Diversity of the Lyme Disease Spirochetes and its Influence on Immune Responses to Infection and Vaccination

Jerilyn R. Izac, BS, Richard T. Marconi, PhD*

KEYWORDS
- Lyme disease • Canines • OspC • Ixodes ticks • Lyme vaccines • Borrelia
- Lyme diagnostics • OspA

KEY POINTS
- The Lyme spirochetes are a unique and genetically diverse group of bacteria.
- To complete the enzootic cycle, the Lyme spirochetes must adapt to the radically different environmental conditions encountered in ticks and mammals.
- Outer surface proteins A and C play distinctly different but critical roles in the biology and pathogenesis of Lyme disease.
- OspC is an immunodominant antigen produced during early infection. Antibody responses to diverse OspC proteins are OspC type specific and driven by variable domains of the protein.
- Understanding the diversity of the Lyme spirochetes and its surface proteins is essential for interpreting immune responses elicited by infection or vaccination.

THE DISCOVERY OF LYME DISEASE

Lyme disease (LD), as a clinical entity, was first described in the United States in the late 1970s (reviewed by Steere1). The path toward defining the basis of this debilitating infection began when concerned parents living in Lyme, Connecticut, contacted the Connecticut State Department of Health and reported an unusual clustering of juvenile rheumatoid arthritis cases in their area.1 A joint investigation launched by the
Connecticut State Department of Health and Yale University School of Medicine identified 51 cases of oligoarthritis of unknown cause in children and adults living in Lyme, Old Lyme, and East Haddam, Connecticut. Approximately 25% of the affected individuals recalled developing an enlarging rash in the weeks before disease onset. The general characteristics of the rash were similar to a rash described in 1909 in Sweden by Arvid Afzelius that was referred to as erythema migrans (EM).

Afzelius made several seminal contributions to our current day understanding of the epidemiology of LD, including the establishment of a connection between the bite of *Ixodes ricinus* ticks (a European *I.* species) and the development of EM. It was not until 1976 that a connection was made between the bite of *I. scapularis* ticks (formerly classified as *I. dammini*) and the development of EM and Lyme arthritis in patients in the United States. Shortly thereafter, researchers at the Rocky Mountain Laboratories (NIH) cultured a previously uncharacterized spirochete from *I. scapularis* ticks that was designated as *Borrelia burgdorferi*. A direct link between *B. burgdorferi* and Lyme arthritis was established with its cultivation from the blood of patients with LD. The first case of canine Lyme arthritis was diagnosed shortly thereafter.

Lyme disease is the most common arthropod-borne disease of canines and humans. The Companion Animal Parasite Council (CAPC) reported that there were 319,000 positive LD antibody tests in canines in 2018; up from 160,000 in 2012 (www.capcvet.org). As explained by CAPC, these values are underestimates because data are collected for only ~30% of the tests that are run. The Centers for Disease Control and Prevention estimates that the probable number of clinician-diagnosed cases of human LD each year in the United States is ~329,000.10 The incidence and endemic regions for LD and *I.* ticks are expanding in the United States, Canada, Europe, and Asia.

**CLASSIFICATION OF TICK-BORNE SPIROCHETES**

Before the identification of the LD spirochetes, the genus *Borrelia* consisted primarily of species associated with tick-borne relapsing fever (TBRF). The TBRF is a spirochetal infection transmitted by the soft-bodied *Ornithodoros* ticks. *Ornithodoros* ticks are anatomically distinct from the hard-bodied *I.* ticks that transmit LD. They also have different feeding strategies and developmental processes. Ticks that transmit TBRF are nocturnal feeders that reside in nesting materials in caves, rustic (unmaintained) cabins, and other similar structures. They feed rapidly and can transmit spirochetes within minutes. A hallmark feature of TBRF is a high-grade relapsing fever that coincides with the appearance of a remarkable number of spirochetes in the blood (10^6 to 10^8 mL^-1 blood) (Fig. 1A). The molecular basis of the cyclic spirochetemias can be traced to an elaborate antigenic variation system. Tick-borne relapsing fever occurs in isolated pockets in the United States but is widespread in other parts of the world. Its health consequences in parts of Africa are staggering.

Lyme disease is transmitted by tick species belonging to the genus *Ixodes*. *I. scapularis* and *I. pacificus* are the primary species that transmit LD in the United States and Canada, whereas *I. ricinus* and *I. persulcatus* are the primary vectors in Europe and Asia. *I.* ticks inhabit wooded areas, unkept brush, tall grasses, and leaf litter. They feed over the course of several days with transmission of the LD spirochetes typically requiring a feeding period of 24 to 72 hours. Transmission time can vary depending on the strain of the LD spirochete, the health of the tick and inherent variation among hosts. In contrast to TBRF, high-density spirochetemias are not a characteristic of LD. A notable exception is *B. miyamotoi*, which can cause transient spirochetemias.
Although *B. myiamotoi* causes a TBRF-like illness, this species is transmitted by *I.* ticks and is more closely related to the LD spirochetes than it is to the TBRF spirochetes. Several reviews have detailed the biology, health toll, and pathogenesis of TBRF in humans and canines.18,21,22 Soon after the discovery of *B. burgdorferi*, comparative studies of LD spirochete isolates from North America, Europe, and Asia revealed significant genetic and antigenic diversity. Based on these analyses, *B. burgdorferi* was divided into 3 distinct species: *B. burgdorferi*, *B. garinii*, and *B. afzelii*.23–25 *B. burgdorferi* is the primary species found in North America, whereas in Europe, all 3 species are present. Further exploration of the phylogenetic relationships among LD spirochete species and isolates led to the delineation of several additional species.26–29 The potential significance of these species in veterinary and human health remains to be defined.

