Borrelia burgdorferi: The Deer Tick's Dark Secret

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Abstract

Since its recent discovery in the late 1970s, Lyme Disease (LD) has been a growing public health concern, especially in the United States where it accounts for the majority of vector-borne infections each year. The causative agent, *Borrelia burgdorferi*, is transmitted to humans through the bite of an infected *Ixodes* tick. This pathogen uses many unique mechanisms to both shield itself from the host immune response and cause disease. Clinically, LD presents in successive phases, with each increasing in severity as the bacterial cells migrate to new tissues and organ systems. On the epidemiological and ecological fronts, limitations in reporting, ecological changes, and a lack of public support hinder accurate surveillance and enhance the spread of the disease. The goal of this literature review is to increase public knowledge of *B. burgdorferi*, its vector, and the disease it causes, along with suggesting preventative measures to protect individuals who reside in high-risk areas. A collective and coordinated public health effort represents our greatest chance of restraining the LD-causing pathogen.

Discovery of the Causative Agent of Lyme Disease

In 1976, the Connecticut State Department of Health reported an outbreak of an unusual form of arthritis near Lyme, Connecticut (37). The affected individuals experienced recurrent bouts of pain and swelling in large synovial joints (such as the knee) without prior injury. Other clinical presentations included flu-like symptoms and unusual cutaneous lesions called erythema migrans (EM), which had first been described by the German physician Alfred Buchwald in 1883 (75). Although a causative agent had not yet been discovered, this unique combination of signs and symptoms was termed Lyme Disease (LD) (37). Most patients with the disease lived in heavily wooded areas away from the centers of towns, and the onset of their symptoms was often in the summer and early fall. The spatial and temporal distribution of cases hinted that the disease was likely transmitted through an insect vector, but the State Department of Health did not have any additional information. It would take six more years to identify the LD-causing pathogen.

In 1982, Wilhelm "Willy" Burgdorfer isolated a spirochete from *Ixodes scapularis*, the black-legged deer tick (7). This microorganism was soon shown to be responsible for the unique disease in Lyme, Connecticut. When ticks harboring this pathogen fed on New Zealand White rabbits, long-lasting EM-like lesions developed. Indirect immunofluorescence also confirmed that the rabbits produced antibodies specific to these spirochetes. A causal relationship between the newly discovered spirochete and LD in humans was established when the serum of clinically diagnosed patients revealed antibodies specific to the pathogen, which was subsequently named *Borrelia burgdorferi*. Despite its recent characterization, the earliest confirmed case of LD occurred in the 5,300-year-old Similaun Iceman ("ÖTZI") found preserved frozen in the Italian Alps (31). Arthritis was observed upon clinical examination, and DNA sequencing of samples from the Iceman confirmed the presence of *B. burgdorferi*.

While a great deal of work has focused on characterizing the pathogen, many challenges exist on the clinical and epidemiological fronts. Diagnosis and treatment of LD is complicated by *B. burgdorferi's* wide array of virulence strategies that allow it to infect multiple organ systems, lie dormant for long periods of time, and resist and suppress the host immune response (20, 47, 48). Additionally, the clinical manifestations can vary widely, which further heightens the challenge for clinicians to make a timely and accurate diagnosis. While a vaccine represents the most effective preventative measure, there are not any currently available on the market.

Due to limitations in disease surveillance, the reported number of LD cases in the United States is thought to be significantly lower than the Centers for Disease Control and Prevention's (CDC) annual estimate (34). Despite this, LD represents more than 80% of vector-borne illnesses making it the most common vector-borne disease in the country (3). Making matters worse, the home range of *B. burgdorferi's* vector and its reservoirs are expanding as the ecological landscape continues to change

(73). Approximately 90 million Americans live in states deemed "high risk" by the CDC, and even this high value is likely an underestimate of the total number of individuals at risk (13). Disease prevention depends on increasing public knowledge and awareness of *B. burgdorferi* and its disease-causing ability, *Ixodes* genus ticks and their life cycle, LD and its clinical manifestations, and of available precautionary measures for individuals in high-risk areas.

Introduction to *B. burgdorferi*

B. burgdorferi is a Gram-negative bacterium with an inner and outer membrane. Unlike most gram-negative organisms, *B. burgdorferi* lacks lipopolysaccharides (LPS) in the outer membrane and instead displays other immunogenic glycolipids (70). The spirochete bores through host tissues using internal periplasmic flagella that confer swimming motility and flat-wave morphology (32). While this flat-wave structure dominates, other pleomorphic forms exist under certain environmental conditions (see "Pleomorphic forms" and Figure 6) (44).

The *B. burgdorferi* genome consists of a single 910,725 base pair linear chromosome with 853 open reading frames whose products are involved in the basic processes of DNA replication, transcription, translation, solute transport, and energy metabolism (23). Due to a lack of biosynthetic genes, the spirochete is an obligate parasite and depends on an arthropod or mammalian host for survival. Aside from hemolysins and drug efflux pumps, *B. burgdorferi* lacks common virulence factors and instead relies on dynamic gene regulation to evade and suppress the host immune response (3).

As an obligate parasite, *B. burgdorferi* can be difficult to maintain in common laboratory cultures that do not closely mimic the host environment (1). To overcome this challenge, optimized Barbour-Stoenner-Kelly (BSK) media that contains 6% rabbit serum and a collagen matrix is used to support the growth of *B. burgdorferi* (62). *In vitro* cultivation consists of a two-step process whereby a rapidly growing starter culture is used to initiate the growth of a long-term culture with a high bacterial yield. While *in vivo* studies most accurately represent the conditions that *B. burgdorferi* naturally encounters, the ability to work with isolated, parasitic bacteria in the laboratory increases the feasibility of research.

Lyme Disease

The unique virulence and immune evasion strategies of *B. burgdorferi* manifest clinically in humans as a complex, multi-stage disease. In this section of the review, the life cycle of the arthropod vector, transmission to the mammalian host, and clinical manifestations of LD will be described.

1. Vector life cycle

Vectors are organisms, often insects, that harbor and transmit pathogens to other hosts. Within the *Ixodes* genus, black-legged deer ticks (*Ixodes scapularis*) are the main vectors that transmit *B. burgdorferi* to reservoirs such as mice and hosts such as humans. These reservoirs vary in their degree of competency to transmit *B. burgdorferi* back into an uninfected arthropod vector.

Ixodes ticks have a complex, two-year, four-stage life cycle, which consists of egg, larva,

nymph, and adult forms (Figure 1) (19). In the spring, adult female ticks at the end of their life cycle lay eggs that hatch into six-legged larvae within about 60 days. The females lay the eggs on grasses where the larvae will be exposed to mammals like deer and mice after hatching. To progress to the next stage of the life cycle, the larvae require a first blood meal which often comes from the reservoir-competent, white-footed mouse, but may also come from other small animals such as chipmunks, shrews, squirrels, and birds.

After larvae feed in the late summer to early fall, they molt into eight-legged nymphs that remain inactive during the winter months. In the spring, the nymphs take a second blood meal which allows them to develop into adults in the summer.

As adults, a third feeding, often from the reser-voir-incompetent white-tailed deer (*Odocoileus virginianus*), is required to reproduce. After feeding, adult male and female ticks copulate on the deer, and females lay their eggs the following spring to complete the life cycle.

