effective models for *Borrelia* infection, and spirochetes have been shown to disseminate and colonize peripheral tissues such as the skin, joints, and heart (Barthold et al., 1990; Weis et al., 1997; Zeidner et al., 2001). Despite successfully emulating the human pathology associated with Lyme arthritis and carditis, the mouse model does not reproduce human neurologic manifestations associated with Lyme (Garcia-Monco & Benach, 2013). This phenomenon has been a major detriment to studying borreliosis in the peripheral and central nervous systems.

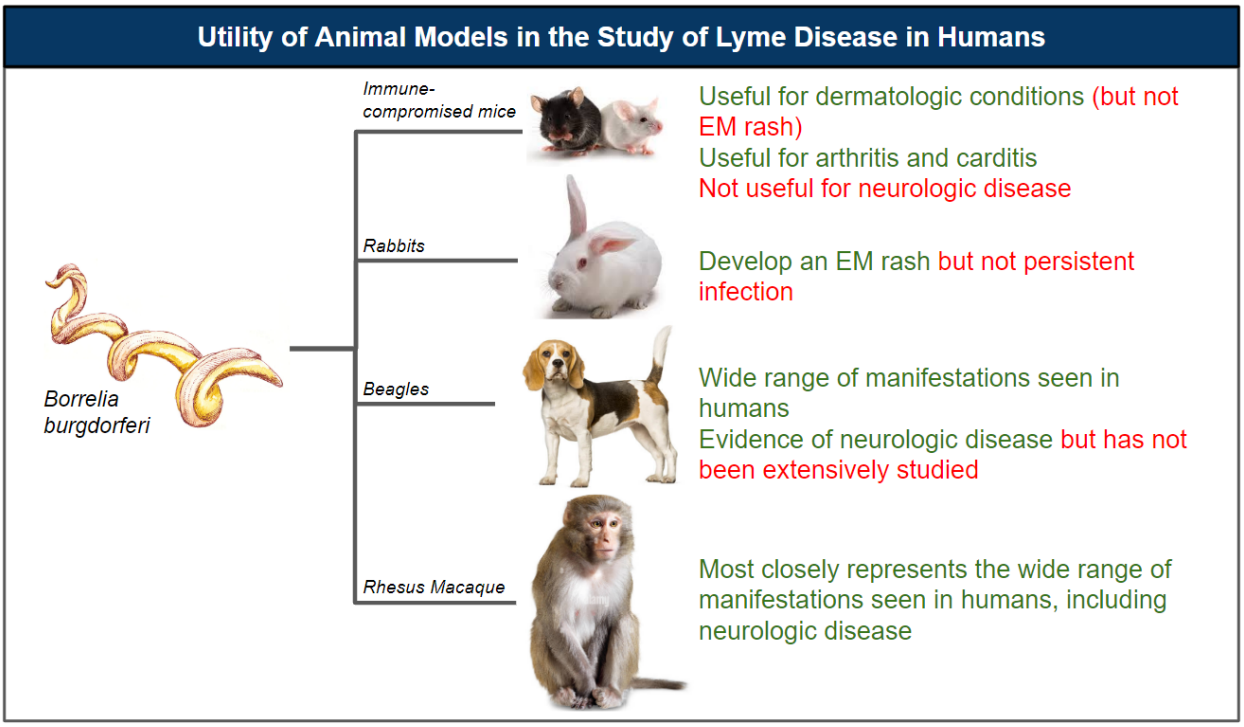


FIGURE 25: The utility of animal models in studying various manifestations of human Lyme disease. Several models can be utilized; however, the rhesus macaque is the best-known animal model for the study of neurological disease.

Larger animal models, such as dogs and monkeys, exhibit evidence of neurologic disease from *Borrelia* and serve as candidates to understand disease pathogenesis and treatment. Neurological symptoms have been reported in dogs with Lyme disease, but the subject has not been extensively studied, and the linkage of neurological symptoms to *Borrelia* remains controversial (Krimer et al., 2011). On the other hand, the rhesus macaque, colloquially known as the rhesus monkey, is an excellent model of Lyme disease and closely parallels the infection in humans for disseminated disease (Philipp et al., 1993). In this non-human primate

model, *Borrelia* has been shown to infect the brain, brainstem, cerebellum, spinal cord, and meninges (Cadavid et al., 2000; Roberts et al., 1995).

However, studies with non-human primates are expensive, and currently, only one center in the U.S. is actively utilizing the model to study Lyme disease. Given the extensive cost and societal harm caused by neurocognitive and mental health disorders, expansion of this critical research is required.

Tools and Resources - Key Needs

An infusion of research funding is needed to address the Lyme disease epidemic, and this funding must also develop the tools and resources required to support that research. Initiatives to provide these tools and resources are summarized in Table 15 and are adapted from the recommendations of the Tick-Borne Disease Working Group.

TABLE 15: Critical areas in tools and resources needed to support Lyme disease research. Actions are adapted from the recommendations of the Tick-borne Disease Working Group.

Tools and Resources Needed to Support Lyme Disease Research
Build a national biorepository of human samples for Lyme disease and other tick-borne illnesses supported by a network of qualified laboratories and physician clinics.
Fund translational research programs with well-characterized patients suitable for treatment trials of future therapies and to answer the questions posed in the pathogenesis section.
Expand the capacity to study Lyme disease in other tick-borne illnesses in animal models that more accurately emulate human disease (such as non-human primates).

CONCLUSION

Enacting the NIH Strategy for Lyme Disease and Tick-Borne Illness

Every year, at least 476,000 Americans are diagnosed and treated with Lyme disease, leading to annual direct medical costs of approximately $1-2 billion and an estimated economic impact between $50-100 billion. These patients face a lack of reliable diagnostics, ineffective therapies for persistent disease, and no available vaccine. They suffer from debilitating conditions due to infection of the skin, joints, heart, and nervous system, including the brain.

In 2019, a trans-NIH strategic planning team released a comprehensive plan to improve the diagnosis, prevention, and treatment of tick-borne diseases, of which over 80% are caused by Lyme. This comprehensive and well-researched plan outlined five areas of opportunity in tick-borne disease research, including improving fundamental knowledge, detection, prevention, treatment, and research tools and resources. HHS has also coordinated an initiative to address Lyme and tick-borne illness with the multi-year Tick-Borne Disease Working Group to advise on the growing public health issue.

Despite these government efforts and their associated recommendations, NIH funding for Lyme disease is dwarfed by other infectious diseases, receiving less than 2% of the funds invested in HIV/AIDS and less than 1% of the funding per case received for other vector-borne diseases like Malaria and West Nile. In 2022, NIH invested only $50 million in Lyme disease research, with only $8 million invested in new diagnostics and treatments, two critical needs for the millions suffering from Lyme disease.

In 2020, the Center for Lyme Action published a policy paper calling for a cure for Lyme and other tick-borne diseases by 2030. Achieving this goal will require research funding of $500 million to $1 billion annually, which can be used to execute the NIH Strategic Plan. Congress is called to action to address this critical need and solve one of America's fastest-growing infectious disease epidemics.

Call to Action for U.S. Congress and Federal Agencies:

Fund $500M - $1B per year for tick-borne disease research.

Execute the NIH Strategic Plan for Tick-Borne Disease Research to solve one of America's fastest-growing infectious disease epidemics.

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The author, Nicole Danielle Bell, is a scientist. and engineer who became interested in tick-borne illness when she realized her husband's dementia was caused by multiple tick-borne pathogens. To read more about Nicole's journey please visit www.nicoledaniellebell.com or purchase her book, *What Lurks in the Woods*. This paper was made possible through her efforts and coordination.

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