The genus *Borrelia* has been recently divided into 2 genera: *Borrelia* and *Borreliella*.30 Consistent with taxonomic precedent, since the TBRF species were described first, they retain the *Borrelia* genus designation. The LD spirochetes and *B. myiamotoi* were assigned a new genus designation, *Borreliella*. Sharp differences of opinion exist concerning the practical implications of this reclassification.31,32 Although the use of the designation *Borreliella* is voluntary, readers should be aware of this change because it has been fully applied in public databases and is beginning to appear in the literature.

**UNIQUE FEATURES OF SPIROCHETES**

Spirochetes are distinct from other bacteria in several fundamental and fascinating ways. A feature shared by all spirochetes is their unique flat wave or spirallike ultrastructure (see Fig. 1A).33 This characteristic morphology results from the presence of endoflagella, which are found in all spirochetes. The flagella arrangement in *Treponema denticola*, the periodontal disease pathogen, is shown in Fig. 1B.
endoflagella are organized into 2 separate flagella bundles, each of which is anchored
to the inner membrane at opposite ends of the cell. The flagella bundles sit within the
periplasmic space and extend ~ three-quarters of the length of the cell.34

Distinguishing features specifically of the LD and TBRF spirochetes include the
composition of their cell wall and a unique genome arrangement (reviewed by Barbour
and Hayes21). Like Gram-negative bacteria, they possess an inner and outer mem-
brane, but lack lipopolysaccharide. Lipopolysaccharide is replaced by a diverse array
of lipidaded outer surface proteins (Osps) that play important roles in the host-
pathogen interaction. Some of these Osps are described in detail below. The LD
and TBRF spirochetes are distinct from all other bacteria, including other spirochetes,
in that they possess a small, segmented genome consisting of a linear chromosome
(0.9 Mb) and a series of linear and circular plasmids.35 Linear DNA is rare in bacteria.
The plasmids range in size from 9 to 200 kb and comprise nearly 40% of the total
genome.36 The total number and size of the plasmids carried by individual isolates
can vary significantly.37 Plasmid variation results from plasmid loss, acquisition and
genetic rearrangement.38 Some plasmids are dispensable,39 whereas others are
essential for infection or survival.40 The unique properties of the LD spirochete
genome are reviewed in.41

DEVELOPMENTAL STAGES OF IXODES TICKS

Lyme disease is maintained in nature in an enzootic cycle involving I. ticks and a
diverse array of mammalian reservoir hosts.42 The first developmental stage of a
tick is the larva. Because transovarial transmission of the LD spirochetes in ticks
does not occur, on emerging from the egg, larvae do not carry the LD spirochetes.
I. ticks can only become infected by feeding on an infected mammal through a pro-
cessed referred to as acquisition. After taking their first and only bloodmeal, the 6-legged
larvae detach from their feeding source and molt into 8-legged nymphs. This anatomic
change has important implications for tick biology and feeding behavior, because it
allows nymphs and adult ticks to climb up into brush where they gain better access
to larger and more mobile mammals. Nymphs also feed just once and then molt
into sexually differentiated adults. The body weight of an adult female tick may in-
crease by as much as 500-fold after the bloodmeal. Because male ticks do not
feed, they play no significant role in transmission of LD. Images of engorged adult fe-
male I. scapularis and Amblyomma americanum (lone star tick) ticks are presented in
Fig. 2. Pets and their owners are merely accidental hosts, and as such, do not play a
significant role in maintaining LD in nature.

ADAPTIVE RESPONSES AND THEIR IMPORTANCE IN THE ENZOOTIC CYCLE

The acquisition of spirochetes by ticks and their transmission to mammals are active
processes that are dependent on tightly regulated adaptive responses.43,44 Akins and
colleagues45 conducted a creative and pivotal study that provided insight into the na-
ture of adaptive responses. They compared the protein content of laboratory grown
spirochetes with that of host-adapted spirochetes. To accomplish this, cultures of
LD spirochetes were placed in dialysis membrane chambers and implanted in the peri-
toneal cavity of rats. Spirochetes maintained in dialysis membrane chambers become
host-adapted and thus, more closely resemble spirochetes during natural infection.45
Comparison of the protein profiles of laboratory-cultivated and host-adapted spiro-
chetes revealed significant differences in the production levels of OspA, OspB, and
OspC (as well as other proteins). OspA and OspB were produced at high levels in
laboratory-cultivated spirochetes but not host-adapted spirochetes.45 In contrast,
OspC production was low in laboratory spirochetes but high in host-adapted spirochetes (Fig. 3). The Akins study also proved central in shaping our understanding of humoral immune responses during infection. The Osp production patterns they reported are consistent with the development of a strong and early antibody response to OspC in mammals and the absence of a response to OspA and OspB. \(^{46}\) Low-level production of OspC during cultivation is well documented in the literature. \(^{47,48}\) Oliver and colleagues \(^{47}\) demonstrated that only 10% of the individual cells in a laboratory culture produce detectable amounts of OspC.