2. Route of transmission

For humans to develop LD, a tick harboring *B. burgdorferi* must bite the human and take a blood meal (7). The tick's first feeding as a larva is unable to cause infection because the bacteria cannot be passed down in eggs and the arthropod can only acquire the spirochete through feeding (19). Therefore, larvae must take a blood meal from an infected, reservoir-competent organism to obtain the pathogen before transmission to humans is possible. If this first blood meal contains *B. burgdorferi*, the pathogen will colonize the tick midgut and the bacterial cells lose their motility. A secondary feeding during the nymph stage is required

Figure 1

The Ixodes genus tick life cycle.
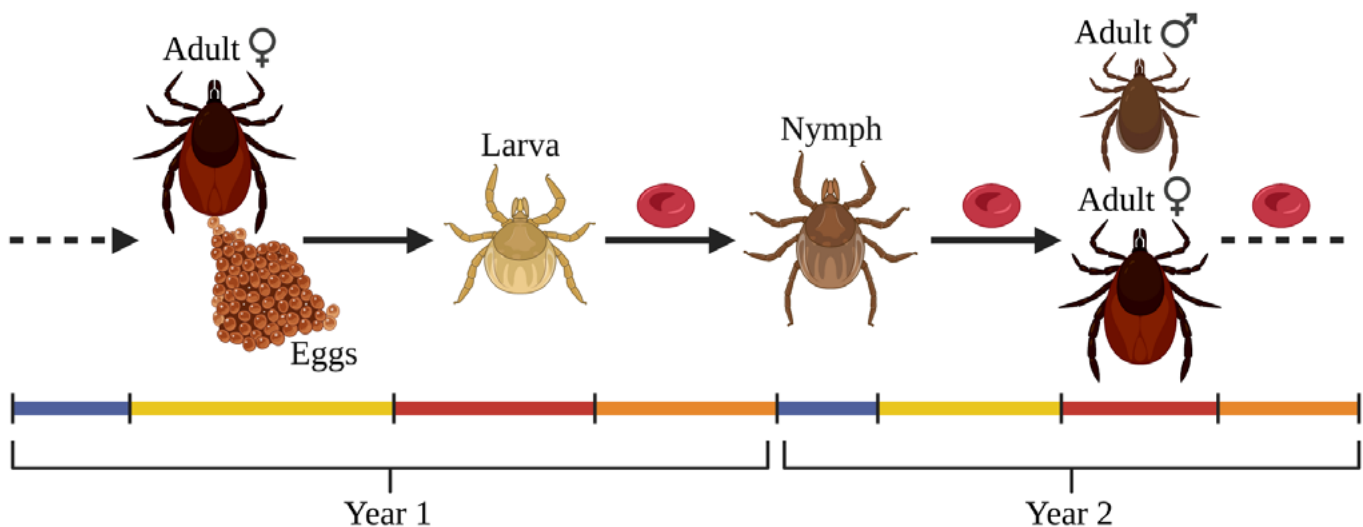
Note. A red blood cell indicates that a blood meal is required to progress to the following stage. The blue, yellow, red, and orange bars represent winter, spring, summer, and fall, respectively. Timeline is based on reference 19.

for *B. burgdorferi* to replicate, restore motility, and migrate to the tick's salivary glands where it becomes primed for transmission. Thus, nymphs represent the earliest stage of the tick life cycle where *B. burgdorferi* transmission resulting in LD in humans is possible.

To locate a host, ticks use a maneuver called questing where they climb to the top of grasses and other small plants, extend their front legs, and latch on to a passing animal (Figure 2B) (18). After finding a host, the tick migrates to a suitable location to take a blood meal. For

humans, one study determined the distribution of tick attachment to be 9% head-neck, 5% arm, 24% stomach/groin, 7% back, 18% chest/shoulder, 25% leg/foot, and 12% hip (Figure 2A) (25).

After a tick begins feeding, transmission of *B. burgdorferi* is not immediate. There is a positive correlation between the duration of vector attachment and the probability of *B. burgdorferi* colonization and disease (57). In a murine study exploring this relationship, infection was established in 7% of mice after 36 hours, 25% after 42 hours, and 75% after 48 hours. This

Figure 2

Tick questing behavior leads to attachment to hosts.
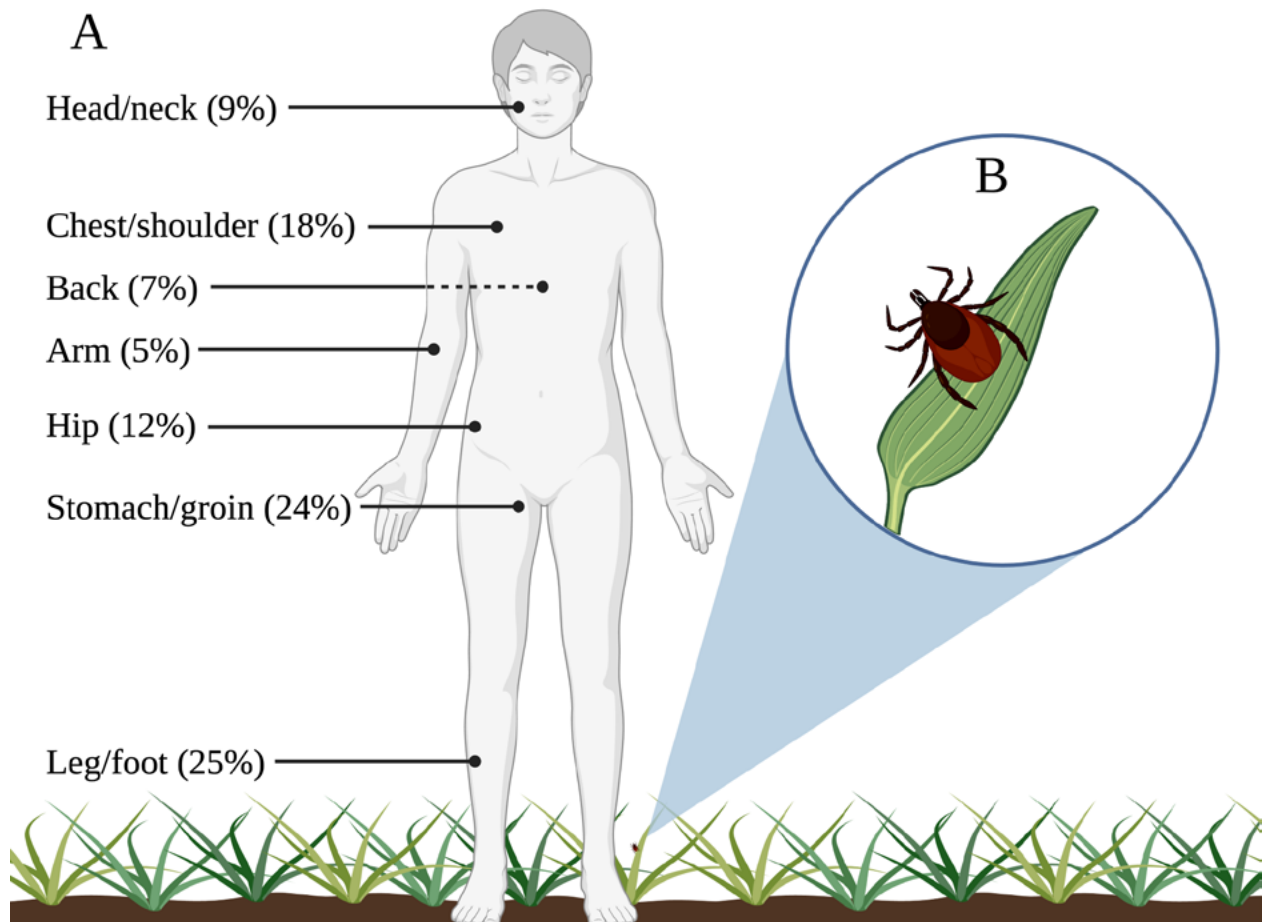
Note. Distribution of tick attachment sites on humans (A) and representation of the tick questing behavior that is used to seize a suitable host for a blood meal (B) (18, 25).

time-dependent transmission demonstrates the importance of rapid tick removal after attachment as a preventative measure against LD.