Adaptation to the distinctly different environmental conditions present in unfed ticks, fed ticks, and mammals also requires changes in Osp production. \(^{49}\) In an unfed tick, spirochetes residing in the nutrient-poor, midgut environment, produce high levels of OspA. Intake of a bloodmeal quickly changes the environment triggering a transition from OspA to OspC production. \(^{50}\) The upregulation of OspC at the tick-host interface is consistent with studies that have demonstrated that OspC is required for transmission and the establishment of an active infection in mammals. Strains that have been modified to not produce a functional OspC are unable to infect mammals. \(^{51-53}\)

**OUTER SURFACE PROTEIN VARIATION: INFLUENCE ON VACCINE AND DIAGNOSTIC ASSAY DEVELOPMENT**

The LD spirochetes produce a diverse array of Osps and the subset produced at any given time is controlled by environmental conditions. A comprehensive review of properties and functions of characterized Osps is beyond the scope of this report. Although several Osps have been investigated for use in vaccine or diagnostic assay development (reviewed by Earnhart and Marconi\(^ {54}\)), the discussion here is focused on OspC. OspC is a lipoprotein that varies in molecular weight (20–24 kDa) among isolates. \(^ {55}\) The ospC gene is carried by a highly stable circular plasmid of 26 kDa\(^ {56}\) referred to as cp26. \(^ {53}\) An individual LD isolate produces only a single OspC protein variant. The antigenic diversity of OspC among LD spirochete isolates is well documented and has been intensively studied. \(^ {57-59}\)
Before our current understanding of OspC phylogenetics, sequence variation seemed an insurmountable hurdle to overcome in efforts to use OspC as a vaccine or diagnostic antigen. It was assumed that ospC variation arises through mutation during infection with subsequent immune selection allowing for the emergence of new antigenic variants. However, OspC is genetically stable during infection. Numerous distinct and stable variants of OspC, referred to as OspC types, have been identified. OspC types are differentiated by a letter or other appropriate designation (reviewed by Marconi and Earnhart). OspC proteins of a given OspC type are conserved with percent amino acid identity values of ~95% or greater. Identity values between OspC types can be as low as 65%. For example, Earnhart and Marconi compared the sequences of 55 OspC type A proteins and found that amino acid identity values among these proteins were greater than 97%.

Studies by Brisson and colleagues have provided significant insight into the biological rationale for the existence and maintenance of multiple, stable ospC types in nature. Although an individual LD spirochete strain produces only a single OspC

Fig. 3. Adaptive responses of Lyme disease spirochetes. The protein profiles of spirochetes cultivated in the laboratory or host-adapted spirochetes that were grown in dialysis membrane chambers implanted within the peritoneal cavity of rats are shown. The proteins were fractionated by gel electrophoresis (SDS-PAGE) and visualized by staining with Coomassie blue. Note, the significant differences in the production levels of OspA, OspB, and OspC. Molecular weight standards are shown on the left. (Courtesy of Dr. Darrin Akins, The University of Oklahoma Health Sciences Center, Oklahoma City, OK.)
type, ticks commonly carry a heterogeneous population of strains that as a whole can produce many different OspC proteins. The existence of multiple OspC types in a given tick may help to ensure that upon feeding, at least a subset of the strains can infect an animal that has been immunologically primed by previous exposure to other OspC types. It has been hypothesized that OspC type identity may also influence mammalian host compatibility. Certain OspC types may facilitate infection of specific mammals. Rhodes and colleagues reported that the most common OspC type detected in infected canines was OspC type F. This is striking, because there have been no reports of the isolation of an OspC type F producing strain from humans. Although more research is required to address the biological rationale for the maintenance of distinct OspC types in nature, OspC diversity is critical to consider when assessing host immune responses to this immunodominant early antigen.

**ANTIBODY RESPONSES TO OspC DURING INFECTION AND ON IMMUNIZATION**

Evidence that antibody responses to OspC are type specific came from studies in which mice were inoculated with individual LD strains producing different OspC types. Immunoblot analyses of the infection serum collected from these mice revealed that IgG responses are OspC type specific. Rabbits immunized with purified recombinant OspC proteins also developed type-specific IgG responses. The lack of antibody cross-reactivity with different OspC types is intriguing because segments of sequence are shared by all OspC proteins (ie, are conserved). The specificity of the antibody response suggests that variable regions of OspC are presented to the immune system.

OspC type-specific antibody responses have also been demonstrated in naturally infected canines. Serum from dogs confirmed to be LD positive reacted with only a limited subset of OspC proteins in immunoblot analyses. The observed specificity of the OspC antibody response is consistent with epitope mapping studies that identified 2 dominant but variable epitopes of OspC. The regions corresponding to these antigenic domains were designated as the L5 and H5 epitopes. Although the sequences of these epitopes vary among OspC proteins, they are highly conserved among proteins of an individual OspC type. The immunodominance of the L5 and H5 epitopes likely explains the basis for type-specific nature of the OspC antibody response.

It has been reported in some studies that a conserved motif of OspC drives antibody responses. This suggestion is difficult to reconcile in light of the type-specific responses detailed above. This motif, referred to as either the C7, C10, or pepC10 motif, is proline rich and comprises the last 10 C-terminal residues of OspC. If a conserved sequence common to OspC types (ie, C10) constitutes a dominant epitope, then antibody to OspC should bind to all OspC proteins. In this report, potential immune responses to C10 were further investigated. All methods used in the experiments presented below have been described previously. Recombinant OspC proteins (types I, F, and T) were generated with or without the C10 motif (OspC-IΔC10, OspC-FΔC10, and OspC-TΔC10), purified and screened with serum from representative LD-positive horses (animal ID number, 1026) and dogs (TF1286). Serum from dog TF1286 bound to OspC type T but not to type F (Fig. 4). Infection serum from horse number 1026 bound to OspC type F but OspC type I. Furthermore, OspC proteins that lack the C10 motif were readily detected by antibody in infection serum. These observations support the contention that the C10 motif is not a dominant epitope and that it is the variable domains of OspC that drive antibody responses.
DOES INFECTION WITH THE LYME DISEASE SPIROCHETES ELICIT PROTECTIVE IMMUNITY?

It is common knowledge among veterinarians who practice in LD endemic areas that a significant percentage of dogs will develop repeated LD infections. This phenomenon is well documented in humans. In 1 study, 15% of patients with LD living in a Lyme endemic area developed 1 or more follow-up infections within 5 years.72 To add to our understanding of LD and protective immunity, we sought to determine if infection of mice with clonal populations of LD spirochetes results in broad, or strain-specific, bactericidal antibody responses. In this report, separate groups of mice were infected with *B. burgdorferi* B31, N40, and 297 and *B. afzelii* PKo using previously detailed methods.51 Sera harvested from the mice were then tested for bactericidal activity.

Fig. 4. Specificity of the antibody response to OspC in infected dogs and horses. Recombinant OspC proteins (types I, F, and T) with or without the putative C10 epitope were produced, purified, and transferred onto membranes for immunoblot analysis. The membranes were screened with serum from an infected dog (ID number, TF1286; top panel) or an infected horse (ID number, 1026; bottom panel), and IgG binding to each protein was assessed using the appropriate secondary antibody and chemiluminescence. Note that although antibody to OspC was detected, the antibody was not cross-reactive with the different OspC types. In addition, deletion of the C10 motif from each protein had no discernible impact on the level of antibody binding.
against each strain using in vitro assays. Representative data are presented in Fig. 5. Serum from mice infected with *B. burgdorferi* B31 efficiently killed B31 but did not kill *B. burgdorferi* N40, 297, or *B. afzelii* PKo (see Fig. 5). Conversely, serum from mice infected with *B. afzelii* PKo efficiently killed PKo but not *B. burgdorferi* B31, N40, or 297. To determine if killing is complement dependent, 1 set of reactions were run with heat-inactivated complement or no complement added. Guinea pig serum served as the exogenous complement source. No killing was observed unless active complement was included in the assay. The data indicate that serum-mediated killing occurs through an antibody-mediated, complement-dependent mechanism. More importantly, it can be concluded that infection with a given LD spirochete does not induce broadly protective antibody responses.

**PREVENTION: THE KEY TO TACKLING THE LYME DISEASE PROBLEM**

Vaccination is widely considered to be the most cost-effective approach for prevention of infectious diseases. Concerns about accurate diagnosis and appropriate treatment strategies for LD could be alleviated to some degree through aggressive...
vaccination. Several licensed LD vaccines are available and approved for use in canines. These vaccines are of 2 general types: bacterin and subunit. Currently available bacterin vaccines are NovibacLyme (Merck), LymeVax (Zoetis) and Ultra Durammune Lyme (Elanco). Available subunit vaccines are VANGUARD crLyme (Zoetis) and Recombitek Lyme (Boehringer Ingleheim).

LYME DISEASE BACTERIN VACCINES

The composition of subunit and bacterin vaccines are inherently different. Lyme disease subunit vaccines consist of highly purified recombinant proteins (OspA and or OspC), whereas bacterin vaccines consist of lysates of 2 laboratory-cultivated LD spirochete strains. The identity of the strains that comprise each commercially available bacterin vaccine is information that is not in the public domain. Because LD bacterin vaccines are generated from cell lysates, they contain a large number of proteins and other cellular constituents. In fact, genome sequencing and proteome analyses have demonstrated that the LD spirochetes can produce in excess of 1600 different proteins. Most of these proteins are produced during laboratory cultivation. The precise proteins that are present in any given bacterin vaccine have not been reported. Importantly, most of the proteins produced by bacteria under any growth scenario are localized within the cell and function in metabolic pathways and other important cellular processes. Although intracellular proteins can elicit an antibody response on vaccination with a cell lysate–based bacterin formulation, they are not likely to elicit productive antibody (ie, antibody that contributes to protective immunity), because in live cells intracellular proteins are not accessible to antibody. The removal of extraneous proteins from bacterins is conceptually beneficial because it would serve to direct and focus immune responses on immunologically relevant proteins.