While there is no evidence that human-to-human transmission of *B. burgdorferi* is possible, there are many published cases of gestational LD with negative outcomes such as miscarriage, death following birth, and congenital abnormalities (72). However, a systematic review of these published cases concluded that most reports contained blinding issues, had missing or limited information on the mother's clinical symptoms, or used diagnostic methods that are no longer considered reliable. Therefore, additional research using reliable methods is necessary to determine the effects of gestational LD and the consequences it may have on women in their childbearing years.

3. Disease progression

LD progresses in three distinct phases termed early localized, early disseminated, and late disseminated infection (48). The basic signs, symptoms, and commonly affected tissues corresponding to each phase are presented in Figure 3, and each phase is described in depth below.

The first clinical manifestation of early localized *B. burgdorferi* infection is usually a slowly expanding cutaneous rash called erythema migrans (EM) (48). EM presents 7-14 days after exposure at the site of tick attachment in 70-90% of cases. The rash begins as a small red papule that takes on a bullseye appearance as it expands to an average diameter of 15 centimeters. EM is usually asymptomatic, but other flu-like symptoms such as headache, fatigue, malaise, and fever may occur. Early localized LD usually lasts for a few days to a month.

Early disseminated infection usually occurs three weeks to several months after the onset of primary EM, and it typically lasts 3-10 weeks (48). During this phase, *B. burgdorferi*

Figure 3

LD signs, symptoms, and commonly affected tissues.

Flu-like symptoms   Erythema migrans   Neuroborreliosis   Carditis   Arthritis
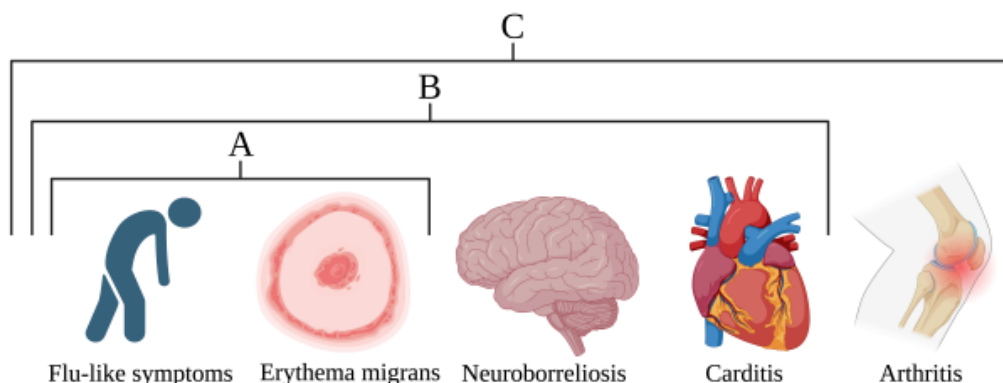
Note. Lyme disease progresses through three distinct stages: early localized (A), early disseminated (B), and late disseminated infection (C) (48). Each stage is characterized by different signs and symptoms that reflect the spread of the pathogen from the initial site of infection.

disseminates to the central nervous system (CNS), the cardiovascular system, and other cutaneous regions which leads to secondary EM away from the initial site. CNS involvement, termed Lyme neuroborreliosis, occurs in about 15% of untreated cases (22). Colonization of the nervous system can occur through penetration of the blood-brain barrier, migration from the peripheral nervous system, or movement through the cerebrospinal fluid. Common signs of neurologic involvement include bilateral cranial nerve palsies of the face, meningitis, and encephalitis. Cognitive impairment, psychiatric disturbances, and even seizures have been observed in patients with early disseminated LD (41). Cardiac involvement, termed Lyme carditis, typically occurs a few weeks to several months after EM onset in about 4-10% of untreated cases (21). Signs of Lyme carditis include prolonged PR interval, atrioventricular (AV) block, myocarditis, intra-ventricular conduction disturbances, bundle branch block, and congestive heart failure (50). Patients presenting with Lyme carditis may require electrocardiogram (ECG) monitoring and temporary or permanent pacemakers for AV block (74). General flu-like symptoms also accompany the early disseminated phase of infection (48).

Late disseminated infection, also called chronic LD, occurs months to years after the onset of primary EM and can last many years if untreated (48). Late-stage disease is marked by chronic, intermittent arthritis which occurs in about 80% of untreated individuals, along with continued neurologic, cardiac, and cutaneous manifestations (76, 30). Lyme-associated arthritis results from acute swelling and erythe-

ma in the joints, most commonly the knee (5). Excessive inflammation, infection-induced autoimmunity, and failure to down-regulate the inflammatory response are factors that contribute to chronic LD.

Virulence of *B. burgdorferi* in Hosts

Bacterial pathogens utilize several methods to spread through and damage host tissues. *B. burgdorferi* is no exception, and in this section of the review a few of the many disease-causing characteristics of this spirochete will be discussed.

1. Plasmid-derived virulence

In addition to the single, linear chromosome, the *B. burgdorferi* genome contains at least 17 linear and circular plasmids (lp and cp, respectively) (23). While some of these extrachromosomal DNA molecules have demonstrated critical roles in disease progression, others remain uncharacterized.

A cell's plasmid profile is its unique collection of plasmids. Long-term *in vitro* cultivation of *B. burgdorferi* results in concurrent changes to both plasmid profile and murine infectivity (64). Over time, the total number of plasmids within each cell decreases, which coincides with a drop in virulence. The degree to which the virulence of *B. burgdorferi* depends on its plasmid profile was expanded upon in a study that examined the infective phenotypes of a collection of clonal mutants, each with a different combination of plasmids (60). High-infectivity was observed in the presence of both linear plasmid 25 (lp25) and 28-1 (lp28-1), intermediate-infectivity was observed in the presence of lp25 and the absence of lp28-1, and

low-infectivity was observed in the absence of lp25 independent of lp28-1 (Table 1).

While some *B. burgdorferi* plasmids have been linked to highly infective phenotypes, others take on different roles (60). For example, cp26 is required to cause disease, but its presence does not affect the degree of infectivity. Other plasmids such as cp9 and lp21 are not associated with infectivity at all. Further characterization of *B. burgdorferi* plasmids linked to infectivity will lead to a greater understanding of the organism's requirements for infection and disease.

2. Temperature-dependent gene regulation

B. burgdorferi relies on both an arthropod vector and a mammalian host for survival. Upon transmission from vector to host, the spirochete experiences a temperature change from 23°C to 37°C. This change activates genetic regulatory mechanisms that allow *B. burgdorferi* to adapt to its surrounding environment and successfully establish a mammalian infection (65).

Outer surface proteins (Osp) are immunogenic lipoproteins found on the cell surface of *B. burgdorferi*. OspC, a known antiphagocytic factor, is required to establish a mammalian infection (8). In unfed ticks at 23°C, *B. burgdorferi* predominantly expresses *ospA* (65). After the tick takes a 37°C mammalian blood meal, the spirochete's *osp* expression profile changes to *ospC*. This was confirmed by examining *B. burgdorferi*-infected murine serum which contained antibodies specific to OspC. One explanation for this temperature-dependent gene regulation relies on the topology of the *ospC*-containing cp26 plasmid. At 23°C, the plasmid is in a supercoiled state which blocks the transcriptional machinery from accessing the *ospC* locus. *In vitro* work demonstrated that upon exposure to warm (37°C) mammalian serum the supercoiling of cp26 is reversed permitting expression of *ospC*.