The differential production of LD spirochete proteins under different environmental conditions may also influence the composition and antigenic content of subunit vaccines. Because bacterins are made from cultivated bacteria, they may lack potentially protective antigens that are produced by the LD spirochetes only during residence in mammals. Similarly, there are additional proteins that are not produced during culture or in mammals that are selectively produced in ticks. Antigens that are produced during infection in mammals or ticks would intuitively be those that are most desirable for inclusion in an LD vaccine. In this context, subunit vaccines offer some advantages in that they are composed of carefully chosen antigens with known production patterns. In addition, subunit vaccines lack extraneous proteins that are not involved in triggering protective immunity.

LYME DISEASE SUBUNIT VACCINES

Recombitek Lyme is a subunit vaccine consisting of lipidated OspA. Anti-OspA antibody inhibits transmission from ticks to mammals by targeting spirochetes in the tick midgut. OspA was also the sole component of LYMErix (SmithKline Beecham), the only human vaccine to have made it to market. LYMErix was introduced in 1998 but then voluntarily removed in 2001. There were many factors that contributed to its demise and detailed assessments of its rise and fall can be found in several excellent reviews. Leaving the more controversial issues aside, LYMErix was compromised by low efficacy (49%) after a 2-dose series. A 3-dose series increased efficacy to 76%. The requirement for multiple boosts is because OspA-mediated protection is strictly dependent on high circulating antibody titers. If titers drop below a critical threshold level, spirochetes are able to transit into a vaccinated animal.
OspA is not produced by the LD spirochetes in mammals, the spirochetes cannot be targeted by anti-OspA antibody after entering an OspA-vaccinated mammal. VANGUARD crLyme (Zoetis), the newest canine LD vaccine to be approved by the United States Department of Agriculture, is a subunit vaccine consisting of OspA and a recombinant chimeric OspC epitope-based protein referred to as a chimeritope. OspC chimeritopes consist of linear epitopes derived from several antigenically distinct OspC proteins that are joined together in a single recombinant protein. The rationale behind the development of OspC chimeritopes was to generate a protein that can elicit antibody that can target OspC proteins produced by diverse strains. The conceptual rationale for chimeritope proteins has been described in detail in earlier reviews and hence is not discussed further in this report. Antibody elicited by OspC chimeritope vaccine antigens can target spirochetes during the process of transmission and during early infection in mammals. A vaccine that induces antibody that can kill spirochetes in both ticks and mammals has the potential to use 2 synergistic mechanisms of protection and thus be less dependent on the maintenance of high circulating antibody titers.

WHERE DO WE GO FROM HERE?

The Lyme spirochetes are a fascinating and remarkably diverse group of bacteria with unique biological properties. In this report, we have focused our discussion on the importance of understanding environmentally regulated protein production, the genetic and antigenic diversity of the LD spirochetes and how that diversity influences immune responses to infection and vaccination. There are many topics not addressed here that are equally worthy of discussion. As we move forward, our ability to critically assess and interpret the results of past and future studies focused on LD will directly affect how successful we are in addressing this important veterinary and human health concern.

REFERENCES

1. Steere AC. Lyme disease. N Engl J Med 2001;345:115–25.
2. Steere AC, Malawista SE, Snyderman DR, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 1977;20:7–17.
3. Dammin GJ. Erythema migrans: a chronicle. Rev Infect Dis 1989;11:142–51.
4. Afzelius A. Verhandlungen der dermatologischen Gesellschaft zu. Arch Dermatol Syph 1910;101:104.
5. Steere AC, Broderick TF, Malawista SE. Erythema chronicum migrans and Lyme arthritis: epidemiologic evidence for a tick vector. Am J Epidemiol 1978;108:312–21.
6. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med 1984;57:521–5.
7. Benach JL, Bosler EM, Hanrahan JP, et al. Spirochetes isolated from the blood of two patients with Lyme disease. N Engl J Med 1983;308:740–2.
8. Steere AC, Grodzicki RL, Kornblatt AN, et al. The spirochetal etiology of Lyme disease. N Engl J Med 1983;308:733–40.
9. Lissman BA, Bosler EM, Camay H, et al. Spirochete-associated arthritis (Lyme disease) in a dog. J Am Vet Med Assoc 1984;185:219–20.
10. Nelson CA, Saha S, Kugeler KJ, et al. Incidence of clinician-diagnosed Lyme disease, United States, 2005-2010. Emerg Infect Dis 2015;21:1625–31.
11. Eisen RJ, Eisen L, Beard CB. County-scale distribution of *Ixodes scapularis* and *Ixodes pacificus* (acari: ixodidae) in the continental United States. J Med Entomol 2016;53:349–86.

12. Heyman P, Cochez C, Hofhuis A, et al. A clear and present danger: tick-borne diseases in Europe. Expert Rev Anti Infect Ther 2018;8:33–50.

13. Levy S. Northern Trek: the spread of *Ixodes scapularis* into Canada. Environ Health Perspect 2017;125:074002.

14. Sykes RA, Makiello P. An estimate of Lyme borreliosis incidence in Western Europe. J Public Health (Oxf) 2017;39(1):74–81.

15. Felsenfeld O. *Borrelia*. Strains, vectors, human and animal borreliosis. St Louis (MO): Warren H. Green, Inc.; 1971.