Another explanation for the temperature-dependent regulation of *ospC* involves small regulatory RNAs (sRNA) (40). RpoS is an alternative sigma factor involved in the initia-

Table 1

B. burgdorferi *infectivity as it relates to linear and circular plasmid profile.*

Infective phenotype*, #	Infectivity-associated		Not infectivity-associated		Always present
	lp25	lp28-1	cp9	lp21	cp26
High	+	+	+/-	+/-	+
Intermediate	+	-	+/-	+/-	+
Low	-	+/-	+/-	+/-	+

Note. * + plasmid must be present for the given infective phenotype, - plasmid must be absent for the given phenotype, +/- plasmid does not affect infective phenotype
#Data from reference 60

tion of *ospC* transcription. Expression of *rpoS* is regulated by DsrAAB, a temperature-sensitive sRNA. At 23˚C, the *rpoS* transcript contains a secondary stem-loop structure that occludes the ribosome binding site (RBS) preventing translation of the sigma factor. Also at 23˚C, DsrAAB exhibits secondary structure which prevents interactions between the sRNA and the *rpoS* transcript that are necessary for high levels of gene expression (Figure 4A). At 37˚C, DsrAAB loses its secondary structure which allows it to post-transcriptionally regulate the *rpoS* transcript. This results in loss of the *rpoS* stem-loop structure, RBS availability, and active translation of RpoS sigma factors (Figure 4B). The high level of RpoS expression at 37˚C greatly enhances the expression of *ospC*, which helps facilitate transmission of *B. burgdorferi* from the arthropod vector to the mammalian host.

Since the discovery of this molecular thermometer, over 1000 other *B. burgdorferi*-sRNAs have been identified, many of which demonstrate temperature-sensitivity (59). These molecules likely contribute to genetic regulatory mechanisms that promote *B. burgdorferi* transmission by allowing the pathogen to adapt to changing environmental conditions.

3. Motility

B. burgdorferi possesses periplasmic bundles

Figure 4

Temperature-dependent expression of ospC.
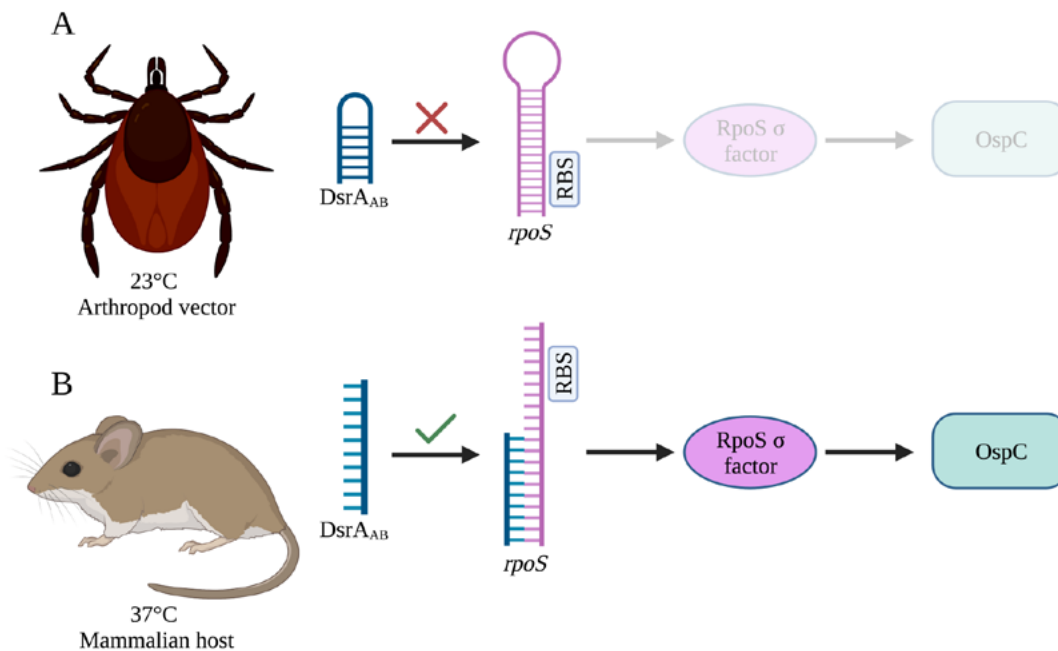
Note. A temperature shift accompanies the transmission of *B. burgdorferi* from the arthropod vector to the mammalian host (40). At 23˚C in the unfed tick, secondary structure of the sRNA DsrAAB prevents translation of the *rpoS* transcript, resulting in low levels of *ospC* expression (A). At 37˚C in the mammalian host, DsrAAB secondary structure is reversed, allowing for the production of *RpoS* and *OspC* (B).

of flagella that originate from basal bodies at its terminal ends (32). These structures confer flat-wave morphology and corkscrew swimming motility, which allow the spirochete to move both forward and backward as it bores through host tissues. This unique ability is integral to pathogenicity because it allows *B. burgdorferi* to disseminate throughout the human body, which results in the wide range of clinical manifestations associated with LD.

The *flaB* and *fliG* genes are required for flagellar functioning (68). The *flaB* gene encodes the major flagellar filament protein FlaB. *flaB* mutants are nonmotile, have a straight, bacillus morphology rather than the classic flat-wave morphology, and exhibit decreased viability in

both the mammalian host and arthropod vector. The *fliG* gene encodes the C-ring at the base of the flagellar basal body, which is important for rotational torque generation. Inactivation of *fliG* results in reduced motility and infectivity despite proper assembly of the flagellar filament (38, 39). These results indicate that periplasmic flagella of *B. burgdorferi* play an important role in transmission and infectivity.

4. Chemotaxis

Some microorganisms use chemotaxis to migrate toward a chemoattractant or away from a chemorepellent in the environment. *B. burgdorferi* utilizes chemotaxis to colonize arthropod vectors and to infect mammalian hosts.

Figure 5

Salp12 salivary protein serves as a chemoattractant for B. burgdorferi.
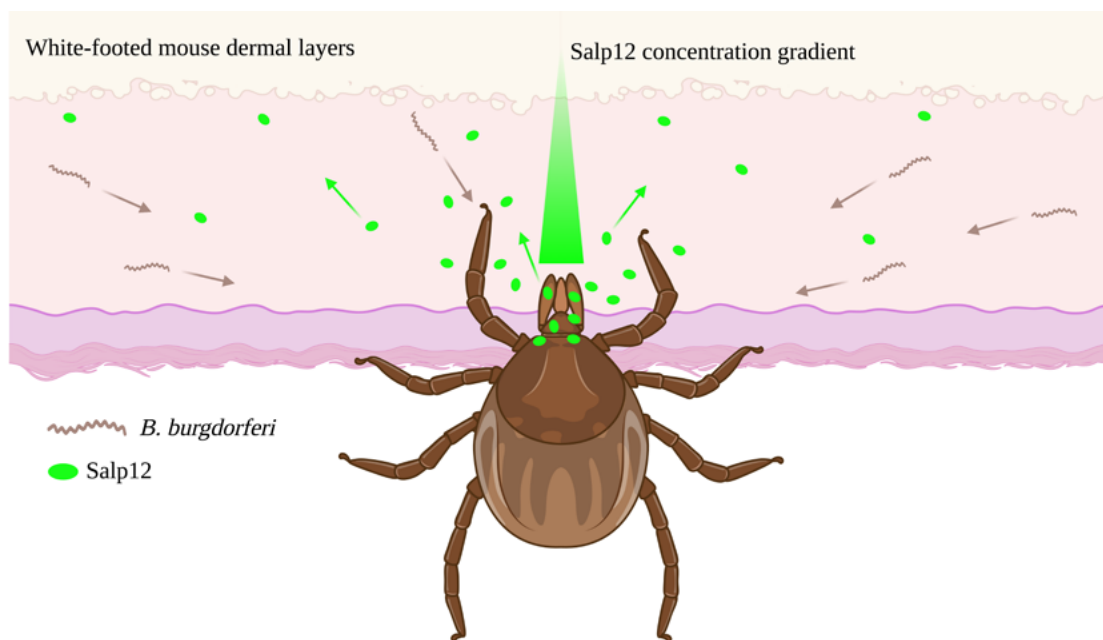
Note. Attachment of the *I. scapularis* tick to a host allows for the release of Salp12 salivary protein into the host (46). *B. burgdorferi* swims up the Salp12 concentration gradient to encounter the tick. This process promotes colonization of the tick midgut, which will allow *B. burgdorferi* to be transmitted to the next mammal that the tick feeds on.