16. Barbour AG. Antigenic variation of a relapsing fever *Borrelia* species. Annu Rev Microbiol 1990;44:155–71.

17. Stoener HG, Dodd T, Larsen C. Antigenic variation of *Borrelia hermsii*. J Exp Med 1982;156:1297–311.

18. Cutler SJ. Relapsing fever Borreliae: a global review. Clin Lab Med 2015;35:847–65.

19. Keirans JE, Hutcheson HJ, Durden LA, et al. *Ixodes* (*Ixodes*) scapularis (Acari:Ixodidae): redescription of all active stages, distribution, hosts, geographical variation, and medical and veterinary importance. J Med Entomol 1996;33:297–318.

20. Fukunaga M, Takahashi Y, Tsuruta Y, et al. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolates from the Ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int J Syst Bacteriol 1995;45:804–10.

21. Barbour AG, Hayes SF. Biology of *Borrelia* species. Microbiol Rev 1986;50:381–400.

22. Piccione J, Levine GJ, Duff CA, et al. Tick-borne relapsing fever in dogs. J Vet Intern Med 2016;30:1222–8.

23. Baranton G, Postic D, Saint Girons I, et al. Delineation of *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol 1992;42:378–83.

24. Marconi RT, Garon CF. Identification of a third genomic group of *Borrelia burgdorferi* through signature nucleotide analysis and 16S rRNA sequence determination. J Gen Microbiol 1992;138:533–6.

25. Postic D, Edlinger C, Richaud C, et al. Two genomic species in *Borrelia burgdorferi*. Res Microbiol 1990;141:465–75.

26. Marconi RT, Liveris D, Schwartz I. Identification of novel insertion elements, restriction fragment length polymorphism patterns, and discontinuous 23S rRNA in Lyme disease spirochetes: phylogenetic analyses of rRNA genes and their intergenic spacers in *Borrelia japonica* sp. nov. and genomic group 21038 (*Borrelia andersonii* sp. nov.) isolates. J Clin Microbiol 1995;33:2427–34.

27. Margos G,S, Vollmer A, Cornet M, et al. A new Borrelia species defined by multilocus sequence analysis of housekeeping genes. Appl Environ Microbiol 2009;75:5410–6.

28. Postic D, Belfazia J, Isogai E, et al. A new genomic species in *Borrelia burgdorferi sensu lato* isolated from Japanese ticks. Res Microbiol 1993;144:467–73.

29. Pritt BS, Mead PS, Johnson DK, et al. Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetemia: a descriptive study. Lancet Infect Dis 2016;16(5):556–64.

30. Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus Borrelia into two genera: the emended genus Borrelia
containing only the members of the relapsing fever Borrelia, and the genus Borreliella gen. nov. containing the members of the Lyme disease Borrelia (Borrelia burgdorferi sensu lato complex). Antonie Van Leeuwenhoek 2014;105:1049–72.

31. Barbour AG, Adeolu M, Gupta RS. Division of the genus Borrelia into two genera (corresponding to Lyme disease and relapsing fever groups) reflects their genetic and phenotypic distinctiveness and will lead to a better understanding of these two groups of microbes (Margos et al. (2016) There is inadequate evidence to support the division of the genus Borrelia. Int. J. Syst. Evol. Microbiol. doi: 10.1099/ijsem.0.001717). Int J Syst Evol Microbiol 2017;67:2058–67.

32. Stevenson B, Fingerle V, Wormser GP, et al. Public health and patient safety concerns merit retention of Lyme borreliosis-associated spirochetes within the genus Borrelia, and rejection of the genus novum Borreliella. Ticks Tick Borne Dis 2018;10(1):1–4.

33. Charon NW, Greenberg EP, Koopman MB, et al. Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella. Res Microbiol 1992;143:597–603.

34. Charon NW, Goldstein SF, Marko M, et al. The flat-ribbon configuration of the periplasmic flagella of Borrelia burgdorferi and its relationship to motility and morphology. J Bacteriol 2009;191:600–7.

35. Barbour AG. Plasmid analysis of Borrelia burgdorferi, the Lyme disease agent. J Clin Microbiol 1988;26:475–8.

36. Xu Y, Kodner C, Coleman L, et al. Correlation of plasmids with infectivity of Borrelia burgdorferi sensu stricto type strain B31. Infect Immun 1996;64:3870–6.

37. McDowell JV, Sung SY, Labandeira-Rey M, et al. Analysis of mechanisms associated with loss of infectivity of clonal populations of Borrelia burgdorferi B31MI. Infect Immun 2001;69:3670–7.

38. Qiu WG, Schutzer SE, Bruno JF, et al. Genetic exchange and plasmid transfers in Borrelia burgdorferi sensu stricto revealed by three-way genome comparisons and multilocus sequence typing. Proc Natl Acad Sci U S A 2004;101:14150–5.

39. Dulebohn DP, Bestor A, Rego RO, et al. Borrelia burgdorferi linear plasmid 38 is dispensable for completion of the mouse-tick infectious cycle. Infect Immun 2011;79:3510–7.

40. Casjens SR, Gilcrease EB, Vujadinovic M, et al. Plasmid diversity and phylogenetic consistency in the Lyme disease agent Borrelia burgdorferi. BMC Genomics 2017;18:165.