The Salp12 salivary protein of the *Ixodes scapularis* tick serves as a chemoattractant for the bacterial cells that helps them colonize the arthropod vector (46). When uninfected ticks take a blood meal from an infected reservoir such as the white-footed mouse (*Peromyscus leucopus*), Salp12 diffuses into the host, attracting resident *B. burgdorferi* cells (Figure 5). This allows the bacteria to enter and colonize the arthropod midgut. Knockdown of *salp12* results in a significant reduction of *B. burgdorferi* colonization of the tick, which demonstrates the importance of chemotaxis for transmission of the pathogen to its vector.

To colonize the mammalian host, a chemotactic response that involves the *B. burgdorferi cheA2* gene is required (69). This gene encodes the histidine kinase of a two-component regulatory system that controls the directional rotation of *B. burgdorferi* flagella. Mutations in *cheA2* result in unidirectional movement and failure to be attracted into the mammalian host upon tick attachment. Interestingly, while *cheA2* mutants are unable to establish an infection in mammals, they retain the ability to colonize ticks. Together, these studies show the key role that chemotaxis plays during transmission of *B. burgdorferi* to the human host.

Innate Immune Evasion

The innate immune system is a nonspecific, noninducible line of defense that protects fungi, animals, and plants against a broad range of pathogens. *B. burgdorferi* uses many methods to evade this first line of defense.

1. Complement inactivation

The complement cascade is a tightly regulated pathway of sequentially activated proteins used to identify and eliminate pathogens through opsonization, phagocytosis, and formation of the membrane attack complex (MAC). *B. burgdorferi* expresses several Osps that disrupt this pathway including the surface lipoprotein BBK32, which binds and inactivates the C1 protease complex (2). This is the initiating component of the complement cascade, and its inactivation prevents all downstream steps. BBK32 mutants exhibit decreased virulence, which demonstrates the importance of complement inactivation for successful infection. Other Osps involved in complement disruption such as OspA, OspC, and CspA function by converting the blood protein plasminogen to plasmin, which is a known inhibitor of the cascade (24, 27, 55).

2. Antimicrobial peptide resistance

Antimicrobial proteins and peptides (AMPs) are produced by the host immune system to defend against pathogenic bacteria. Lactoferrin is an AMP that inhibits microbial growth by scavenging free iron, which is a cofactor required by most bacteria (9). *B. burgdorferi* avoids the effects of lactoferrin by using a manganese cofactor for biological redox reactions instead of iron (2). Cathelicidin is another AMP produced by many mammalian cells. While this molecule usually functions by interacting with cell surface components to disrupt microbial membrane integrity, it exhibits limited binding to the *B. burgdorferi* outer membrane (63). Additionally, the *B. burgdorferi* BBA57 surface protein has demonstrated the ability to downregulate the expression of some AMPs such as bactericidal/permeability-increasing protein (BPI), further promoting its virulence in hosts (6).

3. Phagocyte interference

The host immune response relies on phagocytic macrophages and dendritic cells to engulf and destroy foreign matter. While phagocytes effectively clear *B. burgdorferi* when exposed to purified bacterial cells *in vitro*, the pathogen can successfully evade these effects *in vivo* (16). This is likely due to the upregulation of the anti-inflammatory cytokine IL-10 in phagocytes upon *B. burgdorferi* engulfment. Normally, IL-10 functions to dampen the host immune response after pathogen clearance to prevent endogenous tissue damage. *B. burgdorferi*-induced premature overproduction of IL-10 inhibits the production of proinflammatory immune factors that are critical to the host's defense. Macrophages deficient in the ability to produce IL-10 generate greater levels of proinflammatory cytokines during *B. burgdorferi* infection, which promote phagocytic events that aid in removal of the pathogen (16). These findings demonstrate the important role that reprogramming the host immune response plays during *B. burgdorferi* infection.
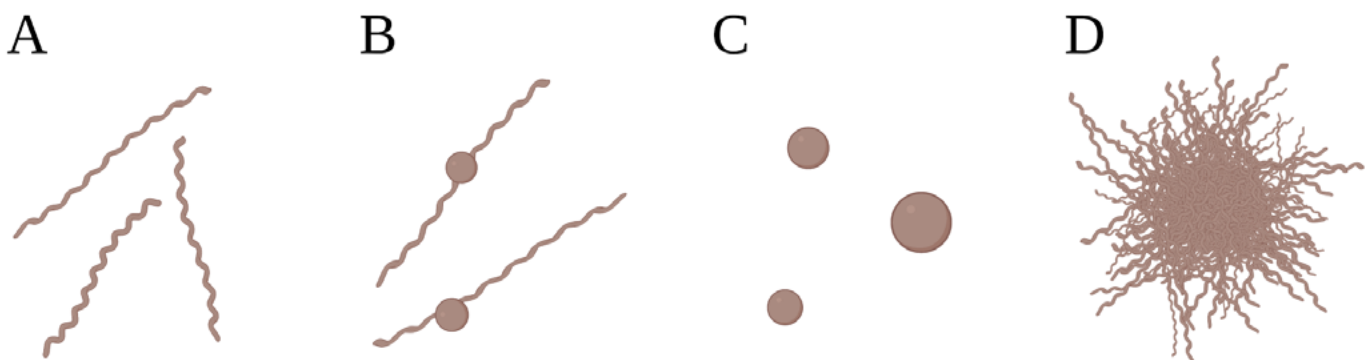
4. Pleomorphic forms

B. burgdorferi shows pleomorphism, which is the ability to alter cellular morphology. This is often used by organisms to survive in extreme environments. In addition to the dominant flat-wave morphology, *B. burgdorferi* has been observed in other forms including blebs, round bodies (RB), and biofilm-like (BFL) aggregates (Figure 6) (44).

At 37°C, nearly all *B. burgdorferi* cells are found in their dominant flat-wave or spirochetal form. Environmental stress signals such as extreme pH, high temperatures, and high levels of reactive oxygen species (ROS) result in a conversion of flat-wave cells to other forms such as RBs, which have reduced metabolic requirements (47). Subsequent removal of *B. burgdorferi* from these unfavorable conditions causes a reversion back to the dominant

Figure 6

B. burgdorferi *cells can be found in four distinct morphologies.*

Note. Schematic representation of *B. burgdorferi* pleomorphic forms based on images obtained from differential interference contrast (DIC) microscopy (44). Flat-wave spirochetes (A), blebs (B), round bodies (RB; C), and biofilm-like aggregates (BFL; D) are not drawn to scale.

flat-wave morphology. Additionally, low levels of BFL aggregates are thought to exist in all environmental conditions where, like biofilms, they promote attachment to host tissues and resist phagocytosis (44). These findings support the idea that *B. burgdorferi* enhances its survival by altering its morphology as environmental conditions change.

The human body contains many microenvironments where *B. burgdorferi* may exist in different pleomorphic forms. To more successfully diagnose and treat LD, it will be important to continue exploring the physiological niches where each morphology dominates. This will guide the pharmacological development of novel treatments that more precisely and accurately target each distinct morphology of *B. burgdorferi*.

Adaptive Immune Evasion

Unlike the nonspecific innate immune system, the adaptive immune system defends against specific pathogens using antibodies and immune cells produced in response to a past exposure. *B. burgdorferi* disrupts the normal functioning of the adaptive immune system in many ways, three of which will be described in this review.

1. Germinal center disruption

During an infection, antigen-presenting cells (APC) display segments of immunogenic proteins from a phagocytosed pathogen on the major histocompatibility complex class II (MHC II) molecules on their cell surface. Next, these APCs present the antigens to B- and T-lymphocytes in secondary lymphoid tissues such as the spleen and lymph nodes. In these tissues, germinal centers form, and it is within these structures that antibody-producing plasma cells and B-lymphocytes develop to confer long-term immunity. During a *B. burgdorferi* infection, the host immune system generates structurally defective, short-lived germinal centers that are unable to generate high quantities of antibody-producing immune cells (20). This leaves the host immunosuppressed and allows *B. burgdorferi* to cause further infection.

2. Antibody class switching

Several classes of immunoglobulins (Igs) exist, each with a unique function in the host immune response. During an infection, the host's ability to shift production from one Ig class to another is useful in targeting pathogenic microorganisms located in multiple tissue types. Upon *B. burgdorferi* infection, pentameric IgM molecules are produced in high quantities, while monomeric IgG production is suppressed (28). IgG is the major circulating Ig found in the blood, and downregulating its production results in a less effective immune response. This is yet another way that *B. burgdorferi* manipulates the host immune response to further propagate an infection.

3. Antigenic variation

B. burgdorferi modifies its immunogenic cell surface proteins in a process called antigenic variation, which promotes evasion from the host's adaptive immune response (15, 79). For example, the immunoreactive VlsE surface lipoprotein encoded on lp28-1 undergoes frequent modification (Figure 7). Upstream of the *vlsE* locus are 15 silent *vls* cassettes that randomly recombine into the expressed region

Figure 7

vls *cassette recombination leads to highly varied VlsE proteins.*
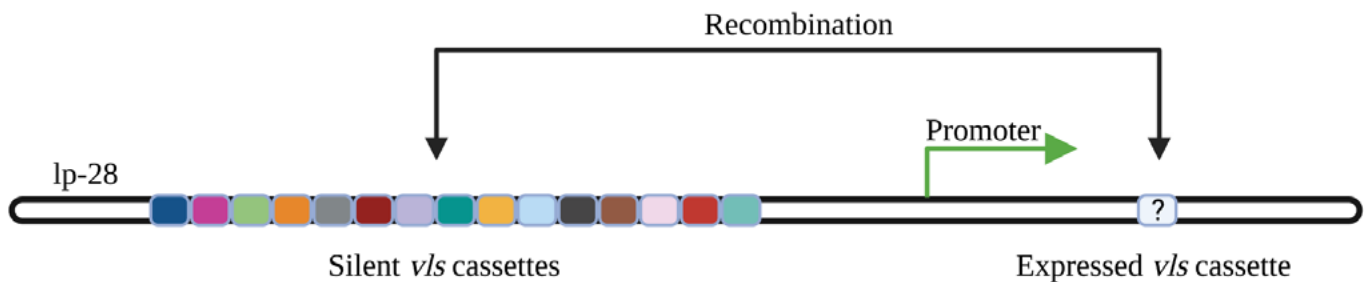
Note. Antigenic variation of the *B. burgdorferi* immunogenic VlsE surface lipoprotein encoded on lp28-1 is accomplished through recombination events between the upstream, silent *vls* cassettes and the downstream expressed region (15). This allows the pathogen to continuously modify the structure of the expressed antigen, which provides a mechanism of immune evasion.

of the gene during *B. burgdorferi* infection. This results in a mosaic VlsE with an estimated 10^{40} possible variants. Continuous modification of the structure of this surface lipoprotein prevents previously generated immune factors from functioning properly.

Incidence and Reporting of Lyme Disease

Understanding the epidemiology of vector-borne diseases such as LD is critical for disease prevention. The incidence rate and geographic distribution of infections provides health officials with pertinent information that can be used to organize public health efforts in high-risk areas. This section of the review will focus on LD surveillance, limitations in reporting, and ecological challenges that exacerbate the spread of disease.

1. CDC case definition

The CDC has published clinical guidelines that define a LD diagnosis (12). The 2022 case definition requires specific clinical and laboratory criteria to be met. Clinically, a patient must present at least one early or late-stage manifestation including EM, arthritis in one or more joints, nervous system abnormalities (lymphocytic meningitis, facial palsy, or unexplainable encephalomyelitis), or cardiovascular involvement (atrioventricular conduction defects). The laboratory criteria include at least one of the following: isolation of *B. burgdorferi* in culture, detection of *B. burgdorferi* by polymerase chain reaction (PCR), detection of *B. burgdorferi* antigens by immunohistochemical assay, or a positive two-tier serology test.

2. Surveillance data

Since its discovery, LD has been recognized throughout the world, particularly in Europe and Asia, and outside of the Northeast or New England region of the United States (67). Although surveillance in other countries is difficult, there are an estimated 85,000 cases annually in Europe, with the majority occurring in Germany, Austria, Slovenia, and Sweden.

In the US, LD is the most common vector-borne disease affecting an estimated 476,000 people annually from 2010-2018 (33, 34, 52). Most cases present in just a few states, with the highest incidences being reported in the Northeast, Mid-Atlantic, and upper Midwest regions (Figure 8) (67). According to the CDC, most cases occur in the summer months from June to August, which coincides with nymphal scavenging (13).

In the Northeast, there have been drastic spikes in disease occurrence, particularly in Maine where the incidence quadrupled between the years 2005 and 2015 (67). Northern expansion of LD is occurring, and this trend is expected to persist as the suitable habitat for ticks continues to expand (54).

In the Midwest, cases are on the rise with the majority concentrated in Wisconsin, Minne-

Figure 8

3-year (2018-2020) average LD incidence by state.
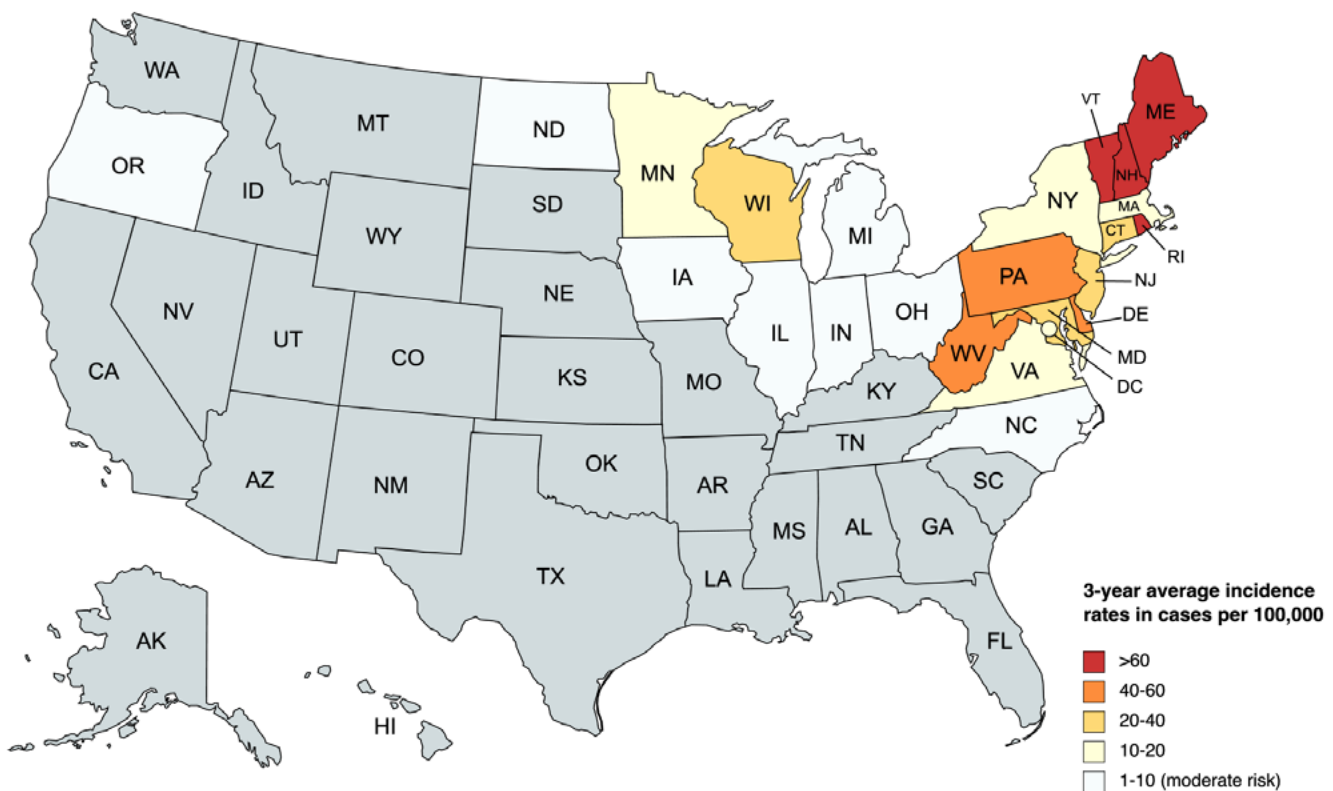
Note. The CDC considers a state "high risk" if it has greater than 10 confirmed cases of LD per 100,000 persons for three reporting years. In this review, states with 1-10 cases per 100,000 persons are considered to have moderate risk. This figure is based on data reported in reference 13.