41. Casjens S. Evolution of the linear DNA replicons of the Borrelia spirochetes. Curr Opin Microbiol 1999;2:529–34.

42. Hovius JW, van Dam AP, Fikrig E. Tick-host-pathogen interactions in Lyme borreliosis. Trends Parasitol 2007;23:434–8.

43. Iyer R, Caimano MJ, Luthra A, et al. Stage-specific global alterations in the transcriptomes of Lyme disease spirochetes during tick feeding and following mammalian host adaptation. Mol Microbiol 2015;95:509–38.

44. Schwan TG, Piesman J. Vector interactions and molecular adaptations of Lyme disease and relapsing fever spirochetes associated with transmission by ticks. Emerg Infect Dis 2002;8:115–21.

45. Akins DR, Bourell KW, Caimano MJ, et al. A new animal model for studying Lyme disease spirochetes in a mammalian host-adapted state. J Clin Invest 1998;101:2240–50.

46. Wilske B, Preac-Mursic V, Jauris S, et al. Immunological and molecular polymorphisms of OspC, an immunodominant major outer surface protein of Borrelia burgdorferi. Infect Immun 1993;61:2182–91.
47. Oliver LD Jr, Earnhart CG, Virgina-Rhodes D, et al. Antibody profiling of canine IgG responses to the OspC protein of the Lyme disease spirochetes supports a multivalent approach in vaccine and diagnostic assay development. Vet J 2016;218:27–33.
48. Xiang X, Yang Y, Du J, et al. Investigation of ospC expression variation among Borrelia burgdorferi strains. Front Cell Infect Microbiol 2017;7:131.
49. de Silva AM, Fikrig E. Arthropod- and host-specific gene expression by Borrelia burgdorferi. J Clin Invest 1997;99:377–9.
50. Schwan TG, Piesman J. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, Borrelia burgdorferi, during the chain of infection in ticks and mice. J Clin Microbiol 2000;38:382–8.
51. Earnhart CG, Leblanc DV, Alix KE, et al. Identification of residues within ligand-binding domain 1 (LBD1) of the Borrelia burgdorferi OspC protein required for function in the mammalian environment. Mol Microbiol 2010;76:393–408.
52. Earnhart CG, Rhodes DV, Smith AA, et al. Assessment of the potential contribution of the highly conserved C-terminal motif (C10) of Borrelia burgdorferi outer surface protein C in transmission and infectivity. Pathog Dis 2014;70:176–84.
53. Tilly K, Casjens S, Stevenson B, et al. The Borrelia burgdorferi circular plasmid cp26: conservation of plasmid structure and targeted inactivation of the ospC gene. Mol Microbiol 1997;25:361–73.
54. Earnhart C, Marconi RT. Lyme disease. In: Barrett AD, Stanberry LR, editors. Vaccines for biodefense and emerging and neglected diseases. 1st edition. London: Elsevier; 2009. p. 1032–60.
55. Fuchs R, Jauris S, Lottspeich F, et al. Molecular analysis and expression of a Borrelia burgdorferi gene encoding a 22 kDa protein (pC) in Escherichia coli. Mol Microbiol 1992;6:503–9.
56. Marconi RT, Samuels DS, Garon CF. Transcriptional analyses and mapping of the ospC gene in Lyme disease spirochetes. J Bacteriol 1993;175:926–32.
57. Earnhart CG, Marconi RT. OspC phylogenetic analyses support the feasibility of a broadly protective polyvalent chimeric Lyme disease vaccine. Clin Vaccin Immunol 2007;14:628–34.
58. Jauris-Heipke S, Fuchs R, Motz M, et al. Genetic heterogeneity of the genes coding for the outer surface protein C (OspC) and the flagellin of Borrelia burgdorferi. Med Microbiol Immunol 1993;182:37–50.
59. Theisen M, Frederiksen B, Lebech AM, et al. Polymorphism in ospC gene of Borrelia burgdorferi and immunoreactivity of OspC protein: implications for taxonomy and for use of OspC protein as a diagnostic antigen. J Clin Microbiol 1993;31:2570–6.
60. Marconi RT, Earnhart C. Lyme disease vaccines. In: Samuels DS, Radolf J, editors. Borrelia: molecular biology, host interaction and pathogenesis. Norfolk (United Kingdom): Caister Academic Press; 2010. p. 467–86.
61. Stevenson B, Bockenstedt LK, Barthold SW. Expression and gene sequence of outer surface protein C of Borrelia burgdorferi reisolated from chronically infected mice. Infect Immun 1994;62:3568–71.
62. Brisson D, Dykhuizen DE. ospC diversity in Borrelia burgdorferi: different hosts are different niches. Genetics 2004;168:713–22.
63. Brisson D, Vandermause MF, Meece JK, et al. Evolution of northeastern and midwestern Borrelia burgdorferi, United States. Emerg Infect Dis 2010;16:911–7.
64. Di L, Wan Z, Akther S, et al. Genotyping and quantifying Lyme pathogen strains by deep sequencing of the outer surface protein C (ospC) locus. J Clin Microbiol 2018;56 [pii:e00940-18].
65. Rhodes DV, Earnhart CG, Mather TN, et al. Identification of *Borrelia burgdorferi* ospC genotypes in canine tissue following tick infestation: implications for Lyme disease vaccine and diagnostic assay design. Vet J 2013;198:412–8.