sota, and northern Illinois (67). According to the CDC, Wisconsin had the fourth highest incidence of LD in 2019 behind Pennsylvania, New York, and New Jersey (13). Past models predicted further spread of *Ixodes* ticks throughout the Midwest into northern Michigan, the Ohio River Valley, and northwest Minnesota, which has indeed been observed in recent years (26).

In the Southeast, LD incidence is relatively low despite vectors being well established in areas such as coastal Florida, South Carolina, North Carolina, and Georgia (67). In this region, *Ixodes affinis* and *I. minor* ticks harbor *B. burgdorferi*, but these species lack the questing ability of *I. scapularis* and rarely feed on humans (4). Reported north to south gene flow of *I. scapularis* raises the possibility of altered southeastern tick behavior to that of the questing northern ticks, which could lead to a greater incidence of LD in this region in the future (78).

3. *Limitations in reporting*

LD has been a nationally reportable disease since 1991 (10). This means that physicians are required to report cases to state and local health departments who relay this information to the CDC. Due to the disease's recent characterization, the CDC points out limitations in surveillance that prevent the accurate estimation of LD incidence. These include both under-reporting in high incidence areas and over-reporting in low incidence areas due to clinical misclassification, inconsistencies in the funding and practices of health departments from one state to the next, and the collection of LD data based on area of residence rather than the location of exposure. The latter leads

to the misinterpretation of surveillance data for tourists who account for a significant proportion of cases. Furthermore, the LD case definition has undergone five modifications since deemed nationally reportable in 1991, which makes it more difficult for clinicians to stay current with reporting guidelines. Due to these limitations, the CDC estimates that the actual number of annual LD cases is about 10 times greater than what is reported.

The recent COVID-19 pandemic has also greatly affected the reporting of many diseases including LD (36). While surveys indicated that Americans spent more time outdoors in 2020 than in 2019 putting them at greater risk for LD, the CDC reported about half the number of confirmed cases (13). One group of researchers successfully predicted this discrepancy before the CDC published their 2020 incidence data (43). Their study looked at the online traffic of the CDC's tick removal website as an indirect quantifier of tick encounters (14). In 2020, the most recent year with published LD data, there were 25% more online visits than in 2019 suggesting that more individuals found themselves at risk for developing LD. Conversely, emergency department visits for tick bites and the frequency of LD diagnostic testing were significantly reduced in 2020. This was likely due to health officials and clinicians being preoccupied during the initial spread of SARS-CoV-2 in the spring and early summer of 2020, which coincided with the peak seasons for tick bites. On top of this, many patients delayed or avoided seeking out healthcare for more minor affiliations, due to fear of contracting COVID-19 or contributing to overburdened healthcare systems (36). This

sudden change in LD reporting was not consistent with long-term, epidemiological trends, which suggests that the COVID-19 pandemic significantly affected reporting. An organized public health effort is needed to address this discrepancy in LD reporting and restore a high standard of surveillance.

4. Climate and ecological challenges

After the colonial period in North America, deforestation, agricultural expansion, and urbanization drastically altered the landscape, which primed the area for LD spread (73). One way this has occurred is through shifts in predator community dynamics (35). Coyotes have replaced populations of both large and small predators such as wolves, bears, and foxes. The reduction in the number of foxes, which are more efficient predators of small mammals than coyotes, has decreased the amount of predation faced by *B. burgdorferi's* main reservoir, the white-footed mouse. This has allowed the white-footed mouse to expand its home range, which has broadened the potential area of *B. burgdorferi* transmission to humans. Additionally, the reduction of large predator populations along with vast agricultural expansion has allowed deer populations to flourish, increasing the reproductive range of LD vectors.

The gradual increase in the temperature of the Earth's atmosphere also plays a key role in host/reservoir expansion. Models used to study these effects suggest dramatic expansions of *Ixodes* tick populations, which will broaden the range of *B. burgdorferi* (56). Over the next four decades, the geographic distribution of the white-footed mouse is predicted to expand northward by about 300 kilometers (61). If this

manifests, it will have significant effects on the distribution of LD.

Disease Treatment and Prevention

Complete eradication of *B. burgdorferi* is implausible given its many hosts, reservoirs, and vectors. Rather, proper treatment of affected individuals and public health efforts to increase awareness of preventative measures in high-risk areas represent our best strategy to minimize the incidence of LD.

1. Treatment

Early localized infection is often treated with oral antibiotics such as doxycycline, amoxicillin, cefuroxime, or azithromycin (76). Pregnant women and children under the age of eight should avoid the use of doxycycline due to its adverse effects on bone development (29).

Early disseminated infection is treated based on the affected tissues. Patients with Lyme carditis or severe Lyme neuroborreliosis often receive intravenously administered ceftriaxone or cefotaxime, followed by one of the oral antibiotic regimens used to treat early localized infections (77). When only mild nervous system involvement such as isolated facial nerve palsy presents, oral antibiotic treatment usually suffices (76).

Treatment for late disseminated, chronic LD also depends on the presented signs and symptoms. If arthritis occurs without neurologic involvement, oral antibiotics are usually administered. If cardiac or neurologic involvement persists from the early disseminated phase of infection, intravenous antibiotic treatment may be necessary (76).

Prophylactic antibiotics are often prescribed in cases of suspected *B. burgdorferi* exposure after a tick bite. In a randomized clinical trial with patients that had removed an *Ixodes* genus tick within 72 hours, a single dose of doxycycline was 87% effective at preventing EM (49). This demonstrates the importance of immediate medical attention after a possible *B. burgdorferi* exposure to prevent long-term LD.

2. Vector-focused approach

While the elimination of *Ixodes* genus ticks is highly unlikely due to their vast, expanding range, local measures can be taken to greatly reduce the chances of human-tick interactions. One such method involves the use of carbaryl, an insecticide that is also lethal to arachnids, which has proven extremely effective at elimi-

nating tick populations (66). Unfortunately, this is a broad-spectrum insecticide that also kills moths, beetles, cockroaches, ants, and mosquitoes, which could have negative ecological effects (51). This downside of the use of carbaryl should be weighed against the positive impact of treating outdoor public gathering spaces and private landscapes to limit *B. burgdorferi* exposures (Figure 9A).