66. Earnhart CG, Buckles EL, Dumler JS, et al. Demonstration of OspC type diversity in invasive human Lyme disease isolates and identification of previously uncharacterized epitopes that define the specificity of the OspC murine antibody response. Infect Immun 2005;73:7869–77.

67. Buckles EL, Earnhart CG, Marconi RT. Analysis of antibody response in humans to the type A OspC loop 5 domain and assessment of the potential utility of the loop 5 epitope in Lyme disease vaccine development. Clin Vaccine Immunol 2006;13:1162–5.

68. Earnhart CG, Buckles EL, Marconi RT. Development of an OspC-based tetravalent, recombinant, chimeric vaccinogen that elicits bactericidal antibody against diverse Lyme disease spirochete strains. Vaccine 2007;25:466–80.

69. Jobe DA, Lovrich SD, Schell RF, et al. C-terminal region of outer surface protein C binds borreliacidal antibodies in sera from patients with Lyme disease. Clin Diagn Lab Immunol 2003;10:573–8.

70. Lovrich SD, La Fleur RL, Jobe DA, et al. Borreliacidal OspC antibody response of canines with Lyme disease differs significantly from that of humans with Lyme disease. Clin Vaccin Immunol 2007;14:635–7.

71. Rousselle JC, Callister SM, Schell RF, et al. Borreliacidal antibody production against outer surface protein C of *Borrelia burgdorferi*. J Infect Dis 1998;178:733–41.

72. Khatchikian CE, Nadelman RB, Nowakowski J, et al. Evidence for strain-specific immunity in patients treated for early Lyme disease. Infect Immun 2014;82:1408–13.

73. Izac JR, Oliver LD Jr, Earnhart CG, et al. Identification of a defined linear epitope in the OspA protein of the Lyme disease spirochetes that elicits bactericidal antibody responses: implications for vaccine development. Vaccine 2017;35:3178–85.

74. Chu HJ, Chavez LG Jr, Blumer BM, et al. Immunogenicity and efficacy study of a commercial *Borrelia burgdorferi* bacterin. J Am Vet Med Assoc 1992;201:403–11.

75. LaFleur RL, Dant JC, Wasmoen TL, et al. Bacterin that induces anti-OspA and anti-OspC borreliacidal antibodies provides a high level of protection against canine Lyme disease. Clin Vaccin Immunol 2009;16:253–9.

76. Barbour AG, Jasinskas A, Kayala MA, et al. A genome-wide proteome array reveals a limited set of immunogens in natural infections of humans and white-footed mice with *Borrelia burgdorferi*. Infect Immun 2008;76:3374–89.

77. Fraser CM, Casjens S, Huang WM, et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature 1997;390:580–6.

78. Ojaimi C, Brooks C, Casjens S, et al. Profiling of temperature-induced changes in *Borrelia burgdorferi* gene expression by using whole genome arrays. Infect Immun 2003;71:1689–705.

79. Schutzer SE, Fraser-Liggett CM, Casjens SR, et al. Whole-genome sequences of thirteen isolates of *Borrelia burgdorferi*. J Bacteriol 2011;193:1018–20.

80. Schutzer SE, Fraser-Liggett CM, Qiu WG, et al. Whole-genome sequences of *Borrelia bissettii*, *Borrelia valaisiana*, and *Borrelia spielmanii*. J Bacteriol 2012;194:545–6.

81. Gilmore RD Jr, Mbow ML, Stevenson B. Analysis of *Borrelia burgdorferi* gene expression during life cycle phases of the tick vector *Ixodes scapularis*. Microbes Infect 2001;3:799–808.
82. Caimano MJ, Dunham-EMS S, Allard AM, et al. Cyclic di-GMP modulates gene expression in Lyme disease spirochetes at the tick-mammal interface to promote spirochete survival during the blood meal and tick-to-mammal transmission. Infect Immun 2015;83:3043–60.

83. Fikrig E, Telford SR 3rd, Barthold SW, et al. Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. Proc Natl Acad Sci U S A 1992; 89:5418–21.

84. Parenti D. Lyme disease vaccine–LYMErix. Conn Med 1999;63:570.

85. Nigrovic LE, Thompson KM. The Lyme vaccine: a cautionary tale. Epidemiol Infect 2007;135:1–8.

86. Zundorf I, Dingermann T. Death of a vaccine – the fall of LYMErix. Pharm Unserer Zeit 2008;37:38–9 [in German].

87. Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. N Engl J Med 1998;339:209–15.

88. de Silva AM, Zeidner NS, Zhang Y, et al. Influence of outer surface protein A antibody on *Borrelia burgdorferi* within feeding ticks. Infect Immun 1999;67:30–5.

89. Schwan TG. Temporal regulation of outer surface proteins of the Lyme-disease spirochaete *Borrelia burgdorferi*. Biochem Soc Trans 2003;31:108–12.

90. Earnhart CG, Marconi RT. An octavalent Lyme disease vaccine induces antibodies that recognize all incorporated OspC type-specific sequences. Hum Vaccin 2007;3:281–9.