Permethrin is another insecticide that can be used against ticks, which poses little to no ecological threat since it is applied to clothing rather than broadly to the environment. Permethrin-based treatment of clothes and shoes has proven effective at preventing tick bites, killing ticks upon attachment, and preventing the transmission of pathogens such as *B.*

Figure 9

LD preventative measures.
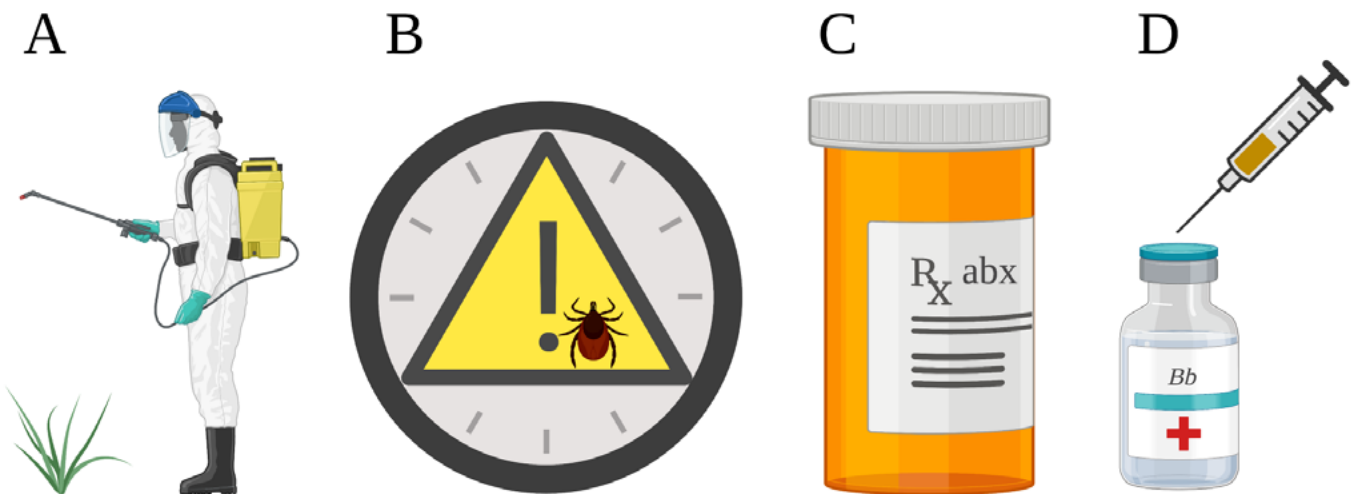
Note. The common methods for preventing LD include targeting reservoirs and vectors as well as best practices for humans who may have encountered ticks. These measures include the treatment of landscapes with insecticides (A), frequent self-examination and prompt removal of attached ticks (B), immediate medical intervention after exposure (C), and vaccine development (D) (17, 49, 57, 66).

burgdorferi (45). In a controlled study of the effectiveness of permethrin-treated outdoor gear, individuals wearing treated clothing received 3.36 times less tick bites than those wearing untreated clothing. Additionally, of the ticks attached to subjects, 97.6% were alive upon removal from individuals wearing untreated clothing, while only 22.6% of ticks were alive upon removal from those wearing permethrin-treated clothing. Outdoor gear can be purchased pre-treated with permethrin, or the insecticide can be applied at home carefully following the CDC's recommendations which include wearing protective gloves, reading the directions before application, allowing clothing to dry before use, and avoiding direct exposure to skin (11).

Other behavioral preventative measures should be taken to minimize the chances of acquiring LD. Frequent self-examination for ticks during and after outdoor activities in high-risk areas is critical (Figure 9B). As previously mentioned, there is a positive, nonlinear relationship between the duration of tick attachment and LD outcomes (57). Therefore, prompt removal of ticks and immediate prophylactic antibiotic treatment significantly decreases the chances of *B. burgdorferi* transmission (Figure 9C). Additionally, tucking pant legs into socks and covering bare skin in the outdoors are good strategies to prevent tick bites. Insect repellents that contain N, N-diethyl-meta-toluamide, commonly known as DEET, may also be helpful, but the effectiveness and duration of efficacy are inconsistent compared to other insecticides like permethrin (45).

3. Reservoir-focused approach

Small rodent reservoirs such as the white-footed mouse play a critical role in transmission of *B. burgdorferi* to its arthropod vector. While culling reservoir populations has been proposed, this would likely have negative ecological consequences. Instead, researchers have turned to the treatment of reservoirs to reduce populations of ticks and therefore the LD-causing spirochete (42). This has been attempted through the dispersal of permethrin-treated cotton in tick-infected areas, which is used by mice as nesting material. While this strategy significantly reduced *B. burgdorferi* transmission, additional research and development is needed to optimize the efficacy of this reservoir-focused treatment.

4. Vaccination efforts

While vector- or reservoir-focused approaches may be beneficial, immunization represents the most effective method of disease prevention (Figure 9D). In 1998, the United States Food and Drug Administration (FDA) approved a recombinant *B. burgdorferi* OspA vaccine called LYMErix (58). This vaccine functions by inducing the production of antibodies that target and eliminate *B. burgdorferi* as it enters the body during a tick bite. Less than four years after becoming available to the public, LYMErix was removed from the market due to low sales, anti-vaccine backlash, safety concerns, and class-action lawsuits. Although the FDA investigated the safety concerns and concluded there was a lack of evidence for the claims, the vaccine has not been available to the public since early 2002 (53).

Currently, companies such as Valneva are developing new LD vaccines designed to specifically protect individuals against North American and European strains of *B. burgdorferi* (17). These VLA15 protein subunit vaccines use the immunoreactive C-terminus of the OspA protein to induce protective immunity. They have proven effective in murine models and are currently undergoing human clinical trials. Wide-spread public acceptance of these vaccines in development represents our greatest potential defense against LD.

Additionally, researchers have begun to explore wildlife vaccination as an approach to LD prevention (71). OspA-based oral bait vaccines for reservoir populations of white-footed mice have demonstrated the ability to reduce the risk of LD in preliminary studies. These vaccines elicit an immune response that protects these animals from infection and reduces transmission of the pathogen to its arthropod vector. Decreasing the number of *B. burgdorferi*-harboring vectors in the environment will reduce the chances of human infection. Once the efficacy of wildlife vaccination is optimized, this may represent a successful long-term strategy to disease prevention.

Conclusion

B. burgdorferi, the causative agent of LD, is a highly complex parasite that uses an arthropod vector for transmission to mammalian hosts. The high rate of infection achieved by this spirochete is mainly derived from its unique ability to both evade and disrupt various aspects of the host immune response as it disseminates throughout the body. Clinically, LD has a variable presentation which makes it difficult

for physicians to diagnose and treat their patients.

In the United States, LD is a major public health concern, especially in the upper Midwest and Northeast regions. Surveillance limitations, a lack of public awareness, and failed vaccination efforts in the past represent some of the major challenges to disease prevention. Enhancing the general public's understanding of the risks associated with outdoor activities and providing individuals in high-risk areas with everyday preventative measures can decrease the incidence of LD in the short-term. In the long-term, widespread acceptance of novel vaccines is likely our most promising solution.

While LD presents several clinical and epidemiological challenges, a collective and coordinated public health effort represents our greatest chance of controlling the deer tick's dark secret.

Glossary of Abbreviations

AMP: antimicrobial protein/peptide

BFL: biofilm-like (aggregates)

BSK: Barbour-Stoenner-Kelly media

CDC: Centers for Disease Control and Prevention

cp: circular plasmid

EM: erythema migrans

FDA: United States Food and Drug Administration

Ig: immunoglobulin

lp: linear plasmid

LD: Lyme Disease

LYMErix: OspA Lyme Disease vaccine

Osp: outer surface protein

RB: round bodies

sRNA: small regulatory RNA

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